LIFE HISTORY AND GENETIC DIVERSITY IN DESERT REPTILES AND AMPHIBIANS

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LIFE HISTORY AND GENETIC DIVERSITY IN

DESERT REPTILES AND AMPHIBIANS

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The overarching goal of my dissertation research is to understand the link between organismal biology and patterns of population genetic structure across temporal and spatial scales. I focus on three vertebrate species that inhabit the deserts of the southwestern United States and that have habitat requirements and life history traits likely to promote population genetic differentiation.

Chapter one examines genetic structure in a lizard endemic to sand dune patches within a shinnery oak dominated landscape in eastern New Mexico. I used mitochondrial DNA and microsatellite markers to examine the effects of historical and contemporary processes on genetic structure across the geographic range of *Sceloporus arenicolus*. I found three main genetic clusters based on mitochondrial data with significant population differentiation within each group at microsatellite loci. These data suggest that specialization on and colonization of the shinnery oak – sand dune landscape may be relatively recent and that the distribution of suitable habitat at fine scales may be less important to population connectivity than the persistence of larger networks of suitable habitat patches.

Chapters two through five address the maintenance and distribution of genetic diversity within and among breeding ponds for two syntopic anurans in Arizona and New Mexico. I examined the consequences of reproductive skew and larval mortality on the persistence of genetic diversity across generations and among breeding aggregations of the Great Plains Toad (*Bufo cognatus*) and Couch's Spadefoot Toad (*Scaphiopus couchii*). Reproductive skew results in reduced genetic diversity in some,

but not all, populations of both species, with a greater effect in larger ponds of *B. cognatus*. Larval mortality influences genetic diversity only when mortality rates are extremely high or when the larval duration is prolonged. I found high effective population sizes and only weak genetic differentiation at large geographic distances in contrast to expectations based on within pond patterns, desert landscapes, and the scale of genetic structure found in other amphibians. In these desert environments, amphibians are remarkably well-connected by gene flow despite a pronounced effect of within pond processes on the maintenance of genetic diversity.

BIOGRAPHICAL SKETCH

Lauren Michele Chan was born on May 31st 1977 to Ronald and Judy Chan the day after they moved into their new house on Helberta Avenue in Redondo Beach, California. She grew up on this quiet southern California street surrounded by family and friends and spoiled by tranquil morning fog, nearly endless sunshine, and a cool ocean breeze.

Despite growing up in a suburb of Los Angeles, Lauren's childhood was full of science and natural history. Each fall she spent weekends exploring the hands-on Discovery Center and wandering the halls of the Los Angeles County Natural History Museum with her parents and brother, Michael. At home, Lauren's mom fostered an appreciation for strange pets. Instead of dogs and cats, Lauren and Michael kept pet newts and hermit crabs, raised silkworms, and bred generations of crayfish that her brother had rescued from a Creole cookout. Lauren looked forward to family trips to the Pollywog Park and the whale watching center, but her favorite outings were the visits to the tidepools where she could wade around for hours touching the anemones and searching for sea hares and brittle stars.

Lauren graduated from Redondo Union High School in 1995 and chose the University of California at Berkeley for her undergraduate studies. She was fortunate to have incredible mentors in Harry Greene, David Wake, and Jim Patton who instilled in her a deep appreciation for natural history, organismal biology, and field biology. During her time at Berkeley, Lauren worked in the Museum of Vertebrate Zoology and did various field research jobs including trapping small mammals, measuring mangrove seedlings in Panama, and monitoring frogs in the backcountry of the Sierra Nevada Mountains in California. She conducted two senior research projects on salamanders at the MVZ. Under the guidance of Kelly Zamudio and David Wake, Lauren used molecular and morphological data to examine the evolutionary

relationships among three genera of Asian salamandrids, one of which was the same species she had as pet. For her honors thesis with advisors Marvalee Wake and David Wake, Lauren used histological sections of testes to characterize annual male reproductive cycles in four species of tropical bolitoglossine salamanders.

When Lauren graduated from college in 1999, she knew that she was headed to Cornell University to work with Kelly Zamudio and study herpetology and population genetics. She delayed the start of her graduate studies for one year and used this extra time to conduct a field season in the Sierra Nevadas, tie up research at Berkeley, and explore South America and China. As a graduate student, Lauren's research focused on North American desert taxa, but she was able to enjoy other field opportunities including instructing a field biology course in Kenya, taking field ecology at Archbold Biological Station with Dick Root and Peter Marks, and traveling to Brazil for field work with Kelly Zamudio and Jeanne Robertson. Following her dissertation, Lauren will conduct postdoctoral research at Brigham Young University in Utah studying the phylogeography and systematics of plants in the phlox family with Leigh Johnson and studying the comparative phylogeography of Patagonian reptiles and amphibians with Jack Sites.

For my parents and grandparents

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Harry Greene has been a friend and mentor and cheerleader since my undergraduate studies and has been central to my development as a biologist. He has always been generous with his time and is a reliable source of sound and thoughtful advice. He has taught me to be a careful observer of nature and has taught me some of the key skills of a field herpetologist. Discussions with Harry, even in casual hallway conversations, have shaped and focused my dissertation research in important ways. Because of Harry, I strive to always be mindful of natural history and behavior and to keep organismal biology at the heart of my research. For that, I am grateful.

Past lab members and other graduate students have contributed to my research. Jeanne Robertson and Kurt Galbreath were my first two Zamudio Lab siblings and have been excellent company. They provided a fresh perspective on my dissertation research and were always willing to help me problem solve or read drafts of manuscripts. Ania Wieczorek, Christine Voyer, and Angie Stevenson kept things running in the wet lab in an orderly manner allowing me to get through lab work in a similar fashion. I also benefited from valuable feedback and support I received from other members of the Zamudio and Greene labs: Jesús Sigala-Rodríguez, Rulon Clark, Jim Austin, Jonathan Richmond, and Anna Savage. I was fortunate to share offices

with Dana Hawley, Justin Schuetz, Jennifer Fox, Brian Barringer, and Jason Andras; they not only brought laughter, absurd stories, and new music to work each day, but they were also good friends who I could bounce ideas off and celebrate / commiserate the latest events in the lab or classroom.

Several faculty members in the department of Ecology and Evolutionary Biology had a significant impact on my experiences as a graduate student. Monica Geber was a member of my dissertation committee and contributed to my dissertation with important and thoughtful questions and suggestions that helped to focus my studies. The graduate field ecology course taught by Peter Marks and Dick Root at Archbold Field Station in Florida reminded me to look and observe. By the their example, I have learned to be a patient and inquisitive naturalist. I feel fortunate to have overlapped with these incredible field ecologists while at Cornell and to have also had the chance to teach the undergraduate field ecology course with Peter.

The staff of the Ecology and Evolutionary Biology department have kept my graduate years running as smoothly as possible. The genetic data in my dissertation are due largely to the expertise and generosity of Steve Bogdanowicz who tirelessly coached me and many others through the development of microsatellite libraries year after year. Linda Harrington looked after the graduate students with undeserved care and I am indebted to her for saving me from the consequences of missed deadlines. I also thank Janeen Orr, Rosie Brainerd, Alberta Jackson, DeeDee Albertsman, LuAnne Kenjerska, Patty Jordan, Carol Damm, Gary Oltz, John Howell, Brian Mlodinski, and Tim Larkin for keeping everything and everybody in Corson Hall ticking along.

My research benefited from interactions I had with colleagues at the Southwestern Research Station in Portal, Arizona. Amanda Talaba, Cedric Hall, and Jeanne Robertson helped with fieldwork during 2003. Robin Greene was an industrious and stellar field companion for two summers who helped me to keep a

sense of humor during dry spells. I must also thank her parents, Erick and Ann Greene, for sending Robin to Arizona to work with me; much of the field data would not have been possible without her assistance. Numerous private landowners granted me access to ponds on their land. Funding for my graduate work was provided by an NSF Graduate Research Fellowship, an NSF Dissertation Improvement Grant, a Budweiser Conservation Scholarship, the Andrew W. Mellon Foundation, a Sigma Xi Grant-in-Aid of Research, an Edna Bailey Sussman Internship, the Upstate Herpetological Association, the American Museum of Natural History, the American Society of Ichthyologist and Herpetologists, and the Liu Memorial Award. The Cornell University Graduate School made it possible for me to attend several professional meetings and the Orenstein Fund and a small scholarship allowed me to attend the Summer Institute of Statistical Genetics. EEB and Kelly made it possible to conduct five field seasons by supporting me financially and Kelly and Harry provided housing and company during several summers in Arizona.

I am fortunate to have an incredible family of friends at Cornell University who have cheered me on, kept me smiling, and made Ithaca my home. They have brought welcomed and much needed distractions as well as moral and academic support and lightheartedness.

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sautéed ramps, and the fern life cycle interpretive dance. Jeanne Robertson has been my best friend and partner in crime throughout graduate school and is quite possibly the world's kindest person. She has been the shoulder to lean on, a sounding board for research ideas, and an endless source of giggles, ridiculousness, smiles, wisdom, and love. I am lucky to have such remarkable friendships.

Gregor Yanega has helped me in finishing this degree with infinite patience, encouragement, and deadly strong cups of coffee. He was a lively companion during a frustrating field season and is the goofball pal in everyday life with contagious and relentless curiosity and enthusiasm. Gregor keeps me smiling and laughing and steadily believes in me.

Finally, and most importantly, thank you to my family: my parents, Ron and Judy Chan, my brother, Michael, my grandmothers Jane Mar and Mary Chan, and the extended Chan and Mar families. Though they may be puzzled by my desire to spend summers catching toads and lizards, they have seen the value in these intellectual pursuits and have supported me without question. I am grateful for their love, support, and confidence.

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CHAPTER ONE

GENETIC DIFFERENTIATION IN THE NORTH AMERICAN SAND DUNE LIZARD (*SCELOPORUS ARENICOLUS*), AN ENDEMIC HABITAT SPECIALIST

Abstract.— *Sceloporus arenicolus* is endemic to the Mescalero and Monahans sand dune systems of eastern New Mexico and western Texas. This species is a habitat specialist and is patchily distributed among sand dune blowouts within shinnery oak (*Quercus havardii*) dominated landscapes. We combine mitochondrial DNA sequences and microsatellite data to examine historical and contemporary patterns of differentiation in this species given the current distribution of suitable habitat. We find three genetic clusters of individuals generally concordant with geographic region; we also find high levels of genetic structure within each of these groups. Despite high habitat specificity, we do not find genetic structure at finer spatial scales. We discuss these results in the context of the scale of landscape heterogeneity, habitat connectivity, and conservation implications for this threatened taxon.

INTRODUCTION

The specialization of organisms to novel environments, prey bases, or ecological resources can facilitate divergence and speciation (Futuyma and Moreno, 1988; Orr and Smith, 1998; Schluter, 2001). Specialists are often dependent on resources or environmental conditions that are rare and/or patchily distributed, thereby increasing the likelihood of differentiation within species due to increased extinction risk and low genetic variation within patches (Hokit and Branch, 2003a; Kelley *et al.*, 2000) and limited gene flow among patches (Brouat *et al.*, 2003; Kelley *et al.*, 2000). Indeed, empirical studies have found that specialist taxa have higher levels of genetic

structure than closely related and co-occurring generalist taxa (e.g. Brouat *et al.*, 2003; Kelley *et al.*, 2000; Zayed *et al.*, 2005).

Because habitat specialists are explicitly linked to landscape features that are patchily distributed, habitat specialization may be particularly likely to result in geographically isolated populations that are genetically differentiated. The size and distribution of suitable habitat patches, the nature of the surrounding matrix of unsuitable habitat, and the vagility of the organism are factors that will ultimately determine the degree of connectivity and genetic structure (Arnaud, 2003; Funk *et al.*, 2005; Spear *et al.*, 2005). Identifying the scale at which habitat heterogeneity begins to limit gene flow will help us understand the degree to which patch size, patch connectivity, and landscape characteristics influence population persistence and the maintenance of genetic diversity (Manel *et al.*, 2003; Wiens, 2001).

The North American sand dune lizard, *Sceloporus arenicolus*, is a habitat specialist in the southwestern United States that evolved from within the more generalized *Sceloporus graciosus* species group (Flores-Villela *et al.*, 2000; Frabotta, 2002; Wiens and Reeder, 1997). This species is endemic to the Mescalero and Monahans sand dunes of eastern New Mexico and western Texas which are comprised of a mosaic of open sandy depressions (referred to as blowouts) and vegetated patches dominated by shinnery oak (*Quercus havardii*) scrub (Degenhardt *et al.*, 1996). Sand dune patches are diverse and unevenly distributed throughout the landscape. While individuals of *S. arenicolus* are found almost exclusively within blowouts, individuals are not uniformly distributed among them. This species prefers large blowouts with particular topography and thermal and physical characteristics (Degenhardt *et al.*, 1996; Fitzgerald *et al.*, 1997). Many seemingly suitable sites are unoccupied possibly due to local extinction and/or isolation (Fitzgerald *et al.*, 1997). Thus, *Sceloporus*

arenicolus is patchily distributed at several hierarchical scales primarily due to habitat heterogeneity and habitat specialization.

Here, we reconstruct the evolutionary history of *S. arenicolus* and infer patterns of differentiation throughout the range of this endemic lizard to understand the genetic consequences of habitat specialization and identify the scale at which gene flow is limited. We expect specialization to shinnery oak – sand dune habitat to result in a genetic signature of reduced population size (i.e. bottleneck) associated with colonization possibly followed by demographic expansion. In addition, we predict that contemporary demographic factors and dispersal rates should contribute to differences among populations and reflect the importance of contemporary landscape features. Using mitochondrial DNA (mtDNA) sequences and multilocus microsatellite genotypes, we examine the geographic distribution of genetic variation across the range of this endemic species and interpret our results in light of the features of the Mescalero and Monahans sand dune landscapes. Our goal is to determine how historical processes and contemporary landscape characteristics have influenced differentiation in this habitat specialist.

Sceloporus arenicolus is listed as an endangered species by the New Mexico Department of Game and Fish and is a candidate for federal listing with a priority number of two by the U. S. Fish and Wildlife Service (Department of the Interior, September 12, 2006). Habitat destruction by herbicide application to remove shinnery oak and fragmentation due to oil and gas development pose threats to sand dune lizard populations (Snell *et al.*, 1997). Because of the patchy distribution of suitable habitat throughout its range and its dependence on sand dune blowouts, attributes of the landscape at several spatial scales may be critical to the maintenance of connectivity among populations. Therefore, we identify the scale of genetic differentiation and

consider the potential consequences of anthropogenic alterations to the landscape for the persistence of *S. arenicolus*.

METHODS

Population Sampling and Laboratory Protocols

We obtained tissues samples throughout the range of *S. arenicolus* from ethanol preserved specimens in the Museum of Southwestern Biology (MSB) and individuals captured during the field seasons of 2003 – 2006 (Figure 1.1). Tissues from live-caught individuals were sampled as toe and/or tail clips preserved in 200 proof EtOH; individuals were released at the point of capture. We included as outgroups two samples of *Sceloporus graciosus* from the *graciosus* clade sister to *S. arenicolus* (Frabotta, 2002) collected from northwestern New Mexico. For mtDNA sequencing, we isolated DNA from tissues with the QIAquick DNeasy Extraction Kit following manufacturers protocols. For microsatellite genotyping, we isolated DNA with Chelex extractions consisting of incubation of tissues for 180 min at 55° C and 10 min at 99° C in 150 µL of a 5% Chelex solution (Chelex-100; BioRad) and 19 µg proteinase K.

We sequenced two mtDNA gene regions for 54 individuals of *S. arenicolus* and two *S. graciosus* (Figure 1.1; Appendix 1). Amplifications via the polymerase chain reaction (PCR) were conducted at a total volume of 25 µL and consisted of 1 µL DNA template, 1 % Hi-Di formamide, 1 x *Taq* buffer, 1.5 mM $MgCl_2$, 1.9 µM dNTPs, 1.5 µM of each primer (forward and reverse), and 0.625 U *Taq* polymerase. We targeted a portion of the cytochrome-b gene (cyt-b) with the primers MVZ 05 (5'-CGAAGCTTGATATGAAAAACCATCGTTG -3') and MVZ 16 (5'-AAATAGGAARTATCAYTCTGGTTTRAT -3') and the entire NADH dehydrogenase subunit 1 (ND1) and flanking tRNAs (Leu and Ile) and 16s ribosomal

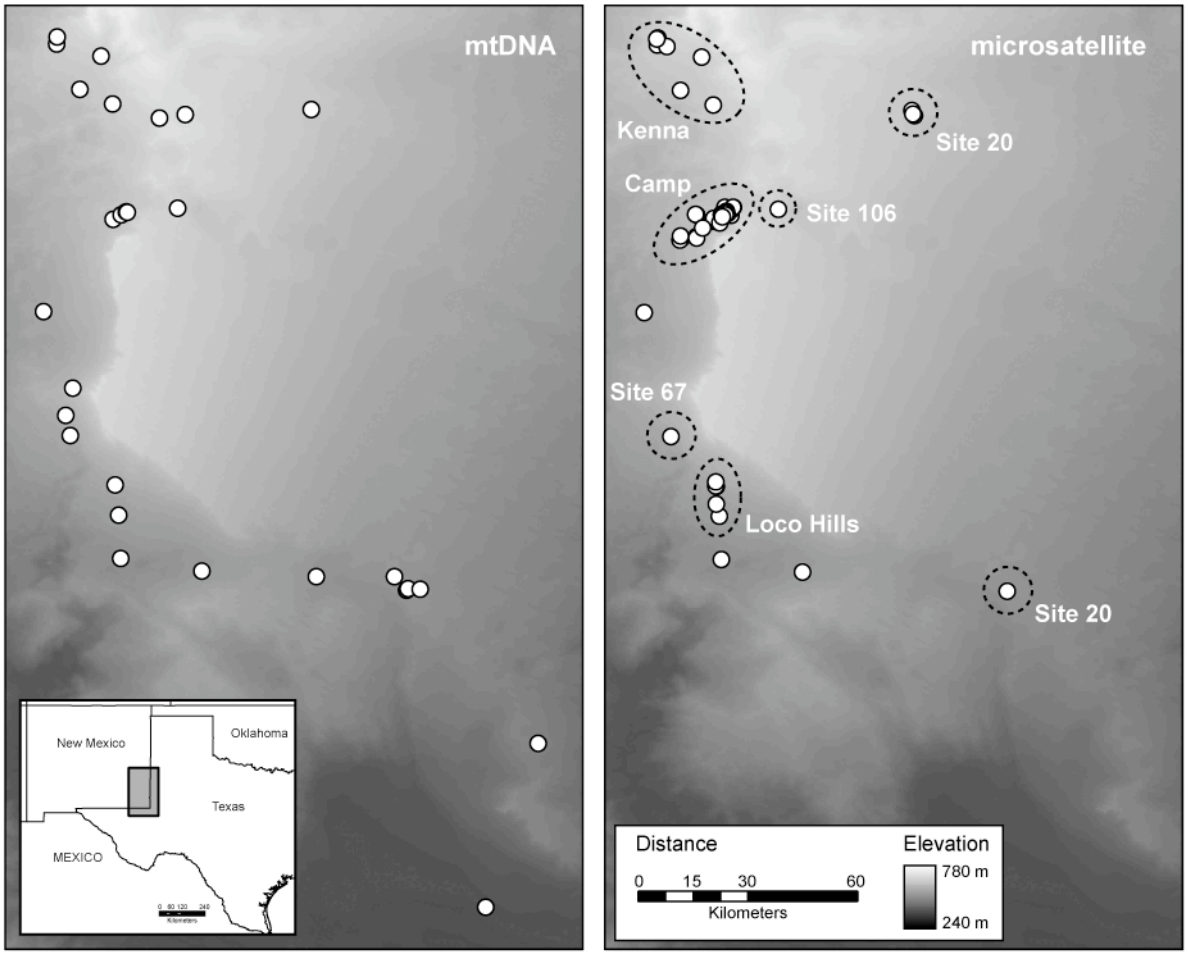

Figure 1.1. Topographic map of southeastern New Mexico and adjacent Texas with geographic collection localities for individuals in the mtDNA dataset (left) and the microsatellite dataset (right). The seven groupings used for population based microsatellite analyses are delineated with dashed lines.

RNA (16S) with the primers tMet and 16dR (Leaché and Reeder, 2002). PCR profiles consisted of an initial denaturation for 5 min at 94° C, 35 cycles of 30 s at 94° C, 30 s at 45° C or 54° C (cyt-b and ND1, respectively), and 1 min at 72° C, followed by a final 15 min extension at 72° C. PCR products were incubated with Exonuclease I (10 units) and SAP (1 unit) to remove unincorporated nucleotides/primers and cycle sequenced in both directions using Big Dye termination sequencing chemistry and the primers used in initial amplifications. Sequencing reactions were done in a total volume of 5 μL with 1 μL cleaned PCR product, 0.24 μM primer, 1 μL Ready Reaction Mix, and 0.5 μL Sequencing Buffer (Applied Biosystems). Cycle sequencing products were purified with Sephadex G-50 and visualized on ABI 3100 and ABI 3730 automated sequencers. Electropherograms were checked by eye prior to constructing contigs for each gene region for each individual in the program SEQUENCHER v4.5 (GeneCode).

To investigate genetic structure at finer geographic scales, we genotyped 233 individuals from seven populations throughout the range (Figure 1.1; Appendix 1) at seven polymorphic microsatellite loci using primers and amplification conditions described in Chan *et al.* (2007). Genotypes were collected on an ABI 3100 Automated Capillary DNA Sequencer with GeneScan-500 LIZ size standard; fragment sizes were scored using the software GENEMAPPER v3.5 (Applied Biosystems).

mtDNA data and phylogenetic history

We aligned sequences to the mtDNA genome of *S. occidentalis* (GenBank AB079242) in MACLADE v4.08 (Maddison and Maddison, 2003) to verify gene regions and check for stop codons and nonsense mutations. Alignments were straightforward with a single base pair insertion-deletion occurring between the outgroup and ingroup haplotypes in the tRNA-Ile. We created separate alignments for

the cytochrome-b (cyt-b) fragment and the ND1 plus flanking regions sequence (ND1-tRNAs-16S) as well as a combined alignment with both gene regions concatenated. We identified identical combined sequences and eliminated redundant haplotypes from the final mtDNA dataset. Unique haplotypes for each gene region were deposited in GenBank (accession no. EF558623-EF558664).

We inferred evolutionary relationships among *S. arenicolus* mitochondrial haplotypes using network based methods, Bayesian inference (BI), and topology tests in a maximum likelihood (ML) framework. We constructed haplotype networks in TCS 1.21 (Clement *et al.*, 2000) using a 95% parsimony connection threshold disregarding ambiguities between sequences. For BI and ML phylogenetic inference, we first used MRMODELTEST (Nylander, 2004) and the Akaike Information Criterion (AIC) to infer the model of sequence evolution that best fit partitions of our dataset (cyt-b and ND1-tRNA-16S considered as separate partitions including ambiguities). We conducted partitioned BI in MRBAYES 3.1.2 (Ronquist and Huelsenbeck, 2003) with rates for the cyt-b and ND1-tRNA-16S regions allowed to vary independently. Likelihood models with two and six substitution rates were applied to the cyt-b and ND1-tRNA-16S partitions. respectively. For both partitions, the prior state frequencies were set as flat Dirichlet distributions with a proportion of sites invariable. MRBAYES analyses consisted of two runs each of 10 chains sampling every 100 generations for 5,000,000 generations. We verified convergence by examining the trends in natural log likelihood scores for all parameters and disregarded the initial 10,001 trees from each run prior to estimating the 50% majority-rules consensus topology, branch-lengths, and posterior probabilities in MRBAYES.

To test the robustness of inferred haplotype clades, we used ML parametric bootstrap topology tests. Unconstrained ML topology searches for the combined mitochondrial dataset were implemented in PAUP 4.0b10 (Swofford, 2002) using the

preferred model from MRMODELTEST, a general-time-reversible model of sequence evolution with six substitution rates, a proportion of sites invariable, and equal rates among variable sites (GTR+I). We rooted topology searches with *S. graciosus* as the sister clade to all *S. arenicolus* and conducted 100 heuristic search replicates. Starting trees were obtained by random stepwise addition sequence and we used the TBR branch-swapping algorithm. We tested three alternative hypotheses regarding the monophyly of haplotypes from each of three geographic regions (Table 1.1). For each alternative hypotheses, we estimated the most likely topology and branch lengths for the mtDNA dataset using 100 heuristic search replicates constrained to the particular skeletal constrained topology. We then used MESQUITE 1.1 (Maddison and Maddison, 2006) to generate 200 random DNA sequence datasets of 1759 base pairs simulated on the preferred tree from the constrained search using the same model of nucleotide evolution assumed for phylogenetic inference. For each of the randomized datasets, we conducted one constrained and one unconstrained analysis of 100 heuristic search replicates. We used the differences in the tree scores between the two analyses for each dataset to construct the null distribution for comparison with the test statistic from the actual data using a one-sided *t*-test (Goldman, 1993).

Microsatellite data and population level genetic structure

Sample sizes for our seven collection localities for which we had microsatellite data were not equal. Therefore, we used the program HP-RARE (Kalinowski, 2004) to estimate overall and private allelic richness at each locus correcting for variation in sample size by standardizing counts for a population of five individuals. We tested multilocus genotypes for departure from Hardy Weinberg equilibrium (HWE) and evidence of linkage disequilibrium using randomization tests in GENEPOP v3.4 (Raymond and Rousset, 1995). A Markov chain method was used (Guo and

Table 1.1. Topologies and tree scores for unconstrained and constrained topologies used for parametric bootstrap tests of alternative topologies. We were not able to reject any of these constrained topologies.

Topology	Score
Unconstrained	3062.9724
(Northern) (Central + Southern)	3066.4058
((Northern + Central) Southern)	3065.3907
(Northern (Central + Southern))	3062.9724

Thompson, 1992) with 5,000 dememorization steps and 1,000 batches of 10,000 iterations each to determine significance for global and within population comparisons.

We used distance and model-based approaches to infer population structure, connectivity, and admixture from our microsatellite data set. First, we calculated pairwise F_{ST} (Weir and Cockerham, 1984) among the seven localities for which we had five or more samples using FSTAT 2.9.3 (Goudet, 1995) and used permutation tests with 10,000 replicates to determine levels of significance after Bonferroni adjustment for multiple comparisons.

To insure that our *a priori* population designations were not obscuring patterns of differentiation, we also examined population structure using Bayesian assignment tests. Bayesian approaches to estimation of population differentiation do not require assumptions about the source of sampled individuals. We conducted Bayesian assignment tests using Markov chain Monte Carlo (MCMC) sampling methods implemented in STRUCTURE 1.2 (Falush *et al.*, 2003; Pritchard *et al.*, 2000) to infer the number of genetic clusters (K) and genetic discontinuities among sampled individuals. For each K from $K = 1$ to 13, we conducted ten independent MCMC runs of 1,000,000 steps following a 250,000 step burn-in. For each run we assumed no correlation of allele frequencies among populations , did not use information on population of origin, and followed an admixture model with a single value of lambda inferred for all populations. We examined changes in the mean and variance in log likelihood scores across increasing value of K to identify the most probable number of clusters (Evanno *et al.*, 2005). For the most probable K, we examined individual membership coefficients (q) and the associated 90% probability intervals for assignment to each of the K clusters. Values of q less than 0.90 had wide 90% probability intervals, thus, we chose $q = 0.90$ as a cutoff for admixed individuals. For each population, we

calculated the proportion of individuals that showed evidence of admixture (all values of $q \leq 0.90$). We used DISTRUCT (Rosenberg, 2004) to plot the membership coefficients, q, of individuals to each of the K clusters with individuals ordered geographically from the northeastern to the southeastern parts of the range.

We additionally conducted tests for spatial autocorrelation implemented in GENALEX 6 (Peakall and Smouse, 2006) to identify the scale at which individuals exhibited non-random genetic similarity. We calculated indices of spatial autocorrelation (r_C) at 10 km distance class intervals and used 999 bootstrap replicates to estimate 95% confidence intervals. We conducted 999 permutations of individuals across distance classes to determine whether r_c was significantly different from zero at each distance class.

Historical and contemporary demographics

To test for population expansion in geographic regions, we calculated F_S (Fu, 1997) in ARLEQUIN v3.1 using 10,000 randomizations of the data to determine the significance of the test statistics. F_S calculates the probability of k or more alleles given the average number of pairwise differences individuals (Fu, 1997) and is particularly sensitive to population expansion and more powerful than other moment estimators (Ramos-Onsins and Rozas, 2002).

We also estimated historical demographics under a model of isolation with migration in the program IM (Hey and Nielsen, 2004; Nielsen and Wakeley, 2001) for two datasets (mtDNA sequences and mtDNA sequences plus multilocus genotypes). IM uses a Bayesian coalescent framework to estimate demographic parameters for a pair of populations. For two pairs of adjacent regions (northern and central; central and southern), we estimated time since divergence ($t = t\mu$), the effective size of each region ($\theta_1 = 4N_1\mu$ and $\theta_2 = 4N_2\mu$), the ancestral effective size ($\theta_A = 4N_A\mu$), and the

migration rate between regions ($m_1 = m_1 / \mu$ and $m_2 = m_2 / \mu$). We performed initial searches varying MCMC search parameters to assure adequate mixing and convergence. Final runs for the mtDNA data set consisted of ten chains heated linearly, an initial burn in of 250,000 steps, and sampling every ten steps for over 20,000,000 generations. Final runs for the combined dataset (mtDNA plus microsatellite genotypes) consisted of 20 chains heated with the two step option, an initial burn in of 250,000 steps with sampling every 25 steps for 10,000,000 generations. We compared the posterior probability densities for *t* from each of the four runs to determine the sequence of divergence among these regions and examined the posterior probabilities for unequal effective sizes ($\theta_1 > \theta_2$), population expansion within regions ($\theta_1 > \theta_A$; $\theta_2 > \theta_A$), and asymmetrical migration ($m_1 > m_2$) for each run.

We examined demographic parameters at finer scales among the seven populations using microsatellite data and the program MIGRATE 2.1.3 (Beerli and Felsenstein, 1999, 2001). MIGRATE uses MCMC chains to simultaneously estimate the effective size of each population ($\Theta_i = 4N_e\mu$) and immigration rates ($M_{ij} = m / \mu$) between population pairs. Because unbalanced sample sizes can skew estimations, we randomly selected twenty individuals from Camp to decrease the variance in sample size among populations. We used a Bayesian approach for our analyses with a stepwise mutation model and exponential priors for Θ and *M*. We conducted an initial search using start parameters for Θ and *M* estimated from F_{ST} with one chain of 1,000,000 steps sampled every 50 genealogies after an burn in of 100,000 generations. The estimates of θ and M from this run were then set as the start parameters for subsequent runs which varied in chain length, the number of chains, and the chain heating parameters. We examined the acceptance ratios and posterior probability densities for all values of Θ and *M* to determine the search parameters of the final run.

The final run consisted of two long chains (each of four heated chains) sampled every 20 steps for 1,000,000 generations following a burn in of 200,000 generations.

Finally, we examined our data for evidence of recent population bottlenecks. We used ARLEQUIN 3.1 (Excoffier *et al.*, 2005b) to calculate the Garza-Williamson index, *M*, which compares the number of alleles and the range of allele sizes (Excoffier *et al.*, 2005a; Garza and Williamson, 2001). Population bottlenecks should reduce the number of alleles, but not necessarily the range of allele sizes resulting in a small *M*; in contrast, stable populations will have an *M* close to 1.

RESULTS

Molecular data

We collected a total of 1759 base pairs (bp) of mtDNA sequence data for *S. arenicolus* and *S. graciosus*. The mitochondrial dataset corresponds to partial cytb-b (608 bp), complete ND1 (969 bp) and tRNA-Leu (75 bp) sequence, and fragments of 16S (29 bp) and tRNA-Ile (68 bp). We found 31 unique haplotypes (29 for *S. arenicolus* and two for *S. graciosus*), seven of which differed from other haplotypes only by ambigious base calls. *Sceloporus arenicolus* haplotypes contained 33 variable sites.

Regional diversity of mitochondrial haplotypes differed across the seven sampling localities. In the north, the four samples from Site 20 contained the same mitochondrial haplotype whereas we found six unique haplotypes among the thirteen individuals sampled from Kenna (Figure 1.2). Among central populations, we detected a single unique haplotype among three individuals at Site 106 and four distinct haplotypes among seven individuals at Camp (Figure 1.2). In Southern populations, six of seven individuals at Site 67 had the same haplotype while individuals from Loco Hills and Site 28 had multiple haplotypes. The three

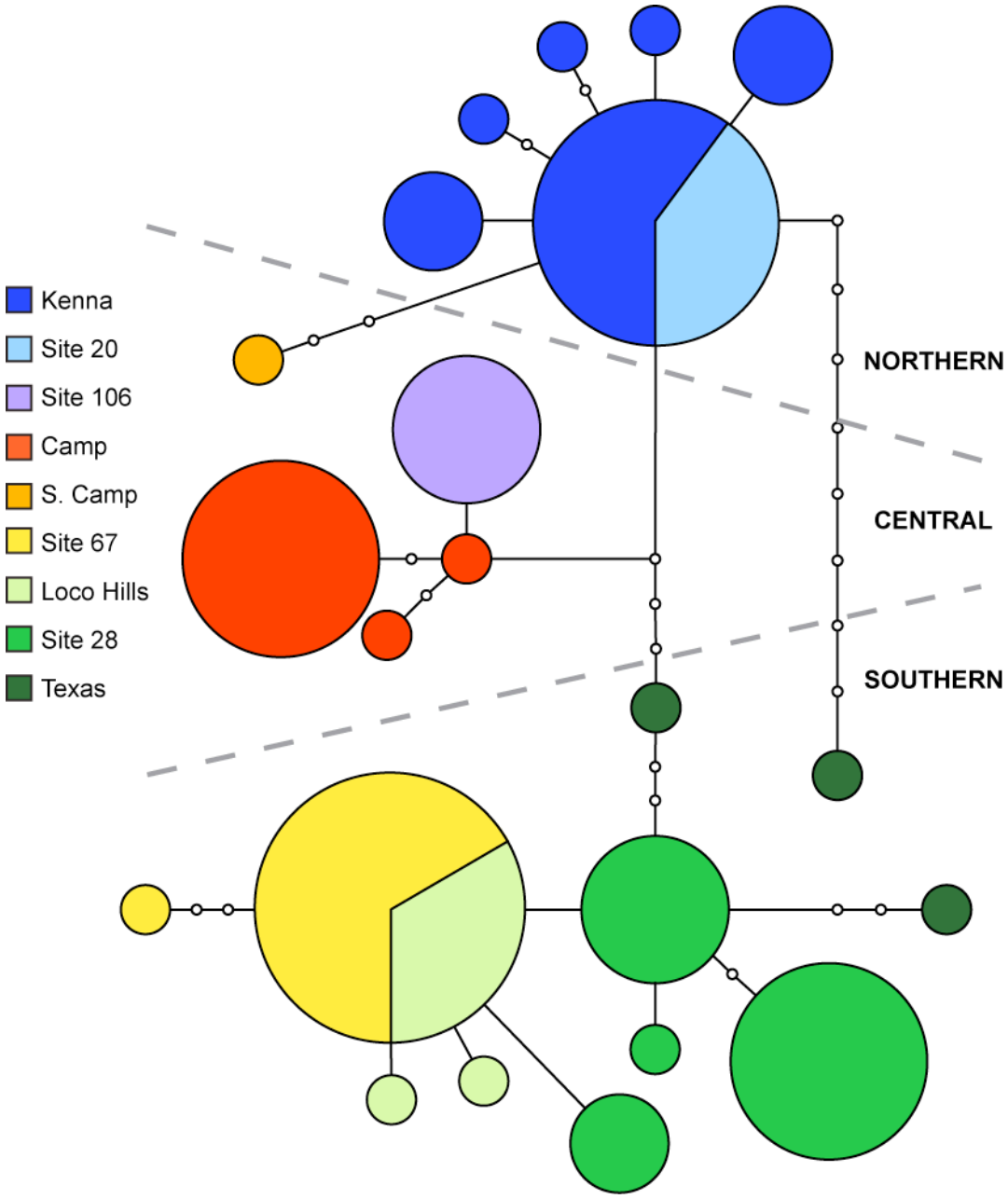

Figure 1.2. Haplotype network based on 1759 base pairs of mtDNA sequence for *S. arenicolus*. Colored circles represent unique haplotypes coded by collection locality. The size of circles is proportional to the number of sampled individuals with that haplotypes. Small open circles represent unsampled haplotypes and line connect haplotypes that are one mutational step apart.

individuals from Texas had unique and distant haplotypes (Figure 1.2, 1.3). Allelic diversity at seven microsatellite loci varied from 5 to 24 alleles per locus (average = 15.1). Population specific allelic richness for the seven sampling localities adjusting for differences in samples sizes varied from an average of 2.98 to 5.96 alleles/locus (Figure 1.4). The average number of private alleles per locus across populations after rarification ranged from 0.10 to 1.12. Expected heterozygosity within populations ranged from 0.507 at Site 20 to 0.789 at Camp. We found no evidence for departure from HWE at any locus in any of the seven localities at $\alpha = 0.05$ after Bonferroni correction for multiple comparison. We also did not detect significant linkage disequilibrium for any locus pairs within or among populations (all $p > 0.177$).

Phylogenetic history

Mitochondrial haplotypes differed by one to nine mutational steps with an average of 1.86 steps (Figure 1.3). Both haplotype networks and Bayesian phylogenetic inference resolved three distinct groups generally concordant with geographic locality (Figure 1.2, 1.3). All haplotypes from individuals collected from northern sites (Kenna and Site 20) are in the same Northern haplotype group. Haplotypes from individuals collected at central sites (Camp, Site 106, and individual MSB57684) and southern sites (Site 67, Loco Hills, Site 28, and Texas) mostly fall within the Central and Southern haplotype groups, respectively. One central haplotype from near Camp, and one southern haplotype from Texas, were more closely associated with Northern haplotypes. The other two Texan samples were associated with Southern haplotypes (Figure 1.2, 1.3), although one haplotype fell outside all other southern samples (Figure 1.3). The Bayesian posterior probabilities for the Central and Southern groups were relatively high (0.9611 and 0.9240).

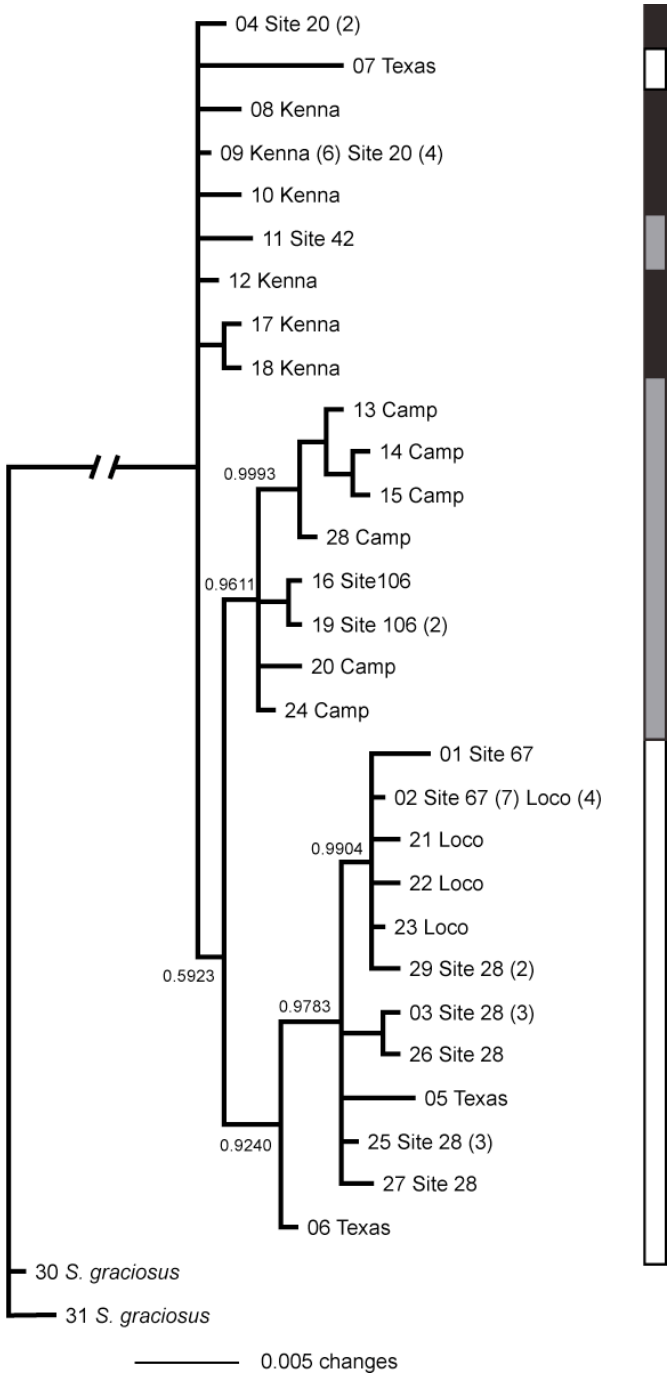

Figure 1.3. 50% majority rules consensus phylogeny from Bayesian analysis of mtDNA sequences. Posterior probabilities are indicated at the notes. Sampling localities are given at the tips with the number of individuals with that haplotype in parentheses. The vertical bar is shared with the geographic region that each haplotype was sampled from (northern – black, central – gray, southern – white).

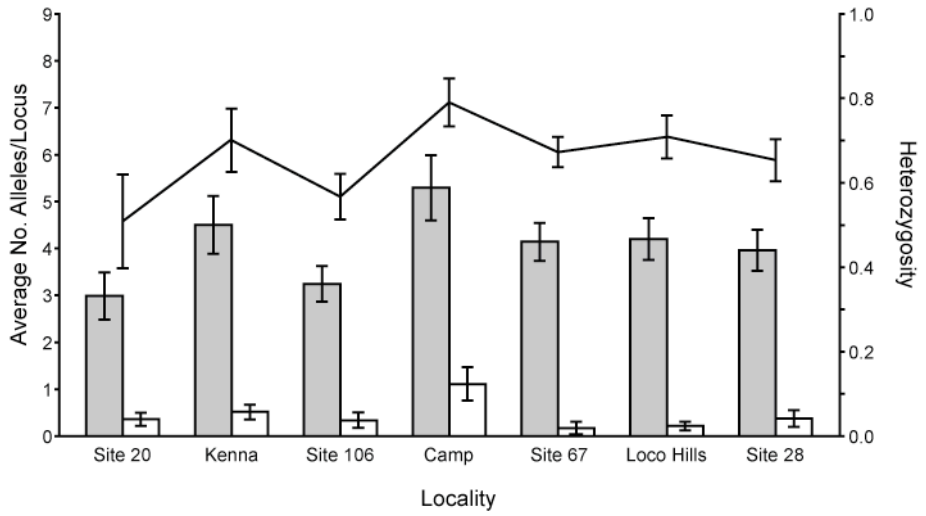

Figure 1.4. Average allelic richness (gray bars) and private allelic richness (white bars) corrected for sample size and expected heterozygosity (line graph) with SE bars for each of seven genotyped populations.

Support for relationships among the three haplotype groups was much weaker; the posterior probability for a Northern clade was only 0.0930. Likewise, the posterior probability for a clade containing both Central and Southern groups was 0.5922. Our inferred phylogenies using BI and ML found that northern haplotypes, plus one central and one southern haplotype, formed a basal polytomy relative to the remaining haplotypes sampled. We were unable to reject our alternative hypotheses in topology tests constraining individuals based on geographic locality (Table 1.1).

Population level genetic structure

Pairwise estimates of F_{ST} based on microsatellite data for the seven collection localities revealed significant differentiation among all but one pair with statistically significant values of F_{ST} ranging from 0.0572 to 0.3471. In general, pairwise values of F_{ST} were greater between populations from different mitochondrial haplotype clades (Table 1.2). The average F_{ST} within regions was 0.1000 (range: 0.0752 to 0.1169) whereas the average F_{ST} for pairwise comparisons of populations in different regions was 0.1969 (range: 0.0572 to 0.3471).

I found greatest support for three genetic clusters in Bayesian assignment based on microsatellite data (northern, central, and southern clusters; Figure 1.5) corroborating the results from mtDNA. The degree of admixture varied across populations. All 21 genotyped individuals from Site 20 unambiguously fell within a northern cluster. Only eight of 21 individuals from Kenna were unambiguously assigned to this cluster whereas the remaining individuals were assigned to both northern and central clusters, or to all three clusters (Figure 1.5). All individuals from Site 106 and most individuals from Camp were assigned with high probability ($q >$ 0.9) to a central group. The other individuals from Camp showed evidence of admixtures ($q_{max} < 0.9$) to northern and/or southern clusters as well (Figure 1.5). Most

Table 1.2. Pairwise F_{ST} estimates based on genotyped individuals from seven collection localities. Gray boxes unite population within the same geographic region. $^{*}\ p \leq 0.05$, $^{**}\ p \leq 0.01$, $^{***}\ p \leq 0.001$.

	Site 20	**Kenna**	**Site 106**	**Camp**	**Site 067**	**Loco Hills**	**Hobbs**
Site 20	-						
Kenna	0.1160 ***	-					
Site 106	0.3471 ***	0.1910 ***	-				
Camp	0.1833 ***	0.0572 ***	0.0917 ***	-			
Site 067	0.3127 **	0.1624 ***	0.2507 *	0.1413 ***	-		
Loco Hills	0.2501 ***	0.1154 ***	0.2527 ***	0.1307 ***	0.0459	-	
Hobbs	0.2550 ***	0.1133 ***	0.2577 ***	0.1303 ***	0.1169 **	0.0752 ***	-

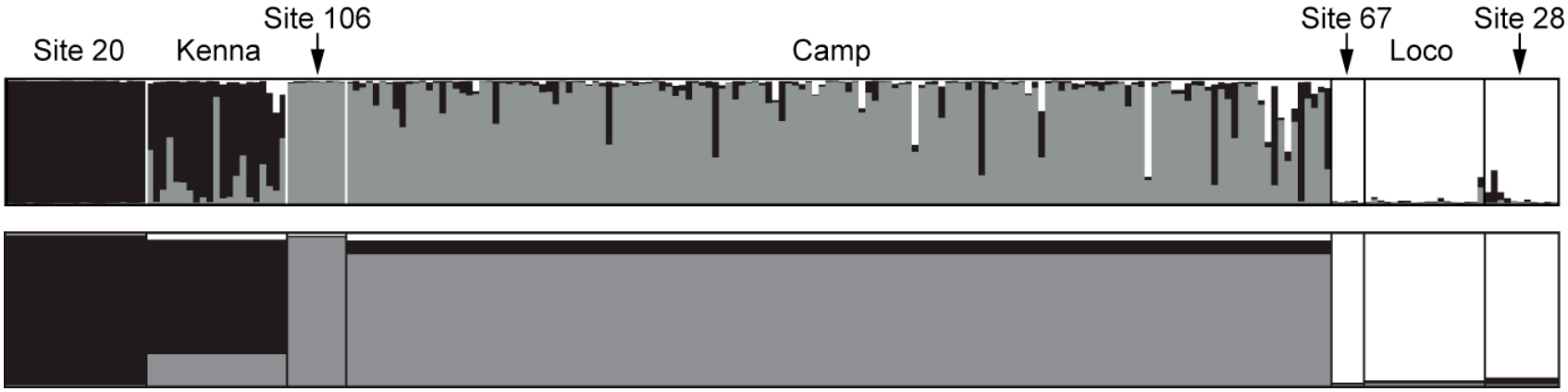

Figure 1.5. Genetic membership for all genotyped *S. arenicolus* inferred from Bayesian assignment tests in STRUCTURE. Membership to each of three regions (northern – black, central – gray, southern – white) are plotted for each individual (top) and as a population average (bottom).

of the individuals from southern localities fall exclusively within the southern cluster, the exception being several individuals collected between Loco Hills and Site 28 that show evidence of admixture.

In the spatially explicit, individual-based test of population structure, we found significant spatial autocorrelation at linear distances of less than 34.35 km for all individuals (Figure 1.6). Estimates of spatial autocorrelation, r_C, range from -0.067 to 0.098.

Historical and contemporary demographics

F_S did not support population expansion in the central or southern regions (central: $F_S = 0.271$, $p = 0.558$; southern: $F_S = -2.676$, $p = 0.097$) and showed some evidence of population expansion in the northern region ($F_S = -2.467$, $p = 0.022$), although it was only borderline significant following the $p < 0.02$ criteria for significance at $\alpha = 0.05$ (Fu, 1997). Bayesian estimation of historical demographic parameters under the isolation with migration model in IM converged on the same posterior distributions over multiple runs. Current population sizes (θ_1 and θ_2) were not significantly different from one another for the mtDNA or combined dataset. However, for the combined but not the mtDNA dataset, θ_1 and θ_2 were significantly smaller than θ_A, supporting population contraction. The posterior probabilities for migration (scaled by θ) into the central region from the northern and southern regions were high (0.79 and 0.86, respectively; Table 1.3) suggesting, but not strongly supporting, the possibility of asymmetrical migration. The divergence time between the northern and central regions and central and southern regions were inconsistent between the datasets (Figure 1.7).

Effective population size estimates (Θ) using Bayesian methods in MIGRATE ranged from a mode of 0.00925 in Site 106 to 0.02175 in Kenna. Posterior probability

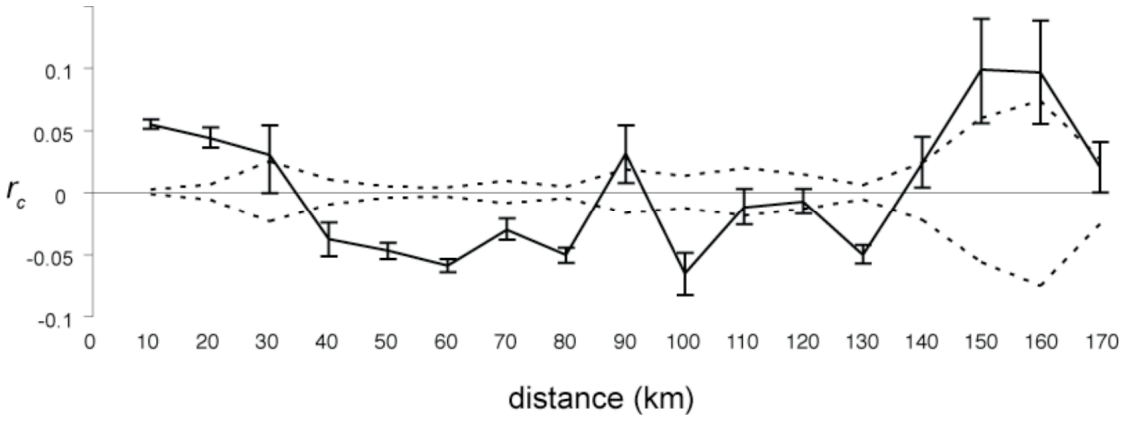

Figure 1.6. Spatial autocorrelation at 10 km distance classes for genotyped individuals. Solid line is the mean and 95% confidence interval (CI) generated with 999 bootstrap replicates and dashed lines are he upper and lower 95% CI for the null distribution of no spatial genetic structure generated by 999 permutations of the data across distance classes.

Table 1.3. Parameter estimates and posterior probabilities of comparisons among parameters from IM. Reported are estimates with the highest posterior probability and the lower and upper bounds of the 90% highest posterior densities for each of the parameter. Bounds for posterior distributions with long, non-zero right hand tails are indicated by "?". For each region, subscripts next to the region name indicate the subscript of estimates for that particular region.

	θ_1	θ_2	θ_A	m_1	m_2	t	$\theta_1 > \theta_2$	$\theta_1 > \theta_A$	$\theta_2 > \theta_A$	$m_1 > m_2$
mtDNA only										
Northern$_1$ – Central$_2$	8.826 (2.434 - 28.949)	9.335 (1.707 - 36.068)	0.055 (0 - 109)	0.005 (0.005 - 1.195)	0.005 (0.005 - 2.045)	1.35 (0.610 - 18.390)	0.437	0.238	0.266	0.293
Central$_1$ – Southern$_2$	25.030 (12.871 - 45.986)	13.130 ?	4.754 ?	0.005 (0.005 - 0.285)	0.005 (0.005 - 0.515)	4.71 ?	0.745	0.268	0.197	0.354
mtDNA and microsatellite data										
Northern$_1$ – Central$_2$	5.919 (3.000 – 10.136)	3.675 (1.670 - 7.684)	79.064 ?	0.105 (0.005 - 1.015)	0.965 (0.155 - 2.645)	1.805 ?	0.787	< 0.001	< 0.001	0.133
Central$_1$ – Southern$_2$	3.409 (1.486 - 6.381)	3.055 (1.471 - 6.675)	76.363 (37.899 - 164.378)	0.005 (0.005 - 0.625)	0.475 (0.005 - 1.495)	0.665 (0.215 - 2.045)	0.551	< 0.001	< 0.001	0.170

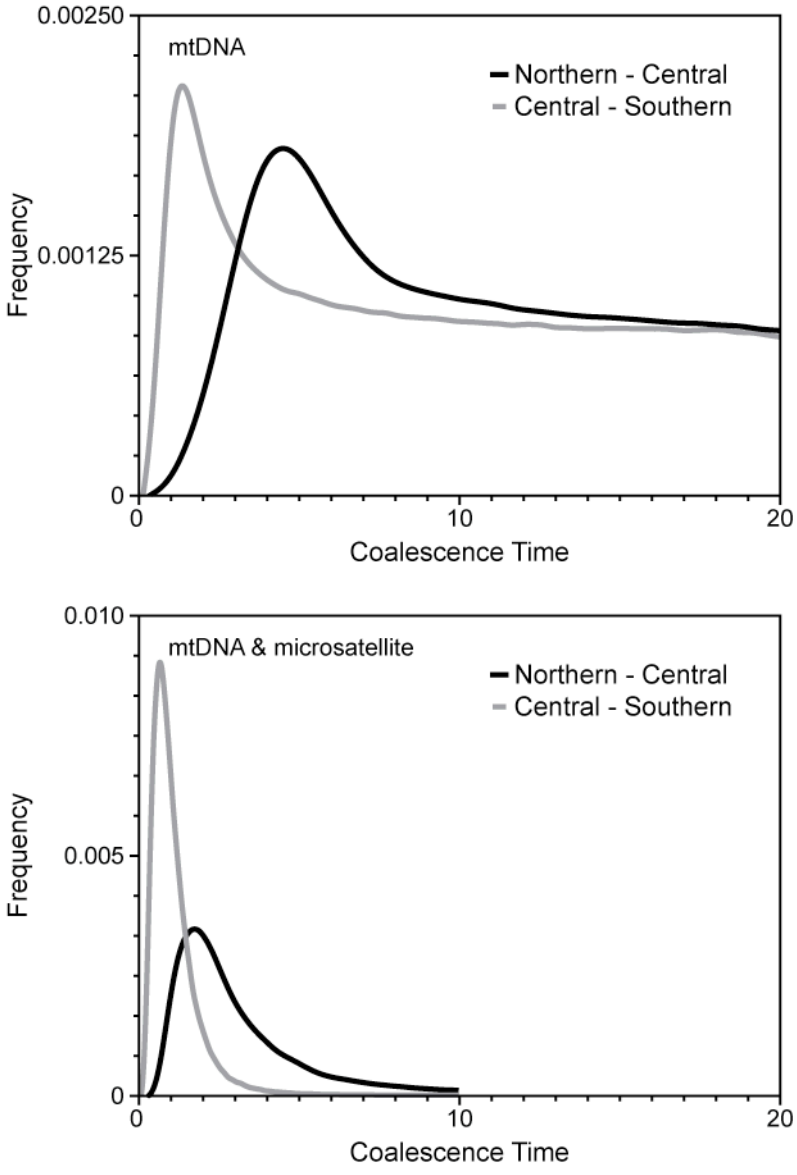

Figure 1.7. Posterior probability densities for coalescence time under an isolation-by-migration model based on mtDNA (top) and mtDNA and microsatellite data (bottom).

densities of Θ for Kenna and Camp were the highest and did not overlap substantially with the estimates of Θ for Site 20 and Site 106 (Figure 1.8). Migration rates had broad 95% credible intervals and ranged from 17.6 to 98.4. They were not different across population pairs, nor were they strongly asymmetrical between pairs.

Garza and Williamson (2001) compared measures of *M* in taxa with stable demographic histories and those with recent population reductions. In their review, *M* was greater than 0.82 in stable populations and less than 0.70 for populations with known reductions in size. Our seven populations showed *M* between 0.567 and 0.884 with a mean of 0.682 (Table 1.4). Three populations (Site 106, Site 67, and Site 28) show evidence of population reduction and Camp shows evidence of population stability. The remaining populations (Site 20, Kenna, and Loco) have *M* between 0.70 and 0.82, and thus, it is unclear what the demographic histories of these regions are.

DISCUSSION

Sceloporus arenicolus inhabits large, deep, and unvegetated sand dune blowouts with intermediate sand grain coarseness within the shinnery oak dominated landscape (Fitzgerald *et al.*, 1997). We hypothesized that the patchy distribution of habitat could restrict movement and result in high levels of genetic structure at small spatial scales. Our results suggest that populations are differentiated, but that not all regions and populations are equally divergent. We detect genetic differentiation in *S. arenicolus* at several spatial scales throughout its limited range that is attributable to historical patterns of isolation as well as reduced dispersal imposed by habitat specialization to specific patches of habitat in a matrix of unsuitable sites.

It is clear that *S. arenicolus* evolved from within the *graciosus* group (Flores-Villela *et al.*, 2000; Frabotta, 2002; Wiens and Reeder, 1997); low levels of sequence divergence and haplotype diversity at mitochondrial loci for samples of *S. arenicolus*

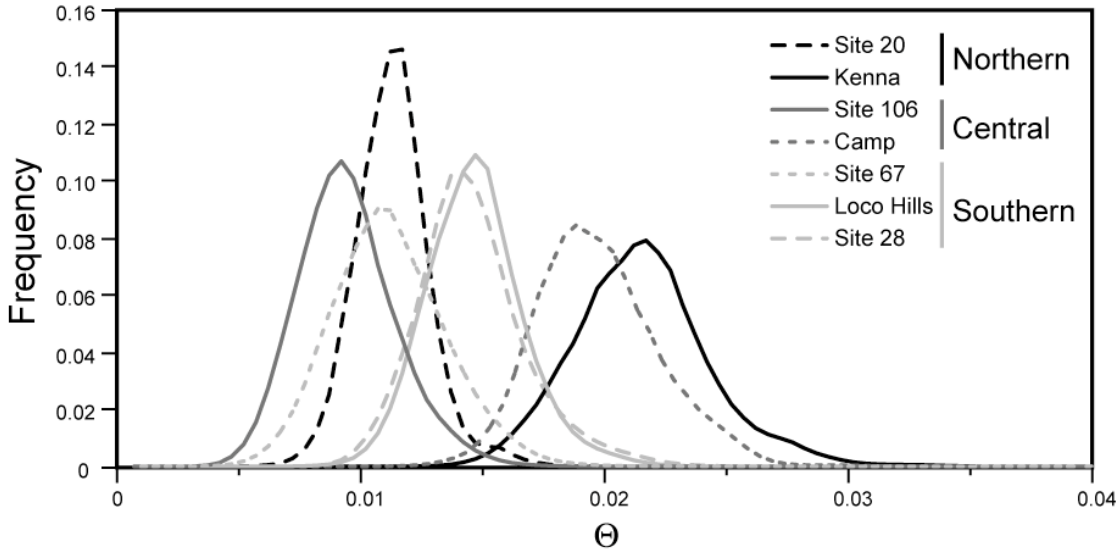

Figure 1.8. Posterior probability densities for Θ in each of seven populations estimated by MIGRATE.

Table 1.4. Average *M* based on microsatellite data for each population.

Locality	Average M	Variance
Site 20	0.740	0.095
Kenna	0.704	0.030
Site 106	0.567	0.091
Camp	0.884	0.010
Loco Hills	0.702	0.042
Site 67	0.598	0.071
Site 28	0.577	0.028
Overall	0.682	0.047

from throughout the range suggest a relatively recent common ancestor for these individuals. The shinnery oak – sand dune habitats of the Mescalero and Monahans sand dune systems formed in the late Pleistocene and Holocene in the Pecos River Valley (Green, 1961; Hawley *et al.*, 1976; Holliday, 2001). Two evolutionary hypotheses potentially explain the low levels of genetic diversity and patterns of divergence among populations. Kerfoot (1968) suggested that the origin of *S. arenicolus* preceded the formation of this sand dune habitat with extinction and range contraction occurring at the end of the Pleistocene subsequent to their divergence from *S. graciosus*. Alternatively, divergence and specialization may have occurred in concert with the formation of Mescalero and Monahans sand dunes more recently. Unless range contraction was severe, evidence of demographic stability would lend support to the former hypothesis; in contrast, a genetic signature of population expansion (e.g. following a founder event) would support a more recent origin.

We resolve three genetic groups corresponding to northern, central, and southern portions of this species' range, but are unable to unambiguously resolve historical patterns of divergence among these groups given our data. The mtDNA data weakly indicate that the northern population is the youngest of the three groups and F_S weakly supports population expansion in the northern regions with demographic stability in the central and southern regions. Based on these mtDNA results, central and southern regions may represent relict populations from the end of the Pleistocene in line with Kerfoot's (1968) hypothesis, with a subsequent colonization and population expansion in the northern portion of the range. Results from mtDNA and microsatellite data, however, support a slightly different evolutionary history of *S. arenicolus*. In contrast to our results based only on mtDNA, analysis of the combined data indicate that the northern-central divergence preceded the central-southern divergence and that none of the three regions have undergone population expansion.

Thus, the combined data suggest historical movement north to south possibly accompanied by reductions in population size from ancestral population sizes within each region through founder effects, although we cannot say with certainty. The conflict between the mtDNA and combined datasets is likely due to differences between the mitochondrial and nuclear markers and levels of variation in each of these genetic markers and, given these data, we are unable to unambiguously resolve the evolutionary history of *S. arenicolus*. *Sceloporus graciosus*, the generalist congener of *S. arenicolus* occupies a number of habitat types, including sand dunes in the Pahvant Valley of Utah (Stuart, 1932). It is possible that *Sceloporus arenicolus* evolved from *S. graciosus* populations that occupied other sandy habitats which existed prior to the formation of the shinnery oak – sand dune habitat and subsequently became specialized and restricted to this habitat in the Mescalero and Monahan sand dunes.

Independent of the sequence of regional population divergences and historical demographics, the three well-resolved groups supported by mtDNA and microsatellite data correspond with geological and ecological landscape features and with breaks in the known distribution. The range of *S. arenicolus* extends to the northeast and southeast along the western edge of a caliche caprock of the Llano Estacado plateau (Figure 1.1; Hawley *et al.*, 1976). Fitzgerald et al. (1997) surveyed potential sites throughout the range of *S. arenicolus* to determine the extent of the species' range and identify gaps in the distribution. The genetic differentiation among the northern and central populations matches discontinuities in suitable habitat and patterns of occurrence underscoring the effects of habitat continuity on the genetic structure of habitat specialist in fragmented landscapes (Branch *et al.*, 2003). Short-grass prairie, tall-grass prairie, shinnery oak vegetation not associated with sand dunes, and human-altered landscapes are unsuitable habitat types for *S. arenicolus* resulting in physical

separation of Kenna and Site 20 (northern sites) from Camp and Site 106 (central sites). Individuals from the southern portion of the range are well-differentiated from central individuals despite presumably contiguous shinnery oak – sand dune habitat throughout this portion of the range. This area between the central and southern groups coincides with the western-most extent of the caprock (Figure 1.1) that constricts suitable habitat to a narrow north-south band approximately eight km wide. *Sceloporus arenicolus* were not found at several sites within this strip of habitat (Fitzgerald *et al.*, 1997), thus, this genetic split is also in line with a potential break in their current distribution.

The fine-scale patterns of genetic structure that we find among populations within regions corroborate our findings at larger scales of generally reduced movement and connectivity in *S. arenicolus*. Landscape characteristics are particularly likely to be more important to patterns of isolation than geographic distance when landscapes are heterogeneous (Arnaud, 2003; Michels *et al.*, 2001; Storfer *et al.*, 2006). The grassland and mesquite dominated regions which presumably contribute to the differentiation between the northern and central regions are also likely to be responsible for the among population differentiation we recover. In contrast, patches of non-shinnery oak habitat are smaller in the southern portion of the range and this may increase connectivity among populations. Pairwise F_{ST} values among the four populations of the northern and central regions are high compared to those among the three southern populations (Table 1.2) despite the larger geographic distances among populations in the south indicating that habitat fragmentation does indeed impact levels of gene flow.

Variability in levels of genetic diversity and effective population size among populations may additionally reflect habitat characteristics and fragmentation. Populations on the periphery of a species range are subject to lower effective

population sizes because habitat quality is usually marginal away from the center of the range (Edenhamn *et al.*, 2000; Vucetich and Waite, 2003). Among the four northern and central localities, Site 20 and Site 106 are nearly invariable at mtDNA loci (Figure 1.3) with low allelic diversity at microsatellite loci as well (Figure 1.2). Individuals from these two sites have high assignment probabilities in Bayesian clustering analyses (Figure 1.5) and low estimates of effective population size (Figure 1.8). These localities are the northeastern-most populations in the range, east of Kenna and Camp, at higher elevation. A soil suitability model that was successful at predicting the occurrence of *S. arenicolus*, found that presumably suitable habitat is most continuous along the base of the western edge of the caprock and more fragmented near Site 106 (Allen and Bird, 2007). While data are not available for Kenna, Site 20, and many of the Camp collection localities, this predictive model is consistent with the distribution of sand dune habitat outlined by Holliday (2001). These data suggest that Site 20 and Site 106 may be in areas with habitat of marginal quality lending support to the range periphery hypothesis. Among the southern populations, within population levels of diversity (Figure 1.2), membership coefficients (Figure 1.5) corroborate our conclusions that less habitat fragmentation results in greater overall similarity among these populations.

Patterns of genetic differentiation occurring at the range-wide scale as well as at the population level may be the product of extinction and recolonization patterns, habitat specificity and isolation, and limited, but not negligible, gene flow. Although there is large agreement at the range-wide scale between geographic regions and the genetic groupings, both mtDNA and nuclear loci show evidence of shared genetic diversity across regions. Based on the mitochondrial data alone, we are unable to distinguish between a scenario of incomplete lineage sorting in the central and southern regions and one of introgression from the north into the central and southern

regions. Bayesian assignment tests suggest that introgression may occur from the northern and southern regions into the central region, however, migration estimates do not support asymmetrical migration for the combined dataset (Table 1.3) nor the microsatellite dataset. We find support for reduced contemporary θ compared to ancestral θ in these three regions (Table 1.3) and variation in effective size (Table 1.4) and evidence of recent bottlenecks among the seven populations. Bottlenecks and founder effects can contribute to higher than expected levels of differentiation (Wade and McCauley, 1988) and likely explain the reduced diversity in some, but not all populations. While evolutionary history has influenced genetic structure in *S. arenicolus*, the patterns of genetic differentiation are also the product of recent demographic changes either through colonization of new habitat, or local extinction events.

Spatially explicit theoretical models have found that species of intermediate dispersal ability may experience increased connectivity in dynamic landscapes because the rearrangement of the landscape may connect previously isolated populations (Matlack and Monde, 2004; Wimberly, 2006). Common examples of discordance between hypothesized landscape influences and patterns of genetic structure can be found in fresh water fishes where stream rearrangements over long periods of time can result in altered patterns of genetic connectivity (Poissant *et al.*, 2005). Sand dune systems are dynamic and the quality and distribution of blowouts can shift over relatively short periods of time due to wind and rain (Holliday, 2001; Muhs and Holliday, 2001). The non-static nature of the habitat likely contributes to historical patterns of connectivity and gene flow among populations that are now isolated (Wimberly, 2006). In addition, increased connectivity due to natural dynamics of the sand dune landscape over short time scales may help to prevent the loss of genetic diversity by increasing effective population size. Currently, a large part

of the Mescalero sand dunes are inactive (i.e. non-shifting) and relatively stable; however, the Monahans sand dunes in Texas are more active and altered by wind moving from west to east along the base of the Llano Estacado plateau (Holliday, 2001; Muhs and Holliday, 2001). If dune activity does promote gene flow at fine scales within the southern region, genetically distant mtDNA haplotypes found here (Figure 1.3) may represent persistent ancestral diversity maintained through greater overall effective population sizes. Alternatively, these haplotypes may result from unidirectional dune-mediated gene flow at the range-wide scale from northern and central populations.

Understanding the scale of genetic structure and the relationship of this to landscape characteristics helps us predict how particular anthropogenic modifications will impact populations of this habitat specialist. Herbicide spraying and oil drilling have occurred throughout the range of *S. arenicolus* (Painter *et al.*, 1999; Snell *et al.*, 1997). Application of tebuthiuron, an herbicide that converts shinnery oak habitat to grassland for cattle grazing, is the predominant anthropogenic activity threatening *S. arenicolus* populations in the northern and central portions of the ragnes. In contrast, oil drilling is the largest habitat disturbance in the south. These practices cause the fragmentation of existing habitat and alter the network of habitat patches and the stability of sand dunes. Removal of shinnery oak vegetation decreases habitat heterogeneity and increases the susceptibility of sand dune blowouts to alteration by wind and rain, thereby eroding the topographic relief of blowouts and changing the matrix of shinnery oak and sand dune blowouts (Snell *et al.*, 1997). *Sceloporus arenicolus* requires sand dune blowouts of particular characteristics for reproduction, foraging, and thermoregulation (Degenhardt *et al.*, 1996; Fitzgerald *et al.*, 1997; Snell *et al.*, 1997). Changes in patch quality are likely to also affect vital rates such as

survivorship, growth, and fecundity (Hokit and Branch, 2003a, 2003b) which, in turn, will influence effective population sizes and levels of connectivity.

Given the high levels of differentiation we find among and within the three regions and lack of population structure at finer spatial scales, the specific arrangement of sand dune blowouts may not be as critical to the persistence of *S. arenicolus* as is the quality and availability of patches within stretches of intact shinnery oak – sand dune habitat. Patch characteristics influence population densities, effective population size, as well as resistance to local extinction in this dynamic landscape. The overall variability among populations in levels of genetic diversity serves as an indication that genetic processes are not equivalent across the landscape and that habitat specialists may be particularly sensitive to patch characteristics and dynamics within these heterogeneous landscapes.

Although we do not detect fine scale genetic structure in association with human disturbances, we should not discount the impact of anthropogenic activities. First, it is possible that *S. arenicolus* populations have not had sufficient time to reflect the effects of recent habitat alteration. Second, the demographic consequences of shinnery oak destruction (Snell *et al.*, 1997) are likely to decrease levels of genetic diversity even if populations do persist. The absence of *S. arenicolus* from many presumably suitable sites (Fitzgerald *et al.*, 1997), the sharp genetic break between central and southern populations despite the presence of shinnery oak – sand dune habitat, and the low genetic diversity of some, but not all populations underscore the fact that unrecognized characteristics of the landscape may also be important to connectivity and persistence. Our data suggest that although the habitat network of shinnery oak and sand dune patches may not need to be managed at fine scales, the preservation of large areas containing a network of suitable habitat is necessary to

maintain historical levels of connectivity, prevent local extinction, and avoid the loss of genetic diversity due to genetic drift in reduced populations.

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CHAPTER TWO

ECOLOGICAL AND DEMOGRAPHIC DETERMINANTS OF REPRODUCTIVE SUCCESS IN TWO DESERT ANURANS

Abstract.— Reproductive success is determined by individual traits as well as the broader social and ecological context in which mating occurs. We are beginning to identify the proximal determinants of individual fitness, but know significantly less about how variation in ecological conditions alters patterns of reproductive success within populations. I examined how reproductive success within two pond breeding anurans varies with breeding context. *Bufo cognatus* and *Scaphiopus couchii* co-occur in the deserts of southwestern North America and breed in ephemeral ponds, but differences in life history and mating system characteristics are likely to affect the impact of ecological and demographic feature on levels of reproductive success in each species. I sampled breeding adults and offspring and quantify changes in genetic diversity at microsatellite loci across generations. For each offspring sample, I estimated genetic diversity, relatedness, and the number of contributing parents and examined these data with respect to pond size attributes. I find evidence for limited reproduction and loss of genetic diversity across generations in both species for some, but not all, ponds. Small ponds have disproportionately fewer breeding adults and lower genetic diversity than large ponds for *S. couchii*. However, in the zone of ecological overlap with *B. cognatus*, pond size does not have a significant effect on reproduction. These results indicate that reproduction is influenced by the ecological context of breeding and that pond size can contribute to maintenance of genetic diversity within populations by influencing breeding success.

INTRODUCTION

Reproduction is rarely, if ever, distributed evenly among members of a group (Shuster and Wade, 2003). Studies of dominance hierarchies (e.g. Altmann *et al.*, 1996; de Ruiter and van Hooff, 1993), alternative mating strategies (e.g. Neff *et al.*, 2004; Sinervo and Zamudio, 2001), and genetic mating systems (e.g. Gopurenko *et al.*, 2006; Tennessen and Zamudio, 2003), have identified determinants of individual fitness and have contributed to our understanding of sexual selection. Such studies have underscored the importance of organismal traits and the social context of breeding to individual reproductive success (e.g. Jones *et al.*, 2004; Sullivan, 1989b). Individual condition and behavioral repertoires influence mating success (Garant *et al.*, 2001; Gross, 1996; Krupa, 1989; Sullivan, 1989a; Tennessen and Zamudio, 2003) and this is particularly true in species with discrete mating strategies. (e.g. Neff *et al.*, 2004; Vieites *et al.*, 2004). However, individual fitness is often context dependent and habitat characteristics, habitat quality, breeding densities, sex ratios, and temporal aspects of reproduction (e.g. breeding asynchrony; Emlen and Oring, 1977) ultimately influence the degree to which particular phenotypes are successful (Richter *et al.*, 2003; Sinervo and Lively, 1996). Despite their importance, the influence of these factors on reproductive success has been under-explored.

The collective reproductive success of individuals within a population determines the maintenance of diversity across generations. Thus, organismal, demographic, and ecological traits are important for individual fitness, but also influence the magnitude of variance in reproductive success across a population and the persistence of within population genetic diversity (Avise *et al.*, 2002; Kokko and Rankin, 2006). If reproduction is highly asymmetrical with small number of individuals producing all offspring, genetic diversity may decrease within populations (Chesser, 1991; Hedgecock, 1994; Hedrick, 2005), thereby increasing the importance

of gene flow among population in preventing divergence. Despite theoretical studies examining the effect of reproductive skew on effective population size (N_e) and population genetic structure (Chesser, 1991; Hedrick, 2005; Nomura, 2002), the population level consequences of reproductive skew have not been thoroughly explored empirically. Quantifying reproductive success within groups of individuals and correlating these data with natural variance in organismal, environmental, and demographic factors allows us to examine the impact of the ecological and social context of breeding on reproductive success at the individual level and patterns of diversity and genetic structure at the population level.

The relative ease of assaying multiple independent genetic markers has led to an increase in our understanding of the true distribution of reproductive success among individuals and the characterization of genetic mating systems (e.g. Avise *et al.*, 2002; Garant *et al.*, 2001; Gopurenko *et al.*, 2006). Genetic methods to examine reproductive fitness have been particularly useful for taxa where direct observations of mating behaviors and rates of fertilization are not always feasible (e.g. Liebold *et al.*, 2006; Tennessen and Zamudio, 2003; Vieites *et al.*, 2004). Furthermore, they allow quantification of population level measures of total reproduction, genetic diversity, and relatedness providing us with the opportunity to tie individual reproductive success to patterns of genetic diversity at the level of populations.

Recent evidence from salamanders (Gopurenko *et al.*, 2006; Tennessen and Zamudio, 2003) and anurans (Laurila and Seppä, 1998; Lodé and Lesbarrères, 2004) suggests that determinants of individual reproductive success may be more dynamic than previously expected and that high levels of reproductive skew may be common among amphibians. These observations are not surprising; amphibians often have temporally and spatially restricted breeding activities (Wells, 1977) that limit the opportunity for reproduction and increase the intensity of sexual selection (Emlen and

Oring, 1977). Furthermore, the large diversity in mating behaviors (Halliday and Tejedo, 1995; Zamudio and Chan, *in press*), mating systems (Sullivan *et al.*, 1995; Wells, 1977), and reproductive modes (Haddad and Prado, 2005) in amphibians increases the opportunity for complex patterns of individual reproductive fitness and skew within populations. While the proximal determinants of reproductive success are becoming more clear in some vertebrate taxa (e.g. Leary *et al.*, 2006; Roberts *et al.*, 1999; Tennessen and Zamudio, 2003), we have a poor understanding of how reproductive skew changes with different ecological and social settings. Operational sex ratio (OSR), defined as the ratio of fertilizable females to sexually active males (Emlen and Oring, 1977), can influence the opportunity for reproduction and the intensity of sexual selection (Shuster and Wade, 2003). Given that OSR and density can play an important role in determining amphibian mating system characteristics (Jones *et al.*, 2004; Sullivan *et al.*, 1995; Zamudio and Chan, *in press*), it is important to understand the relationship between patterns of reproductive success and the ecological and social contexts of breeding.

Anurans in the deserts of the southwestern United States are well-suited for studies of the context dependence of mating success and reproductive skew. Species in this region have similar temporal and spatial breeding ecologies, but differ considerably in life history characteristics and mating strategies (Sullivan, 1985; Sullivan, 1989b) providing the opportunity to examine the consequences of interspecific variation in taxa with considerable ecological overlap. Two common species, the Great Plains Toad (*Bufo cognatus*) and Couch's Spadefoot Toad (*Scaphiopus couchii*), aggregate at ephemeral ponds for one to three nights of breeding activity at the start of the summer monsoon rains in July and August of each year (Mayhew, 1965; Sullivan, 1985). These species often breed at the same time in the same ponds and therefore rely on the similar conditions for reproduction, however, *S.*

couchii also breeds in small shallow ponds where *B. cognatus* is unable to breed because of its longer larval development time (Bragg, 1937a; Graves and Krupa, 2005; Mayhew, 1965). While males of both species vocalize to attract females, *B. cognatus* has a highly biased OSR with lower overall densities of females in comparison to *S. couchii* (Krupa, 1989; Sullivan, 1982); therefore, the strength of selection and the opportunity for reproductive skew is likely greater for *B. cognatus*.

In this study, I use these two species to investigate how the ecological and social contexts of breeding influence the reproductive success of breeding individuals and the persistence of genetic diversity across generations. I use temporal sampling methods of parents and offspring and multilocus genetic markers to test the hypothesis that breeding behaviors, male-biased sex ratios, and habitat characteristics can substantially constrain the number of individuals within a breeding group that are reproductively successful. Specifically, I compare relatedness, shared parentage, and genetic diversity of offspring samples for these two species to assess the effect of breeding habitat characteristics on the persistence of genetic diversity. These data contribute to our knowledge of how processes operating at the individual level influence genetic diversity across generations and examine the potential importance of species-typical characteristics to patterns of genetic differentiation and microevolution.

MATERIALS AND METHODS

Spatial and temporal sampling of breeding aggregations

I sampled ponds throughout the San Simon Valley and the San Bernardino Valley of southeastern Arizona and southwestern New Mexico at two times during the breeding season to compare genetic diversity between breeding adults and their offspring following explosive breeding events. I collected tissue samples from adults

at 12 *Bufo cognatus* and nine *Scaphiopus couchii* breeding aggregations from July and August of 2002 – 2005 (Appendix 2). The sites included in this study ranged from small puddles and roadside ditches to medium-sized temporary ponds. *Scaphiopus couchii* breeding aggregations occurred at sites of all types and sizes whereas *B. cognatus* bred only in larger ponds and ditches. At each aggregation, I weighed, measured (snout-to-vent length, SVL), sexed, and toe-clipped adult individuals for unique identification and to obtain tissue samples for genetic analyses. Breeding bouts at single ponds are short, usually lasting one to three nights (Sullivan, 1985), therefore, I collected samples on all nights of active chorusing. Eggs of both focal species hatch in 24 – 48 hours (Bragg, 1936, 1965; Mayhew, 1965). I collected 15 – 60 tadpoles two to three days post-hatching from all ponds which had tadpoles. This sampling date was chosen to minimize bias that might occur from sampling too early and missing larvae that were not yet hatched, while still reducing the possibility of bias due to post-hatching mortality from predation, selection, or stochasticity. Not all breeding aggregations successfully produced offspring so I increased the total number of sites with tadpole collections from ponds for which I did not have adults samples. One adult collection locality (Willow, WL) consisted of three discrete ponds (NW, EW, and SW) separated from one another by approximately 20 meters. I pooled adults from the three ponds into a single sample because of adult movement among ponds, but treated each pond as a unique tadpole sample because of the absence of larval movement among sites. Thus, considering each Willow pond separately, the final dataset included six *B. cognatus* and two *S. couchii* sites for which I obtained both adult and tadpoles samples as well as one *B. cognatus* and 12 *S. couchii* sites for which I collected tadpole samples but no adult samples.

For each pond I measured the dimensions of the pond (diameter or length and width) to the nearest meter and estimated the depth at the center to the nearest quarter

of a meter at the time of the sampling of tadpoles. I calculated the approximate perimeter, area, and volume of each site assuming a constant depth and either circular or rectangular surface area. Ponds in desert environments are constantly changing because of high evaporation rates, nonetheless, these measurements are good representations of pond sizes at the time of explosive breeding events.

Laboratory protocols

I extracted whole genomic DNA from adult toe or tadpole tail clips (approximately 1 mm^3) by incubating them in 150 µL of a 5% Chelex solution (Chelex-100, BioRad) and 19 µg ProteinaseK at 55 °C for 180 minutes followed by 95 °C for 10 minutes. The supernatant was used directly as template DNA in polymerase chain reaction (PCR) amplification of microsatellite loci. Individuals of *B. cognatus* and *S. couchii* were genotyped at thirteen and twelve nuclear microsatellite loci, respectively, for a total of 266 adult and 288 larval *B. cognatus* and 216 adult and 469 larval *S. couchii*. Primer sequences and PCR conditions follow those previously published in Chan (2007a) and Chan (2007b). An additional marker (locus ihhh, Gonzales *et al.*, 2004) was also used for *B. cognatus*.

Null alleles can lead to erroneous estimates of genetic diversity, relatedness, and parentage based on genotypic data (Blouin, 2003; Dakin and Avise, 2004; Wagner *et al.*, 2006), therefore, I checked for the presence of null alleles in my dataset prior to estimating diversity and relatedness by testing adult genotypes for deviation from Hardy-Weinberg equilibrium (HWE) both globally and within populations. A Markov chain method (Guo and Thompson, 1992) with 10,000 dememorization steps and 1,000 batches of 10,000 steps was implemented in GENEPOP v3.4 (Raymond and Rousset, 1995) to determine the significance of within population and overall heterozygote deficiencies.

Changes in genetic diversity

I estimated genetic diversity and allelic richness for all adult and offspring samples from each locality, omitting a single locus in *B. cognatus* with a null allele at high frequency (see Results). I used FSTAT 2.9.3 (Goudet, 1995) to calculate locus specific standardized allelic richness (AR) accounting for variation in sample size (Petit *et al.*, 1998) and unbiased estimates of gene diversity (H_S; Nei, 1987). To adjust for a left skewed distribution to values of H_S, I arcsin transformed the square of H_S [$\arcsin(H_S^2)$]. For each species, I compared AR and transformed H_S values of all adults to those of all offspring using a one-sided *t*-test to test the hypothesis that overall diversity is greater in adults compared to tadpoles.

At all localities for which I had both adult and offspring samples, I calculated the locus specific change in AR and non-transformed H_S as the adult value minus the offspring value. If diversity is lost due to reproductive skew then adults should have greater genetic diversity than offspring and the distribution of pairwise differences should be positive. Thus, I used a one-sided t-test to determine whether average changes in genetic diversity across loci at each pond was significantly greater than zero.

Pairwise relatedness

If reproduction is evenly distributed among breeding adults, average pairwise relatedness among pairs of tadpoles should be equal to the average relatedness among adult-adult and adult-tadpole comparisons at each locality. In contrast, if reproductive skew is high and a limited number of adults at each aggregation are reproductively successful, then offspring samples will be composed of a small number of kin groups and average relatedness should be higher among tadpoles than among breeding adults or adult-tadpole pairs.

Maximum likelihood (ML) estimators of relatedness perform better, with smaller standard error and less bias under a broader range of conditions than more commonly used moment estimators (Milligan, 2003). Thus, I used the program ML-RELATE (Kalinowski *et al.*, 2006) to determine ML estimates of pairwise relatedness between individuals. This procedure can account for the presence of null alleles (Kalinowski *et al.*, 2006; Wagner *et al.*, 2006), therefore, I include all data for both species in these estimates. Relatedness estimators, including this ML estimator, are based on the probability of sampling particular genotypes given population level allele frequencies (Wagner *et al.*, 2006). Because offspring may be related and not reflect the true allele frequencies of the population (Blouin, 2003), I pooled each tadpole sample with all adults so that relatedness estimates were based on overall adult allele frequencies rather than those of just the offspring. This minimizes the bias in pairwise relatedness estimates due to tadpole samples that do not reflect population level allele frequencies.

For each of seven *B. cognatus* and 14 *S. couchii* tadpole groups, I estimated pairwise relatedness in ML-RELATE for combined datasets including the tadpoles of interest and all adults. I calculated the mean pairwise relatedness ($\bar{r}$) between tadpole pairs for each locality and used a two-sided *F*-test to determine if the variance in $\bar{r}$ across ponds differed between the two focal species. In addition, for each site I conducted 10,000 replicates of a two-sample permutation test to generate the null distribution of average pairwise relatedness among offspring expected under the hypothesis of equal reproduction among adults and no difference of tadpole and adult pairwise relatedness. For each tadpole group, I determined the *p*-value of the sample mean and standard deviation on the null distribution.

The number of full-sib groups

For both focal species, I estimated the number of full-sib groups within each tadpole sample using clustering methods in the program PARENTAGE (Emery *et al.*, 2001; Wilson, 2006). PARENTAGE uses multilocus genotypic data and implements MCMC search methods in a Bayesian framework to partition individuals into full- and half-sib groups, estimate shared paternity and maternity among offspring, and infer the multilocus genotypes of parents. For the six *B. cognatus* ponds and four *S. couchii* ponds for which I had multigenerational genotypes, I included the genotypes of adults collected at the particular breeding pond as potential fathers and mothers and used a Dirichlet prior on the background allele frequencies, uniform priors on the number of fathers and mothers, and the "all fathers contribute" and "all mothers contribute" options turned off. For an additional 11 PARENTAGE runs for which I had only tadpoles samples and no adult data (one *B. cognatus* and 10 *S. couchii*), I used the same priors on allele frequencies and the number of mothers and fathers, but did not specify potential parents. For each tadpole sample, I first conducted exploratory searches that varied in the number of chains, heating parameters, and chain lengths. I examined the log likelihood plots of the posterior distribution of the full model to determine an appropriate burn-in period, sampling frequency, and the set of search parameters that adequately explored parameter space and converged on the best estimate for each sample.

Final runs used identical priors as above with the mutation prior set to a gamma distribution with a shape parameter of 2 and a mean of 0.001. Because the data and sample sizes differed across tadpole samples, successful search parameters varied across samples (Appendix 3). In general, runs consisted of one to seven heated chains with posterior distributions generated from 5,000 samples taken following an appropriate burnin period. For each final run I determined the mode and even-tailed

90% credible interval on the posterior probability densities for both the number of fathers and mothers. Because offspring sample sizes varied across populations, I calculated a reproduction index (*ri*) for each locality to adjust for differences in sample sizes among ponds:

$$ri = (N_F + N_M) / (2N_O)$$

where N_F and N_M are the number of inferred mothers and fathers, respectively, and N_O is the offspring sample size. Values of *ri* range between zero and one; *ri* equal to zero indicates full sibship among all offspring and *ri* increases to one as fewer individuals within a sample share parents. I used a two-sided *t*-test to determine if average *ri* across sites differed between these species.

Demographic and ecological determinants of genetic diversity

If limited adult reproduction reduces genetic diversity among offspring samples, then estimates of H_S and AR, as well as the difference in H_S and AR across temporal samples, should be predicted by the number of breeding individuals inferred in PARENTAGE analyses. Thus, I examined the correlation between *ri* and locus specific estimates of AR and transformed H_S for all tadpole samples and the change in AR and H_S for those sites where I had adult and offspring samples. I used a standard least squares mixed model in JMP 6.0 (SAS Institute) accounting for variation across loci in measures of AR and H_S; I included locality as a random effect and both *ri* and locus as fixed effects and used a maximum likelihood approach to determine significance. Outlier ponds with extremely low reproductive success may bias the correlation between *ri* and genetic diversity, thus, I also examined the relationship between these variables for a reduced dataset excluding ponds with low reproductive success ($ri \leq 0.10$). To determine the number of breeding individuals necessary to maintain levels of diversity found in among adults, I plotted the average number of

parents inferred for each pond [(N_F + N_M) / 2] against average within pond AR and H_S of tadpoles and compared the distribution of points to the average AR and H_S among adults.

Ecological characteristics, such as pond size, may result in different sized explosive breeding aggregations and potentially underlie patterns of reproductive skew and diversity in these populations. I examined the relationship between *ri* and three pond size attributes (natural log transformed perimeter, area, and volume) using linear regression. To test the relationship between locus specific diversity estimates and pond size attributes, I again used a standard least squares mixed model. For AR and transformed H_S, I included locality as a random effect and locus and one of the three pond size attributes as fixed effects. Potential differences between *B. cognatus* and *S. couchii* in patterns of genetic diversity may be determined by differences in breeding habitats and the ability of *S. couchii* to utilize smaller, more ephemeral ponds. Thus, in addition to the analysis including all ponds, I examined the correlation between these parameters in areas of potential ecological overlap, including only ponds large enough to sustain breeding aggregations of both species.

RESULTS

I found a large heterozygote deficiency at a single locus for *B. cognatus* (BC52.11) both within samples and globally, suggesting the presence of a null allele in high frequency. This locus was omitted from calculations of gene diversity and allelic richness as well as Bayesian assignment tests in PARENTAGE. However, I included locus BC52.11 in estimates of pairwise relatedness with ML-RELATE because this method is able to account for the presence of null alleles (Wagner *et al.*, 2006).

In both species, average gene diversity (H_S; Nei 1987) and allelic richness (AR) across loci were high for adult and tadpole groups. For *B. cognatus*,

standardized AR ranged from 6.56 to 7.44 and 5.93 to 7.40 in adults and tadpole samples, respectively and from 5.59 to 6.41 and 2.66 to 6.06 for adult and tadpole samples of *S. couchii* (Figure 2.1). For *B. cognatus*, average H_S ranged from 0.812 to 0.854 in adult samples and 0.783 to 0.851 in tadpole samples; for *S. couchii* H_S ranged from 0.706 to 0.759 in adult samples and 0.508 to 0.738 in tadpole samples. Average AR across all adults was significantly greater than that for all tadpoles for *S. couchii* ($p = 0.030$), but not *B. cognatus* ($p = 0.080$). Average $\arcsin(H_S^2)$ was not significantly greater among adults in comparison to tadpoles for either species ($p_{Bufo} = 0.101$, $p_{Scaphiopus} = 0.064$).

At ponds for which I had both adult and tadpole samples, within pond locus specific measures of AR and H_S decreased significantly between generations for some, but not all sites (Table 2.1). Out of six ponds for *B. cognatus*, three and two showed a reduction in AR and H_S, respectively. Of the four *S. couchii* ponds, two showed a reduction in both AR and H_S. Across these sites for each species, I found a significant reduction in locus specific estimates of AR and H_S for *B. cognatus* and of AR for *S. couchii*.

Pairwise relatedness

The average relatedness ($\bar{r}$) of all adults was comparable for both species (*B. cognatus,* mean = 0.036 ± 0.0003 SE, n = 266; *S. couchii*, mean = 0.047 ± 0.0005 SE, n = 216). Average relatedness among offspring varied across localities (Figure 2.2), with greater variance among ponds for *S. couchii* compared to *B. cognatus* ($F_{13,6} = 9.0021$, two-tailed $p = 0.0131$). Within pond values of $\bar{r}$ ranged from 0.039 to 0.133 with an overall mean of 0.078 for *B. cognatus* and from 0.049 to 0.453 with an overall mean of 0.116 for *S. couchii*. For six of seven *B. cognatus* samples and 13 of 14 *S. couchii* samples, $\bar{r}$ was greater than that expected under a null hypothesis of random

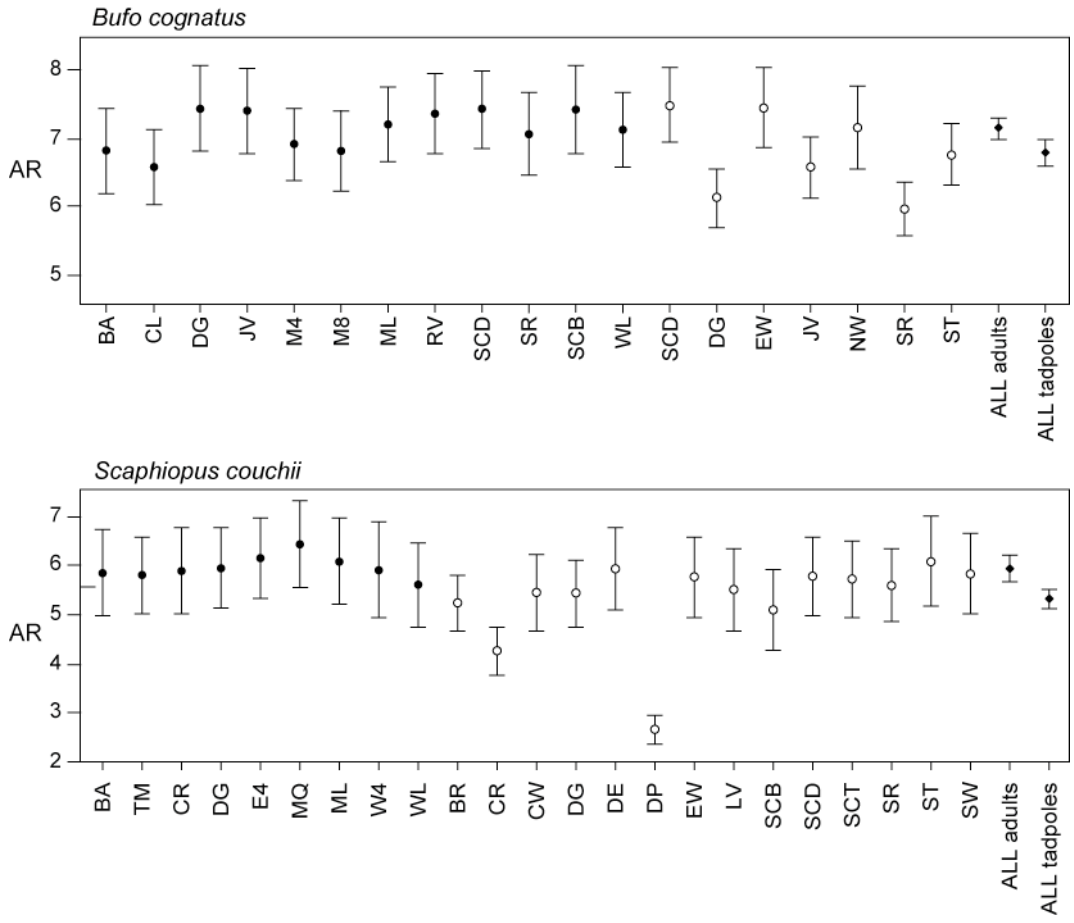

Figure 2.1. Average allelic richness (AR) ± SE across loci for adult (closed circles) and tadpoles (open circles) populations and across all adults and tadpoles (diamonds) for *Bufo cognatus* (top) and *Scaphiopus couchii* (bottom).

Table 2.1. One-sided *t*-test results for a reduction in allelic richness (AR) and gene diversity (H_S) across generations within ponds for *B. cognatus* and *S. couchii* and across all ponds for each species.

		AR		H_S	
	Locality	t statistic	*p*-value	t statistic	*p*-value
Bufo cognatus					
	DG	4.5940	0.0004	4.2054	0.0007
	EW	-1.4345	0.9104	-2.1688	0.9736
	JV	2.6230	0.0119	0.8962	0.2035
	NW	-0.1240	0.5482	-0.9345	0.8149
	SCD	-0.2238	0.5865	-0.1930	0.5748
	SR	3.9789	0.0011	6.1221	< 0.0001
	Overall	4.0240	< 0.0001	1.6860	0.0481
Scaphiopus couchii					
	CR	3.7886	0.0015	2.2356	0.0235
	DG	2.7584	0.0093	1.7249	0.0562
	EW	-0.7897	0.7768	0.2178	0.4158
	SW	-1.0102	0.8330	-1.2061	0.8735
	Overall	2.5224	0.0075	1.6432	0.0535

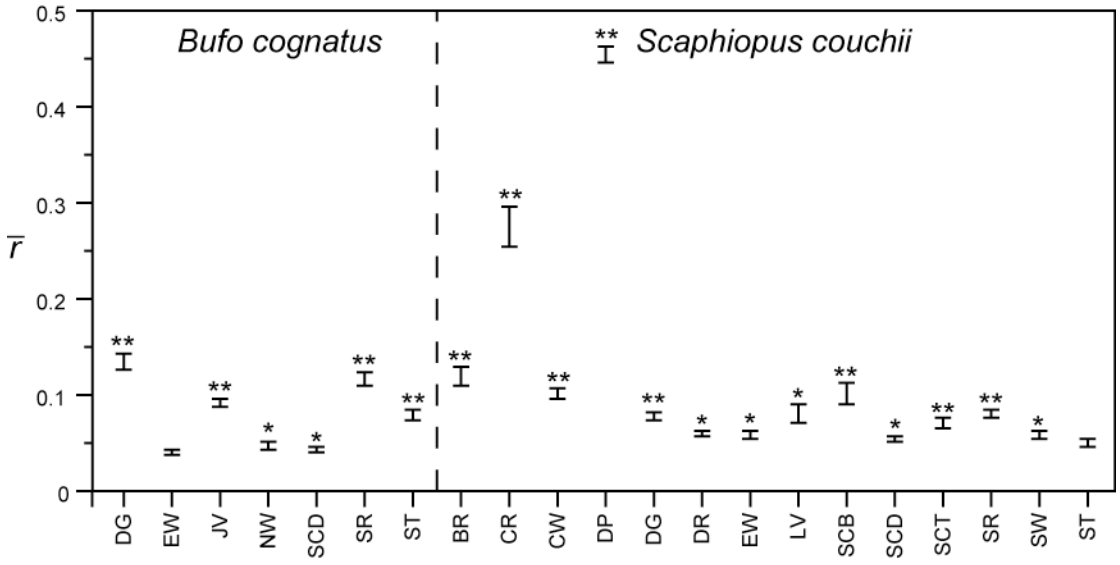

Figure 2.2. Average pairwise relatedness $\bar{r}$ ± SE for tadpole samples. Samples with significantly greater average relatedness than expectations under a null hypothesis of no difference among adult and tadpole pairwise relatedness are indicated by $^{*}p < 0.05$ and $^{**}p < 0.001$.

mating and no reproductive skew (Figure 2.2). *Bufo cognatus* from EW and *S. couchii* from ST were the only two samples with $\bar{r}$ not significantly different from the null distribution ($p = 0.123$ and 0.363, respectively).

Shared parentage clustering

For most offspring samples, multiple runs of sibship clustering analyses in PARENTAGE converged on the same posterior distribution for the number of inferred fathers and mothers. In a single sample (*S. couchii* SCD), runs did not converge on the same mode, but the quantiles of the posterior distribution were consistent across runs. Thus, for this sample, I used the mean of the posterior distributions as the number of inferred parents. These analyses revealed variation among ponds in the number of inferred fullsib groups (Table 2.2, Figure 2.3). The reproduction index, *ri*, ranged from 0.2 to 0.588 among populations of *B. cognatus* and 0.071 to 0.5 among populations of *S. couchii* and the two species did not differ significantly in mean *ri* ($p = 0.270$). Values of *ri* close to zero indicate that few adults reproduced and that most offspring belong to the same kingroup whereas *ri* of one indicates a large proportion of reproductively successful adults and no shared kinship among any two offspring. After examining shared parentage plots, I found that samples with *ri* greater than 0.40 did not have highly resolved kinship relationships. Three *B. cognatus* and five *S. couchii* samples had $ri > 0.40$ suggesting low levels of shared kinship in these populations

Ecological and demographic determinants of reproductive success

The reproduction index, *ri*, was a significant predictor of AR and H_S within ponds for both species (Table 2.3). When a single pond with an extremely low *ri* (*S. couchii* at DP) was omitted from the analysis, the correlation between *ri* and measures

Table 2.2. Results from Bayesian sibship analyses. The inferred number of fathers (N_F) and mothers (N_M) with 90% credible intervals, the number of offspring genotyped (N_O), and the reproduction index (*ri*). * indicate estimates taken as the mean of the posterior distribution rather than as the mode.

Pond	N_F		N_M		N_O	*ri*
Bufo cognatus						
DG	7	(6 – 9)	7	(6 – 9)	40	0.175
EW	26	(24 – 28)	21	(19 – 23)	40	0.588
JV	10	(10 – 11)	11	(10 – 12)	57	0.184
NW	17	(16 – 19)	17	(15 – 19)	29	0.586
SCD	26	(24 – 28)	27	(25 – 29)	53	0.500
SR	8	(8 – 8)	8	(7 – 8)	38	0.197
ST	11	(11 – 14)	11	(11 – 14)	36	0.306
Scaphiopus couchii						
BR	6	(5 – 7)	6	(5 – 7)	26	0.231
CR	4	(4 – 4)	3	(3 – 3)	15	0.233
CW	7	(7 – 9)	7	(7 -9)	42	0.167
DG	17	(16 – 19)	17	(16 – 18)	34	0.500
DP	2	(2 – 2)	2	(2 – 2)	28	0.071
DE	12	(12 – 19)	12	(12 – 19)	42	0.286
EW	19	(18 – 21)	16	(15 18)	40	0.438
LV	7	(6 – 9)	7	(6 – 9)	19	0.368
SCB	5	(4 – 6)	5	(4 – 6)	21	0.238
SCD	18*	(15 – 21)	18*	(15 – 21)	40	0.450
SCT	12	(11 – 13)	12	(11 – 13)	39	0.308
SR	12	(11 – 14)	12	(11 – 14)	43	0.279
SW	15	(13 – 17)	20	(18 – 23)	40	0.438
ST	18	(17 – 20)	18	(17 – 20)	40	0.450

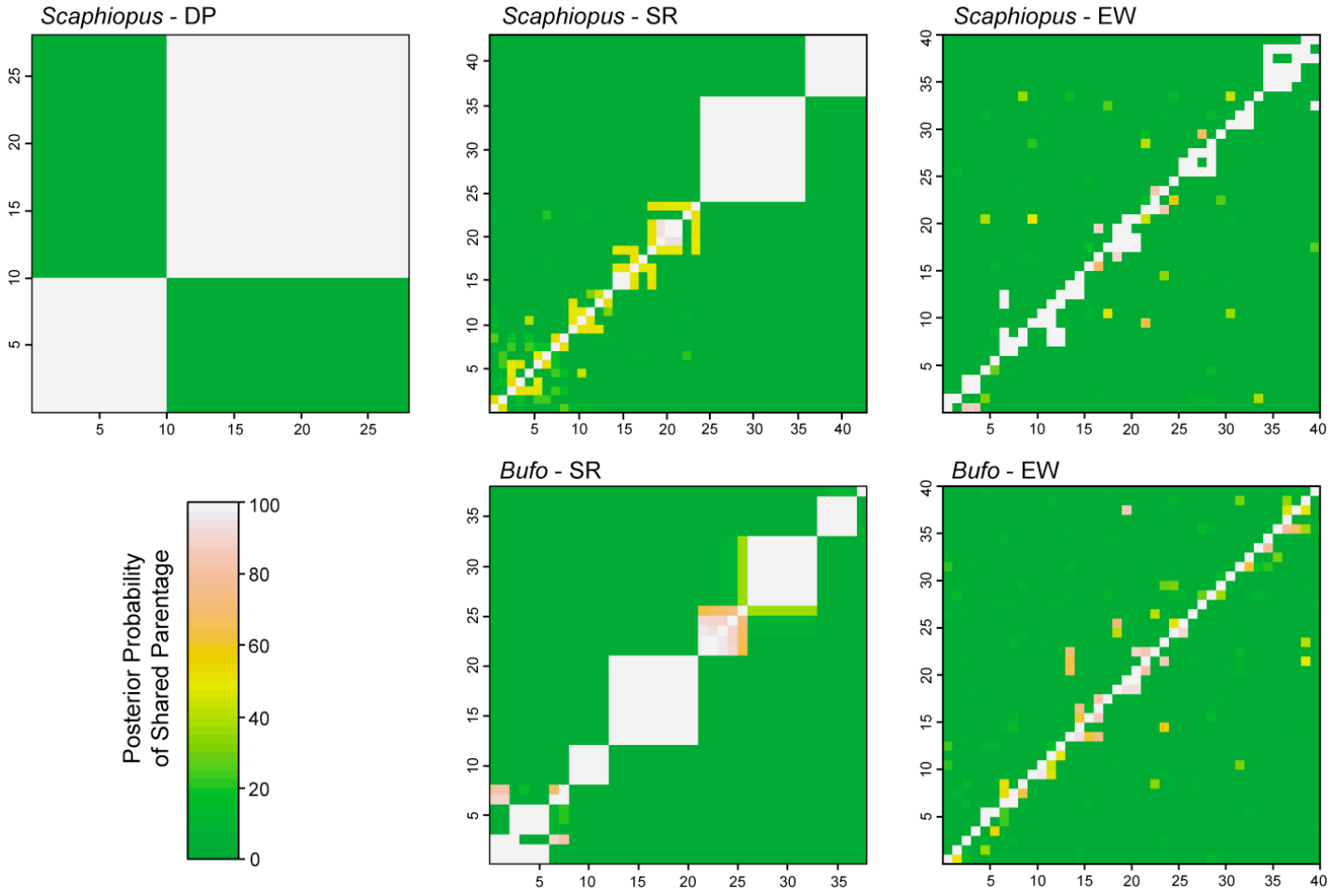

Figure 2.3. Posterior probability plots of shared parentage for three *S. couchii* ponds and two *B. cognatus* ponds ordered from smallest to largest in size (left to right). Individual offspring are along each axis and colors correspond to the posterior probability that two individuals share a father (above diagonal) and a mother (below diagonal).

Table 2.3. Effects of the reproduction and pond size characteristics on allelic richness (AR) and gene diversity (H_S) in standard least squares mixed models.

	Bufo cognatus				*Scaphiopus couchii*				*S. couchii* (partial dataset*)			
	AR		H_S		AR		H_S		AR		H_S	
Source of variation	$F_{1, 5}$	p	$F_{1, 5}$	p	$F_{1, 12}$	p	$F_{1, 12}$	p	F^*	p	F^*	p
Reproduction												
ri	19.086	0.007	18.828	0.007	11.770	0.005	10.830	0.006	4.091	0.068	3.456	0.090
Pond Size												
ln(Perimeter)	5.441	0.067	6.045	0.057	12.856	0.004	12.711	0.004	0.340	0.576	0.001	0.975
ln(Area)	2.141	0.203	2.971	0.145	14.771	0.002	15.836	0.002	0.296	0.602	0.137	0.721
ln(Volume)	0.403	0.554	0.542	0.495	14.139	0.003	13.996	0.003	0.584	0.467	0.183	0.680

* Reproduction analysis excludes DP, df = 1, 11; pond size analyses exclude BR, CR, CW, and DP, df = 1, 8

of diversity were only marginally significant for *S. couchii*. In ponds for which I had both adult and offspring samples, *ri* was negatively correlated with the amount AR and H_S lost across generations for *B. cognatus* (AR – $F_{1,4} = 42.6208$, $p = 0.0028$; H_S – $F_{1,4} = 18.8955$, $p = 0.0122$), but not in *S. couchii* (AR – $F_{1,2} = 3.6896$, $p = 0.1947$; H_S – $F_{1,2} = 2.5537$, $p = 0.2511$). The number of inferred parents showed a clear relationship with AR and H_S and tadpoles in ponds with less than 15 and 10 reproductively successful pairs for *B. cognatus* and *S. couchii* respectively, had lower diversity than adults (Figure 2.4).

Pond size attributes were significant predictors of *ri* in some, but not all instances (Figure 2.5). For *B. cognatus*, no aspects of pond size were positively correlated with *ri* suggesting that pond size is not the primary determinant of reproduction in this species. Pond perimeter was positively correlated with *ri* for *S. couchii* when all ponds were included. However, in analyses examining the region of ecological overlap with *B. cognatus* (excluding the four smallest *S. couchii* ponds), no attributes of pond size were correlated with *ri*. The effect of pond size on levels of AR and H_S among offspring within a locality differed across species. For *B. cognatus* pond perimeter was positively correlated with both AR and H_S although this relationship was only marginally significant (Table 2.3). All aspects of pond size were significant predictors of AR and H_S in *S. couchii* ponds for the full dataset. However, after excluding small ponds and considering only *S. couchii* ponds of ecological overlap with *B. cognatus*, the relationship between these parameters was no longer significant (Table 2.3, Figure 2.6).

DISCUSSION

Variance in reproductive success among breeding individuals can greatly influence levels of genetic diversity in offspring and consequently decrease the

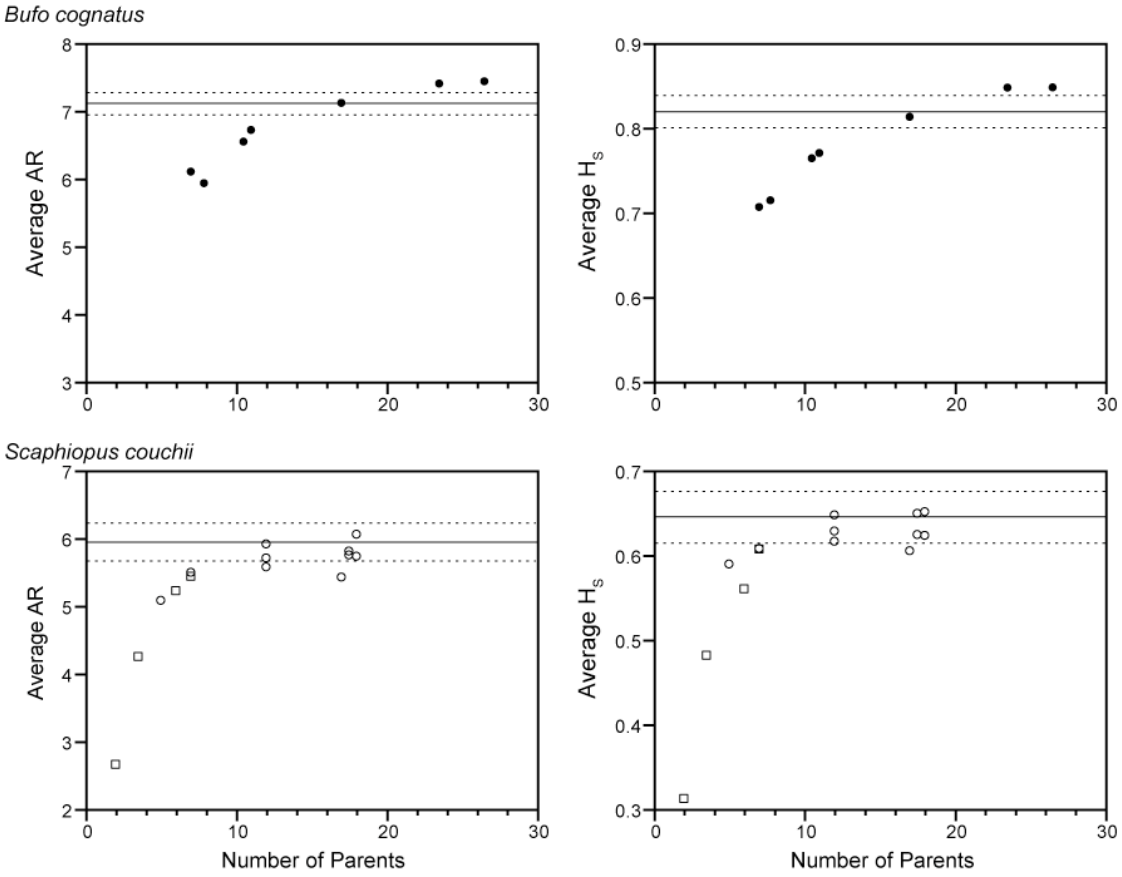

Figure 2.4. The average number of parents inferred in Bayesian assignment tests against average allelic richness (AR; left panels) and gene diversity (H_S; right panels) within ponds for *B. cognatus* (top) and *S. couchii* (bottom). Horizontal lines on each plot indicate average genetic diversity indices (solid) plus and minus one standard error (dotted) across all adult samples. Open squares in *S. couchii* plots indicate the four smallest ponds (BR, CR, CW, and DP).

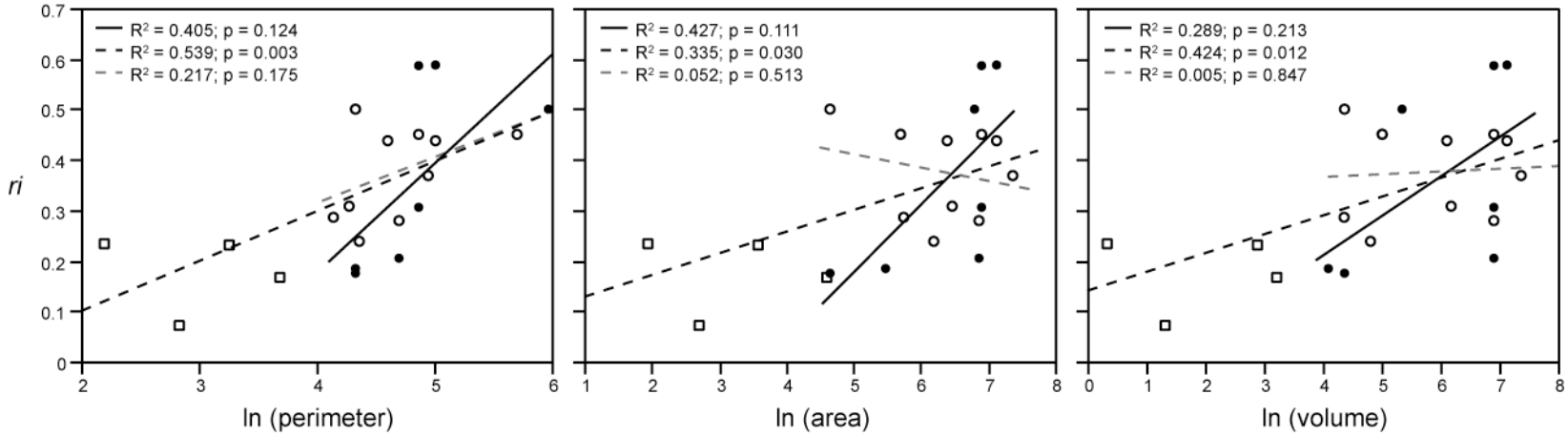

Figure 2.5. Regression of pond size versus the reproduction index (*ri*). *Bufo cognatus* ponds are represented by closed circles and the solid regression line. *Scaphiopus couchii* ponds are represented by open symbols and dashed lines; the black dashed line is the regression for all *S. couchii* ponds and the open circles and gray dashed line are for just the *S. couchii* ponds included in the reduced dataset (excluding BR, CR, CW, and DP represented by open squares). R^2 and *p*-values for tests of slopes significantly different from zero are reported for each regression.

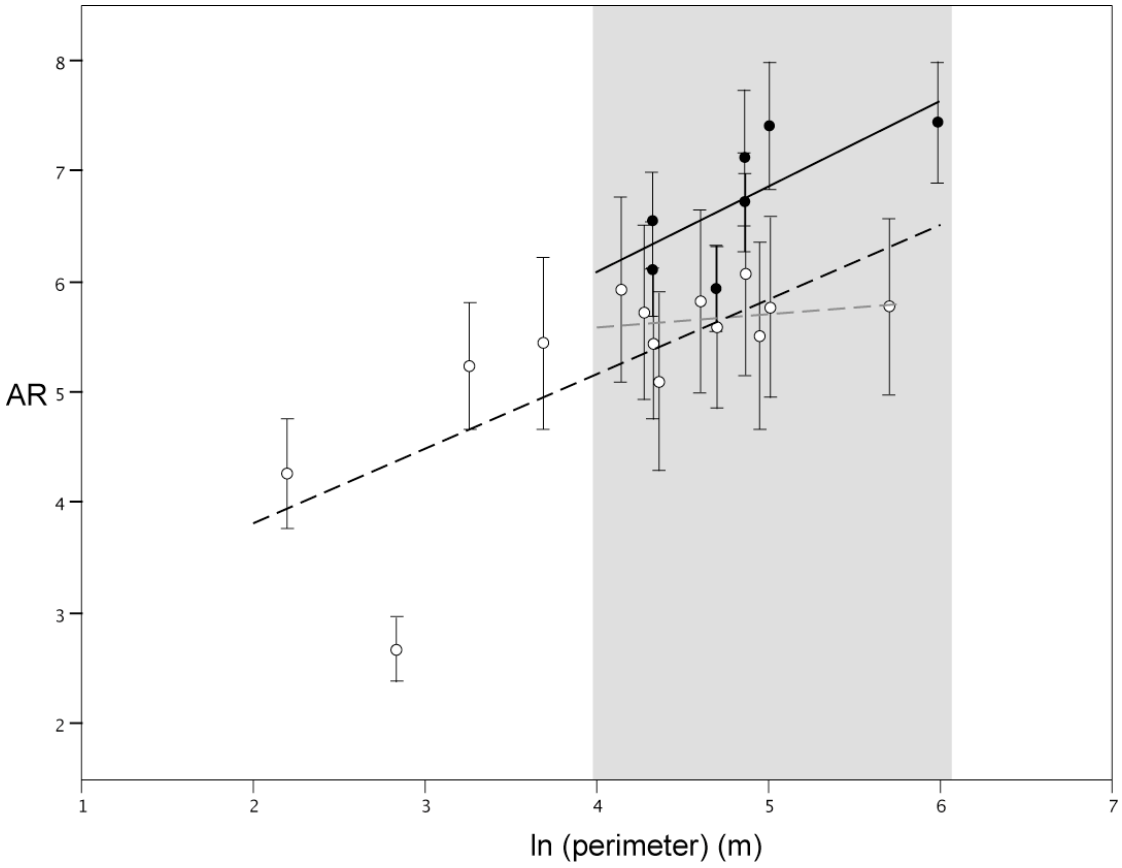

Figure 2.6. Regression of the pond size versus the average allelic richness (AR) ± SE within each pond across loci. *Bufo cognatus* ponds are represented by closed circles and the solid regression line. *Scaphiopus couchii* ponds are represented by open circles and dashed lines; the black dashed line is the regression for all *S. couchii* ponds and the gray dashed line is for only the *S. couchii* ponds included in the reduced dataset (excluding BR, CR, CW, and DP). Gray shading indicates the zone of ecological overlap.

effective size of populations (Hedrick, 2005; Nunney, 1993, 1996; Wright, 1938). However, other factors such as selection, low survivorship, and environmental stochasticity will also decrease genetic diversity in populations (Hedgecock, 1994; Waples, 2002). While temporal estimates of effective size are commonly used to examine the effect of reproductive skew on changes in genetic diversity, they do not explicitly examine the role of reproductive success in lowering population genetic diversity. By focusing specifically on the reproductive success of individuals and changes in genetic diversity over time, I am able to examine population-level consequences of reproductive skew and link individual success under different breeding contexts to patterns of population genetic diversity. This comparison between two ecologically similar species of frogs differing in mating system and life history characteristics reveals that the maintenance of genetic diversity is influenced by both reproductive skew and the ecological and demographic context of reproduction.

The levels of reproductive success, average relatedness, and temporal variation in genetic diversity that I find in *B. cognatus* and *S. couchii*, meet some but not all of the expectations for aggregate and explosively breeding amphibians. Overall, reproduction is limited and genetic diversity decreases between adult and offspring generations as expected in situations with intense competition for mates due to temporal synchrony and short breeding bouts (Emlen and Oring, 1977). However, I also find large variance both between species and across populations in the degree to which reproduction is skewed and genetic diversity is lost, even in areas of ecological overlap, indicating that species characteristics as well as ecological factors play a role in adult reproductive patterns and their genetic consequences.

Explosive breeding amphibians are often characterized as having dense aggregations with intense male-male competition (Wells, 1977) and despite general

similarities between *B. cognatus* and *S. couchii*, they differ in the OSR at breeding aggregations. OSR can affect sexual selection and variance in reproductive success (Emlen and Oring, 1977; Shuster and Wade, 2003; Sullivan *et al.*, 1995), effective population size, and genetic diversity (Caballero and Hill, 1992; Nunney, 1993, 1996). Because *B. cognatus* aggregations have low OSR relative to *S. couchii* (Krupa, 1989; Sullivan, 1982), I expected that the intensity of competition for mates and hence, the degree of reproductive skew and loss of genetic diversity, would be greater in *B. cognatus*. I find evidence for significant reductions in both AR and H_S in both species, but these species do not show a loss of diversity in all populations, nor do they differ in amount of genetic diversity which is lost. Within ponds, changes in genetic diversity are not influenced solely by sex-ratios and mating system characteristics. These factors may instead have a greater effect on the variance across breeding ponds in levels of reproductive skew across sites for each species. Average relatedness among tadpoles within ponds varies more across *S. couchii* sites compared to *B. cognatus* sites (Figure 2.2) showing that within pond reproduction in *S. couchii* can be extremely limited in some, but not all, situations. Determinants of reproductive success may be influenced to a greater degree by the context of mating in *S. couchii* whereas reproduction in *B. cognatus* may be buffered against limits to reproduction.

The number of breeding individuals

Multilocus, multigenerational genetic data, and the development of Bayesian clustering techniques (see Blouin, 2003; Manel *et al.*, 2005) provide the opportunity to examine factors underlying temporal changes in genetic diversity. Previous studies quantifying reproduction at the population level have used paternity assignment methods with sampled parents (e.g. Garant *et al.*, 2001; Matocq, 2004), distance-based clustering methods to infer the number of sibship groups (e.g. Bentzen *et al.*, 2001;

Blouin *et al.*, 1996), and temporal methods to calculate the effective number of breeding individuals, N_b (e.g. Jehle *et al.*, 2001; Scribner *et al.*, 1997). The Bayesian clustering method implemented in PARENTAGE (Emery *et al.*, 2001; Wilson, 2006) has two main advantages over other commonly used methods: it does not rely on complete sampling of adults, nor does it necessitate posthoc delineation of sibgroups. In addition, this method provides estimates of kingroup sizes and relationships among offspring. PARENTAGE can incorporate genotypes of potential adults as prior information, but does not require these data, and provides a quantitative estimate of posterior probability of shared sibship and parentage among all individuals.

Offspring samples of both species vary in the number of inferred parents corroborating that reproduction was limited at some, but not all sites. While the average *ri* across sites did not differ between *B. cognatus* and *S. couchii*, within pond *ri* is lower for *B. cognatus* compared to *S. couchii* at three of the four ponds for which I have samples of both species (DG, SR, ST) such that in identical ecological situations, fewer individuals of *B. cognatus* may breed than do *S. couchii*. The positive correlation between *ri* and measures of offspring genetic diversity provide strong evidence that diversity is lost as a direct result of low reproductive success among adults rather than from larval mortality or genetic drift. However, while this pattern is robust among *B. cognatus* ponds, the correlation is no longer significant at $p < 0.05$ in *S. couchii* when the pond with only two reproductively successful pairs, DP, is omitted. In *S. couchii*, ponds with extremely limited reproduction have disproportionately low genetic diversity. The number of reproductive individuals required to maintain diversity is lower for *S. couchii* than *B. cognatus* and even ponds with a small number of breeding pairs may avoid losing genetic diversity across generations (Figure 2.4). This pattern may simply reflect the fact that *S. couchii* has lower overall levels of both AR and H_S than *B. cognatus*, but these results also support

that differences between the species in reproductive success at ponds of varying sizes influences the maintenance of diversity.

The ecological context of breeding in desert anurans

Examining the specific nature of the relationship between genetic diversity and the number of breeding individuals in light of pond size elucidates the importance of breeding group size for the maintenance of genetic diversity in *B. cognatus* and *S. couchii*. In this study, *B. cognatus* bred only in larger water bodies while *S. couchii* bred in habitats ranging from small puddles to large temporary ponds; this pattern is similar to that found for *S. couchii* and two different species of *Bufo* in other parts of the southwestern United States (Dayton and Fitzgerald, 2001). While Dayton and Fitzgerald (2001) found that larval competition may mediate the co-occurrence of these genera, the absence of *B. cognatus* at smaller ponds in this study may simply be due to differences in larval development time. *Scaphiopus couchii* has a short larval period of 8 – 13 days (Mayhew, 1965) while *B. cognatus* takes significantly longer to complete metamorphosis (18 - 45 days, Bragg, 1936, 1937a). Thus, *Bufo cognatus* are restricted to breeding in larger bodies of water with longer hydroperiods, while *S. couchii* are able to breed in smaller ponds and puddles, and, therefore a greater number of sites overall.

Breeding site characteristics have a strong effect on reproductive skew and levels of genetic diversity across generations; small ponds where only *S. couchii* breed (BR, CR, CW, and DP) drive many of the relationships between pond size and measure of genetic diversity and reproduction (see Figure 2.4, 2.5, 2.6). These ponds have disproportionately few breeding adults (Figure 2.5), whereas within the zone of ecological overlap with *B. cognatus* reproduction, levels of genetic diversity in *S. couchii* are largely independent of pond attributes. Enough individuals of *S. couchii*

are reproductively successful to avoid a reduction in genetic diversity across generations.

Breeding site attributes may also interact with mating system characteristics and influence patterns of genetic diversity across generations. The density of individuals at breeding aggregations, independent of pond size, can affect the intensity of sexual selection and the opportunity for reproduction (Emlen and Oring, 1977; Wells, 1977) thereby altering the distribution of reproductive success among individuals (Kokko and Rankin, 2006). Density-dependent mating tactics and determinants of mating success have been documented in a number of anurans (e.g. Byrne and Roberts, 2004; Krupa, 1994; Woolbright *et al.*, 1990). Because reproduction is constrained both spatially and temporally in the two anurans studied here, breeding densities and the opportunity to mate may influence the degree of reproductive skew and overall breeding success for both *S. couchii* and *B. cognatus*. All male *S. couchii* vocalize while floating in the water from throughout the pond (Bragg, 1945) whereas only a proportion of the population of *B. cognatus* males vocalize, sitting in the shallow areas at the edges of the pond (Bragg, 1937b), while the other "satellite" males attempt to intercept females attracted by the calling males (Krupa, 1989; Sullivan, 1982). Thus, reproductive success of *S. couchii* populations may be limited at small ponds by the amount of space available for calling, but not at larger ponds. If density limits reproduction in *B. cognatus*, pond perimeter should be the most important pond size attribute for patterns of genetic diversity and reproduction. I do find weak and positive correlations between measures of genetic diversity and pond perimeter for *B. cognatus* suggesting that the space available for calling may influence the number of reproductively successful pairs. However, I do not find a positive correlation between *ri* and any of the pond size attributes, rather, the variance in measures of *ri* increases with pond size (Figure 2.4). While a greater

number of breeding pairs may be able to reproduce successfully in large ponds than in small ponds, pond size in itself does not determine levels of reproduction. The absence of a significant relationship between pond size and genetic patterns in *B. cognatus* suggests that for this species, mating tactics, sex ratios, and individual breeding condition may play a larger role in determining the number of individuals that breed at a pond than spatial constraints.

Small ponds may have fewer competitors and predators (Spencer *et al.*, 1999; Woodward, 1983) and may be favorable for *S. couchii* despite short hydroperiods. However, if *S. couchii* reproduction were to occur solely at small ponds, overall genetic diversity at the population-level would decrease substantially because of limited mating opportunities. Given the importance of the number of breeding individuals for inter-generational maintenance of genetic diversity in this species, larger ponds may be a necessary for persistence of genetic diversity over time. A number of studies have shown that small wetlands can be important for migration dynamics and patterns of genetic connectivity among patches (Gibbs, 1993; Semlitsch and Bodie, 1998). My results, in contrast, emphasize the importance of larger ponds for reproduction and the maintenance of diversity across generations. Habitat requirements critical to the spatial distribution of diversity may differ from those important to the persistence of diversity across generations; these results suggest that both small and large ponds may play important roles in amphibian population genetics.

The persistence of genetic diversity across generations depends on the collective reproductive success of individuals within populations and these results underscore the important role that mating system and ecology play in population genetics (Nunney, 1993; Wright, 1931, 1938). By quantifying the number of reproductively successful individuals, levels of genetic diversity in offspring and

changes in genetic diversity across generations, I can directly examine the correlation between reproductive success and genetic diversity. These results emphasize the close relationships among organismal ecology, mating system characteristics, and ecological conditions in determining reproductive success and the persistence of genetic variation. The loss of genetic diversity across generations due to reproductive skew is both species- and context-dependent. Considering the ecological and social context in which reproduction occurs when examining the genetic consequences of reproductive episodes will help us to better understand the proximal determinants of patterns of genetic diversity.

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CHAPTER THREE

LARVAL MORTALITY AND TEMPORAL PATTERNS OF GENETIC DIVERSITY IN POND BREEDING ANURANS

Abstract.— The persistence of genetic diversity across generations is the combined result of reproductive success and offspring survival. Previous studies have shown reductions in genetic diversity and effective size between parents and offspring, but the proximal determinants of these changes are not always clear. Ecological and organismal attributes that affect levels of reproductive skew are likely to be quite different from those that influence patterns of mortality among offspring. In this study, I focus on two species of pond breeding anurans and evaluate microsatellite genotypes for two temporally spaced samples to examine the role of larval mortality in the loss of genetic diversity between generations. *Bufo cognatus* and *Scaphiopus couchii* are explosive aggregate breeding frogs that lay eggs in ephemeral ponds in the desert southwest of North America. *Scaphiopus couchii* is a desert adapted species with a short larval duration of 8 – 13 days; in contrast, *B. cognatus* tadpoles take two to three times longer to develop and therefore spend a greater amount of time vulnerable to mortality in ponds. I do not find evidence for a reduction in genetic diversity from tadpoles to metamorphs in *S. couchii* ponds indicating that mortality does not generally have a strong effect of patterns of diversity for this species. However, I find a significant loss in genetic diversity in *B. cognatus* that is positively correlated with the number of days tadpoles are in ponds suggesting that diversity is lost continually throughout the larval period in *B. cognatus*. *Scaphiopus couchii* are likely to experience reduced genetic diversity across the larval period only when mortality is severe whereas *B. cognatus* loses diversity in a variety of ecological situations. These results suggest that both life history attributes and variation in larval

environments influence the loss of genetic variation within populations and across generations.

INTRODUCTION

Patterns of population genetic structure are the combined result of diversity maintained across generations via reproduction and survival, and the distribution of diversity across landscapes via migration and dispersal. While the importance of gene flow to population differentiation and evolutionary change is well-appreciated (Lenormand, 2002; Slatkin, 1987), the consequences of within group factors to changes in genetic diversity has primarily been explored through theoretical work (e.g. Hedrick, 2005; Waples, 2002; Wright, 1938). Both unequal reproduction among individuals and non-random offspring mortality can each reduce genetic diversity across generations (Chesser, 1991; Hedgecock, 1994; Waples, 2002). Mortality rates can differ dramatically between life stages (Caswell, 2001), particularly in organisms with complex life cycles (Wilbur, 1980) and such stage-specific mortality can exacerbate the effects of reproductive skew and lead to additional reductions in genetic diversity (Hedgecock, 1994; Waples, 2002). We have an understanding of the interaction between survival rates and population dynamics (Crowder *et al.*, 1994; Leslie, 1945; Pearman, 1993), but the impacts of mortality on patterns of genetic diversity has not been examined empirically. The maintenance of genetic diversity within groups is important for overall population genetic structure; migration and gene flow can distribute existing variation among regions, but as genetic diversity is lost within populations the ability of gene flow to restore genetic diversity within groups decreases. If genetic diversity is extremely low within population, no amount of gene flow will be enough to prevent divergence among populations through genetic drift and the negative consequences of inbreeding.

Previous studies of temporal variation in genetic diversity have found low effective population size and reductions in genetic diversity between adult and offspring samples in a number of taxa (e.g. Jorde and Ryman, 1995; Planes and Lenfant, 2002; Rowe and Beebee, 2007; Schmeller and Merilä, 2007). However, both variance among adults in reproductive success and offspring mortality can result in these patterns of reduced genetic diversity within populations; in many studies, changes in diversity due to these two mechanisms are not disentangled. Because the organismal characteristics and ecological circumstances influencing reproductive success are likely to be different from those affecting mortality rates, it is useful to tease apart the contribution of each to changes in genetic diversity for a better understanding of the proximate determinates of population genetic structure.

Mortality is expected to decrease genetic diversity when survival is non-random (Crow and Morton, 1955; Waples, 2002). Hedgecock (1994) suggested that species with Type III survivorship curves, that is, high fecundity and high rates of mortality at early life stages, are more likely to suffer reductions in genetic diversity across generations due to family-correlated mortality. Non-random mortality may result from heritable differences as well as stochastic processes, especially when kin are spatially clumped. For example, larvae hatching from egg masses (Hedgecock, 1994), aggregations due to kin recognition (Waldman, 1982), and habitat selection (Pfennig, 1990) will result in the non-random distribution of individuals. Even mild departures from random survival during the larval period can result in a loss of genetic diversity and decrease in effective population size across generations when mortality rates are high (Waples, 2002).

Terrestrial amphibians are an ideal group for quantifying the genetic consequences of larval mortality because many species have complex life histories (Wilbur, 1980) with aquatic larvae that are confined to water bodies until

metamorphosis. Larval amphibian communities have been well-studied (McDiarmid and Altig, 1999b; Woodward and Mitchell, 1991) and intense competition (Alford and Wilbur, 1985; Dayton and Fitzgerald, 2001), predation (MacKay *et al.*, 1990; Woodward, 1983), and stochasticity in hydroperiod (Miller, 1909; Skelly, 1996) are known to contribute to high levels of mortality during the larval period for many species (Alford and Richards, 1999). Anurans inhabiting the deserts of North America are a unique system and are particularly appropriate for studies of larval mortality and population genetic structure due to their breeding biology and larval environment. Most desert anurans are explosive breeders (Wells, 1977) with one to three nights of reproductive activity occurring at the onset of summer rains (Bragg, 1940; Sullivan, 2005). Reproductive skew in some ponds can be high (Chan, Chapter 2) and decreases genetic diversity between parent and offspring groups. Resources, community composition, and hydroperiod are extremely unpredictable in desert pond environments (Low, 1976); therefore, particularly high rates of larval mortality may occur in some ponds due to competition, predation, and pond evaporation (see Woodward and Mitchell, 1991). Desert pond environments are an interesting opportunity to examine the relative roles of reproductive skew and offspring mortality on the maintenance of genetic diversity. Furthermore, breeding bouts occur during the initial filling of the pond (Sullivan, 1983; Woodward, 1984), therefore, larvae within a pond belong to the same age cohort. This provides the opportunity to focus on changes that occur within the larval period without the confounding effects of overlapping age classes and continued reproduction.

The Great Plains Toad (*Bufo cognatus*) and Couch's Spadefoot Toad (*Scaphiopus couchii*) are two abundant species in the southwestern deserts of Arizona and New Mexico that are expected to differ in the degree to which genetic diversity is lost due to larval mortality. Both species are ecologically similar and breed

explosively at ephemeral ponds, but they vary in life history characters that should influence larval mortality. These anurans have generalized exotrophic, benthic larvae (McDiarmid and Altig, 1999a), but tadpoles of *B. cognatus* require 18 – 40 days to complete metamorphosis (Krupa, 1994) whereas *S. couchii* are truly desert adapted (Blair, 1976; Mayhew, 1965; Woodward and Mitchell, 1991) with a short larval period of 8 – 13 days. Because of this rapid development, *S. couchii* are able to lay eggs in a variety of habitats from small puddles to large ponds whereas *B. cognatus* are constrained to breeding in larger ponds with longer hydroperiods and these habitats typically have higher densities of competitors and predators (Newman, 1987; Spencer *et al.*, 1999; Woodward, 1983). Previous studies of these two species found that habitat selection associated with larval development time may influence the loss of genetic diversity due to reproductive skew; reductions in genetic variability from parents to offspring were detectable in *B. cognatus* ponds but only in small *S. couchii* ponds (Chan, Chapter 2). At ponds where *S. couchii* co-occur with *B. cognatus*, *B. cognatus* will lose more genetic diversity due to reproductive skew and I predict that larval cohorts should additionally lose a greater amount of genetic diversity than *S. couchii* through mortality because of their longer exposure to competition, predation and environmental stochasticity.

Here, I compare temporal variation in genetic diversity within tadpole cohorts of these two species of desert frogs. By taking temporally spaced samples during the larval period, I am able to isolate population changes in genetic diversity that occur due to larval mortality and compare the impact of larval period duration, characteristics of the larval habitat, and differences in mating system for the maintenance of genetic diversity within populations of these two species.

METHODS

Tissue sampling and laboratory protocols

I monitored sites in the San Simon Valley and the San Bernardino Valley of Arizona and New Mexico during the summers of 2004 and 2005 for breeding activity. At 12 breeding aggregations of *B. cognatus* and nine breeding aggregations of *S. couchii*, I uniquely marked and sampled tissue from breeding adults by toe clipping (locality abbreviations listed in Appendix 3). Tissue samples were preserved in 100% EtOH for subsequent genetic analyses and provide an estimate of the overall genetic diversity present among the ponds.

To quantify the effects of larval mortality on levels of genetic diversity, I sampled tadpoles from breeding ponds at two times that bracketed their larval development. Reproduction in *S. couchii* occurs at a greater number of sites than *B. cognatus* because of shorter development times, therefore, I focused at larger ponds in the zone of ecological overlap where both *S. couchii* and *B. cognatus* could reproduce. Tadpoles of both species hatch 24 – 48 hours after eggs are laid (Bragg, 1936, 1965; Mayhew, 1965). Therefore, two to three days following the last night of breeding activity at a site, I randomly collected 15 - 60 tadpoles of each species from each pond and preserved them in 100% EtOH. These initial tadpole samples represent the genetic diversity present among offspring immediately following reproduction and prior to any significant larval mortality. At each site I measured the size of the pond when eggs were laid (depth, perimeter, surface area, and volume), noted the composition of the tadpole and invertebrate community, monitored larval development, and checked ponds for drying every other day. I captured metamorphic individuals as they emerged from the pond and sampled tissue with a small tail clip before releasing them; this second sample represents the genetic diversity of offspring that have survived the larval stage. Individuals of *B. cognatus* and *S. couchii* emerge

from ponds over the course of four to seven and two to four days, respectively, (L. Chan, unpublished data). Therefore, I visited ponds on consecutive days and took tissue from 10 – 20 individuals each day. The final temporal dataset consisted of five pairs of tadpole and metamorph samples for *B. cognatus* and six pairs for *S. couchii*. At a single pond, SR, some individuals reached late Gosner stages (Gosner, 1960), but all tadpoles died from disease prior to metamorphosis (*Spea* spp., *B. cognatus*, and *S. couchii*). Thus, I sampled late stage tadpoles at this site as a second temporal sample. I retained this population in the study to investigate the effects of elevated rates of larval mortality on genetic diversity.

I extracted whole genomic DNA from approximately $1mm^3$ of adult toe clips and tadpole/metamorph tail clips by incubating tissues in 150 µL of a 5% Chelex solution (Chelex-100, BioRad) with 19 µg ProteinaseK at 55 °C for 180 minutes followed by 95 °C for 10 minutes. The supernatant from each extraction was used directly as template DNA in polymerase chain reactions (PCR) to amplify twelve species-specific microsatellite loci. For *B. cognatus,* I used the primers and PCR conditions described for eleven loci in Chan (2007a) and one additional locus (ihhh) described in Gonzales *et al*. (2004). For *S. couchii* I used the primers and PCR conditions described for twelve loci in Chan (2007b).

I tested for deviation from Hardy-Weinberg equilibrium (HWE) and evidence of linkage disequilibrium (LD) between loci for adult samples in GENEPOP V3.4 (Raymond and Rousset, 1995). I used a Markov chain method (Guo and Thompson, 1992) with 10,000 dememorization steps and 1,000 batches of 10,000 steps to determine the significance of within population heterozygote deficiencies and non-independence among loci. Table wide significance at $\alpha = 0.05$ was determined after sequential Bonferroni correction.

Population genetic diversity and the number of family groups

For each species at each locality, I calculated indices of genetic diversity for tadpole and metamorph samples. I used FSTAT 2.9.3 (Goudet, 1995) to calculate locus specific standardized allelic richness (AR) accounting for variation in sample size (Petit *et al.*, 1998) and unbiased estimates of gene diversity (H_S; Nei, 1987). Because all tadpoles at pond SR died from disease rather than from more typical causes, I calculated pond specific estimates of diversity and kinship for these samples but omitted them from all comparative analyses across ponds or between species. I examined whether the variance among ponds in mean AR and H_S for tadpoles and metamorphs was greater among *B. cognatus* samples in comparison to *S. couchii* samples with a two-sided *F*-test.

If mortality is non-random with respect to family group, the number of sibship groups among metamorphs may be lower than that of tadpole groups. Because census sizes at breeding aggregations of desert anurans can be large, standard methods of determining paternity and kinship that require parental genotypes cannot be applied in studies such as this one. Thus, to determine the number of family groups within each tadpole and metamorph sample, I used a clustering method implemented in the program PARENTAGE (Emery *et al.*, 2001; Wilson, 2006) that utilizes Markov chain Monte Carlo search algorithms in a Bayesian framework to partition individuals into groups of shared paternity and maternity. PARENTAGE can incorporate genotypes of potential parents as prior information, but does not require them. I conducted initial PARENTAGE searches varying sampling interval, chain length, the number of chains, and the temperatures of heated chains to determine the appropriate parameters for adequate exploration of parameter space and convergence. For those samples for which I had adults collected from the same pond, I included adult genotypes as potential fathers and mothers and used flat priors for the number of fathers and

mothers with "all fathers contribute" and "all mothers contribute" options turned off. I set the prior on mutation rate to a gamma distribution with a shape parameter of 2 and a mean of 0.001. The search parameters of final runs varied considerably across samples because of differences in sample sizes, levels of genetic variation, and constraints on computational time (Appendix 3).

For each final run, I used the modes of the posterior probability distribution as the number of inferred fathers (N_F) and the number of inferred mothers (N_M) and determined the 90% credible interval around each estimate. For samples where multiple PARENTAGE runs were unsuccessful at finding a unimodal distribution to the posterior densities, I examined the posterior probability distributions for similarity across runs and instead used the mean of each distribution as the numbers of inferred fathers and mothers. I calculated the average number of inferred parents for each sample as $(N_F + N_M) / 2$. Because the number of offspring genotyped differed among samples, I calculated a reproduction index, *ri*, to adjust for variation in sample sizes.

$$ri = (N_F + N_M) / (2\ N_O)$$

where N_F and N_M are the number of inferred fathers and mothers and N_O is the offspring sample size. Values of *ri* range from zero to one; low values indicate few reproductive adults and high kinship among offspring; the degree of shared kinship within a sample decreases as *ri* increases.

Changes in diversity and the number of family groups

If offspring mortality is non-random and influences overall patterns of genetic diversity, I expect indices of diversity to be greater for early tadpoles compared to metamorphs. To examine temporal changes in diversity indices, I subtracted the metamorph estimates of AR and H_S from tadpole estimates for each locus at each locality and used a one-sided t-test to determine whether the loss of diversity was

significantly greater than zero within localities and overall. Likewise, family-correlated mortality during the larval period may result in the elimination of some sibships. Therefore, I expect that *ri* will decrease between these two temporal samples. For each pond I subtracted the $ri_{metamorphs}$ from $ri_{tadpoles}$ and used a one-sided t-test to determine whether the change in *ri* across all ponds of each species was significantly greater than zero.

If a reduction in the number of kingroups is the mechanism underlying the loss of genetic diversity, temporal changes in *ri* should be negatively correlated with changes in AR and H_S across samples. Thus, I estimated the relationship between the change in *ri* and the change in locus specific estimates of AR and H_S accounting for variation across loci in diversity estimates by using a standard least squares mixed model (JMP 6.0, SAS Institute). For each species, AR and H_S were dependent variables, locality was included as a random variable, and both locus and change in *ri* as fixed effects.

Clutch size effects

Clutch sizes vary within the two focal species (Krupa, 1988; Woodward, 1987) and variation in kingroup size may influence the chance that members from a family group survive to metamorphosis. Stochastic processes could eliminate small family groups before larger family groups; in that case, I would expect to find that small sibship groups at the tadpole stage have few individuals persisting to the metamorph stage whereas large sibship groups should have a greater number of individuals detected in the metamorph sample. To examine whether the probability of survival through the larval period is related to family group size at early tadpole stages, I used PARENTAGE to estimate sibship among combined tadpole and metamorph samples for each pond that had evidence of shared kinship among tadpoles in separate analyses

($ri_{tadpoles} < 0.4$). For each PARENTAGE run, priors were identical to those in single sample searches and initial runs were conducted to assure adequate mixing and convergence. Final run parameters are listed in Appendix 3.

For each pond, I examined the pairwise posterior probabilities that individuals share fathers and mothers and delineated sibship groups with posterior probabilities of shared parents greater than 75%. For each group of two or more individuals, I determined the proportion of tadpoles and metamorphs assigned to that group by dividing the number of tadpole and metamorph samples in each group by their respective sample size. For each pond, I plotted the proportion of tadpoles against the proportion of metamorphs within each sibgroup. If survival is not influenced by clutch size and all tadpoles have an equal probability of persisting to the metamorph stage, this relationship should be approximately linear though the origin and a slope of one. Alternatively, if mortality is severe and small clutches are eliminated before large clutches, small kingroups at the tadpole stage should be less frequent at the metamorph stage and large tadpole clutches should make up a larger proportion of the metamorphs; the effect of clutch size on mortality can be assessed by the deviation from a one-to-one linear regression (see Figure 3.3).

Pond size and larval duration as determinants of genetic diversity

Larval community dynamics are complex (Alford and Richards, 1999; Semlitsch, 2003; Wilbur, 1980; Woodward and Mitchell, 1991) and mortality and developmental rates can be influenced by overall larval densities, resource limitations, and the presence of dominant predators and competitors (Alford, 1999; Newman, 1987; Newman, 1998; Wilbur, 1987; Woodward, 1982, 1983). Because community species richness is positively correlated with pond size (Spencer *et al.*, 1999), the size of the larval habitat may predict the selective pressures larvae experience. The larval

duration of *B. cognatus* restricts breeding to larger breeding ponds because of longer hydroperiods (Schneider and Frost, 1996) and potentially greater predator and competitor densities. Thus, I expect that populations in large ponds will lose greater genetic diversity in comparison to populations in small ponds. I used a standard least squares mixed model (JMP 6.0, SAS Institute) to determine whether aspects of pond size (natural logarithm of perimeter, area, and volume) predicted changes in AR and H_S in each species. For each model, I included locality as a random effect and both locus and pond size as fixed effects. Furthermore, rapid larval development in *S. couchii* will minimize the time tadpoles spend in the aquatic environment and the amount of genetic diversity lost in comparison to *B. cognatus*, thus, I expect to find a greater loss of genetic diversity over the larval period in *B. cognatus*. Due to stochastic mortality, larval densities should decrease with time, however, predator activity has been found to increase with lower prey abundance (Abrams, 1991, 1993; Anholt and Werner, 1998) such that I expect to find a non-linear relationship between sampling interval and the loss of diversity. Specifically, proportionally greater diversity should be lost at longer sampling intervals. I examined the relationship between the number of days between tadpole and metamorph sampling squared and locus specific changes in AR and H_S. Again, I used a standard least squares mixed model with the locality as a random effect and locus and the square of the sampling interval in days as fixed effects.

RESULTS

Estimates of genetic diversity and the number of family groups

Following Bonferroni correction for multiple comparisons, I found evidence for a significant heterozygote deficiency at one locus (BC52.04) in one population of *B. cognatus* and at one locus (sco126) in two populations of *S. couchii*. These loci did

not show a significant deficiency in heterozygotes in other populations suggesting that they generally meet HWE expectations. Likewise, I did not find evidence of significant LD in either species indicating that loci are segregating independently.

The mean number of alleles per locus for *B. cognatus* ranged from 5.93 to 7.44 among tadpoles and from 5.60 to 7.25 among metamorphs. On average *S. couchii* had fewer alleles per locus; these values ranged from 4.25 to 6.06 and 4.45 to 5.97. Mean H_S ranged from 0.782 to 0.853 and 0.786 to 0.851 for *B. cognatus* tadpoles and metamorphs, respectively, and from 0.657 to 0.732 and 0.654 to 0.728 for *S. couchii* tadpoles and metamorphs (Figure 3.1). The variance among populations in mean AR and H_S was greater in *B. cognatus* than in *S. couchii* for tadpole samples (AR: $F_{4,5}$ = 21.556, p = 0.005; H_S: $F_{4,5}$ = 6.850, p = 0.058) as well as for metamorph samples (AR: $F_{4,5}$ = 7.545, p = 0.048; H_S: $F_{4,5}$ = 10.368, p = 0.025).

Clustering analyses found a unimodal posterior probability density for the number of fathers and mothers in all but two samples (*S. couchii* tadpoles at SCD and metamorphs at ST). The posterior probability densities for these ponds were bimodal, thus I used the mean of the posterior probability densities as the estimate for the number of parents as these values were consistent across runs. The average number of inferred parents in *B. cognatus* samples ranged from seven to 26.5 for tadpoles and from five to 23.5 for metamorphs. In *S. couchii*, the average number of inferred parents per population ranged from 12 to 18 for tadpole samples and four to 19 for metamorph samples. Estimates of *ri* ranged from 0.200 to 0.586 in tadpoles and 0.210 to 0.650 in metamorphs of *B. cognatus* and from 0.279 to 0.5 in tadpoles and from 0.245 to 0.485 in metamorphs of *S. couchii* (Figure 3.2).

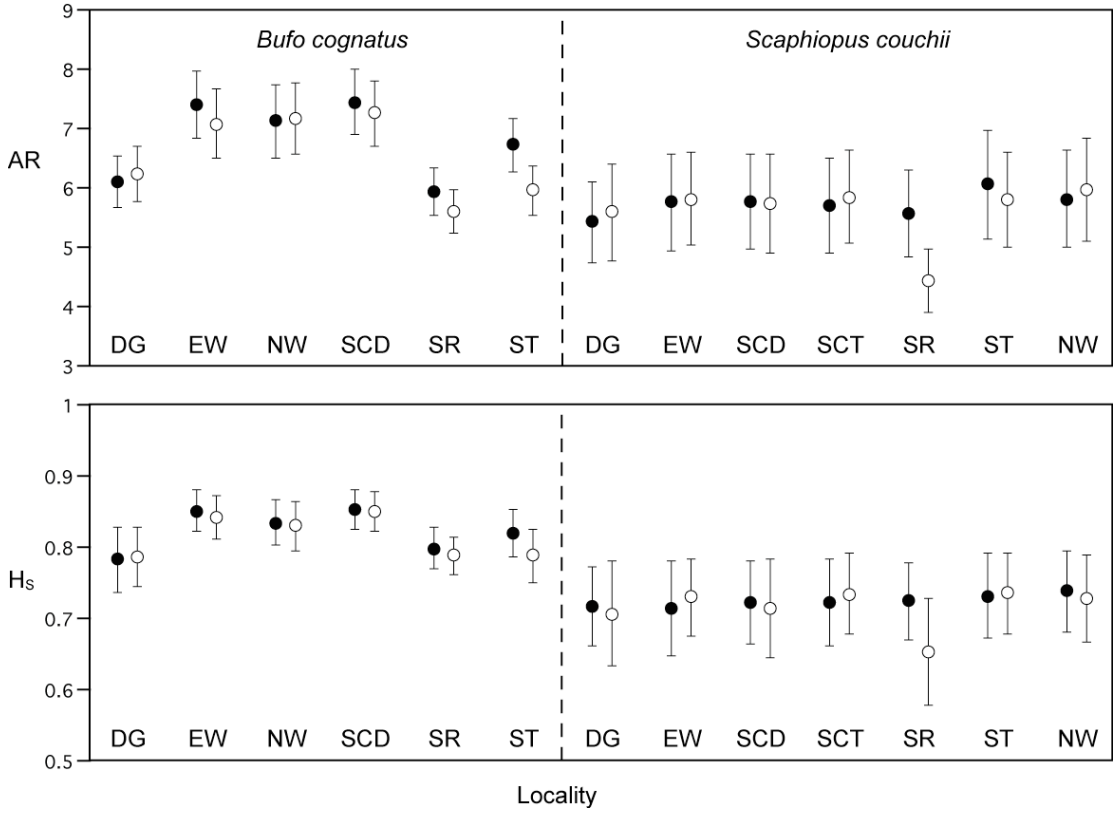

Figure 3.1. Average allelic richness (AR) and gene diversity (H_S) ± SE for tadpole (closed circles) and metamorph samples (open circles) for *B. cognatus* and *S. couchii* populations.

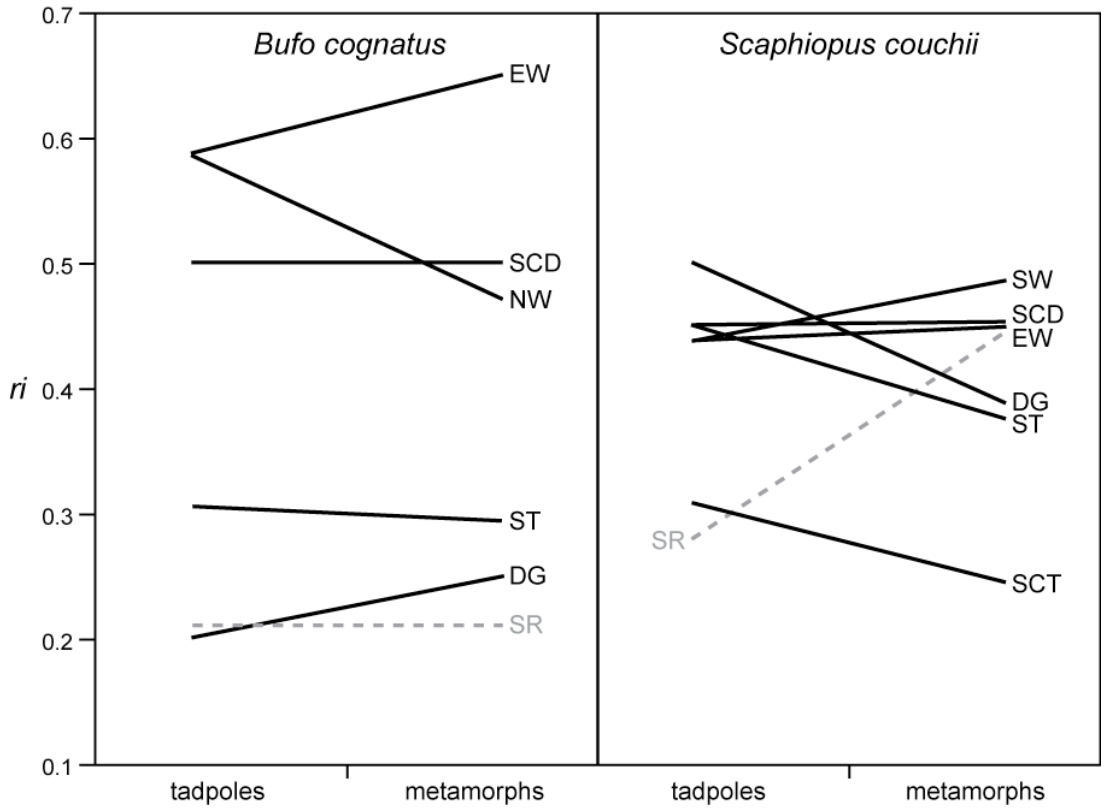

Figure 3.2. Changes in *ri* between tadpole and metamorph samples for each population of *B. cognatus* (left) and *S. couchii* (right). SR is indicated in gray dashed line.

Changes in genetic diversity and the number of family groups

Across populations of *B. cognatus* and *S. couchii* I found significant reductions in AR and H_S from early to late stage samples at two localities (Table 3.1). In ST, *B. cognatus* lost an average of 0.768 alleles/locus and gene diversity decreased by an average value of 0.031 between early and late stages ($p = 0.032$). In contrast, measurements of genetic diversity did not decrease between samples of *S. couchii* from this same locality. At site SR, both species experienced a significant reduction in AR (Table 3.2), though the change in H_S for *B. cognatus* was not significant at $p < 0.05$ ($p = 0.068$). Additionally, for *B. cognatus* samples in two ponds, EW and SCD, there was a trend toward a decrease in AR across samples though not significant ($p_{EW} = 0.0742$, $p_{SCD} = 0.0681$; Table 3.1).

Mean estimates of *ri* were not significantly different across sampling groups for either species (one-tailed $p_{Bufo} = 0.4890$, $p_{Scaphiopus} = 0.2458$) Likewise, changes in *ri* between samples within ponds were not significantly greater than zero across populations (one-tailed $p_{Bufo} = 0.4647$, $p_{Scaphiopus} = 0.1314$). Both the direction and the magnitude of change in *ri* did not show any trends for either species (Figure 3.2). I did not find a significant relationship between change in *ri* or the mean number of parents with changes in AR or H_S for either species (Table 3.2).

Effects of clutch size on probability of persistence

Three *B. cognatus* ponds (DG, SR, and ST) and two *S. couchii* ponds (SCT and SR) had sufficient variation in the number and size of kingroups among tadpoles to examine whether clutch size influences persistence to the metamorph stage. I did not find an effect of clutch size on persistence, but instead found evidence for random mortality. At each sites, the proportion of tadpoles that belonged to kingroups was similar to the proportion of metamorphs assigned to that group (Figure 3.3), thus, the

Table 3.1. One-sided *t*-test results for loss of allelic richness (AR) and gene diversity (H_S) between tadpole and metamorph samples. Changes in genetic diversity significantly greater than zero at $p < 0.05$ are indicated in bold for each population and overall including and omitting population SR.

	AR			H_S		
Locality	Mean Δ	t	*p*	Mean Δ	t	*p*
Bufo cognatus						
DG	- 0.1377	- 0.8986	0.8059	-0.0032	-0.3002	0.6152
EW	0.3259	1.5545	0.0742	0.0092	0.8554	0.2053
NW	- 0.0580	- 0.3616	0.6377	0.0048	0.5640	0.2920
SCD	0.1864	1.6075	0.0681	0.0024	0.3733	0.3580
SR	0.3351	3.9485	**0.0011**	0.0098	1.6045	0.0684
ST	0.7678	4.2770	**0.0007**	0.0313	2.0633	**0.0318**
Overall	0.2366	3.3415	**0.0007**	0.0091	2.1746	**0.0165**
Overall, no SR	0.2169	2.6051	**0.0058**	0.0089	1.8306	**0.0361**
Scaphiopus couchii						
DG	- 0.1605	- 0.6947	0.7492	0.0109	0.3944	0.3504
EW	- 0.0638	- 0.5428	0.7009	- 0.0150	- 0.8352	0.7893
SCD	- 0.0030	-0.0257	0.5100	0.0064	0.3444	0.3685
SCT	- 0.1405	-1.2722	0.8852	- 0.0123	- 1.0736	0.8470
SR	1.1252	3.5346	**0.0023**	0.0705	2.7024	**0.0103**
ST	0.2588	1.3111	0.1083	- 0.0039	- 0.3805	0.6446
SW	- 0.1667	-1.0570	0.8434	0.0098	1.1690	0.1336
Overall	0.1214	1.4361	0.0774	0.0095	1.2852	0.1012
Overall, no SR	- 0.0460	- 0.6959	0.7556	- 0.0007	- 0.0997	0.5396

Table 3.2. Effects of the changes in kingroup parameters (*ri* and the mean number of parents, N_P), sampling interval, and pond size characteristics on the change in allelic richness (AR) and gene diversity (H_S) in stand least squares mixed models. Significant relationships indicated in bold.

	Bufo cognatus				*Scaphiopus couchii*			
	AR		H_S		AR		H_S	
Source of variation	$F_{1,3}$	p	$F_{1,3}$	p	$F_{1,4}$	p	$F_{1,4}$	p
Number of kingroups								
Change in *ri*	0.064	0.816	0.028	0.878	0.166	0.705	0.000	0.988
Change in N_P	5.138	0.108	1.272	0.342	1.132	0.347	0.836	0.412
Sampling Interval								
$(Days)^2$	14.476	**0.032**	11.711	**0.042**	0.010	0.925	0.023	0.886
Pond Size								
ln(Perimeter)	0.121	0.751	0.002	0.970	0.882	0.401	0.014	0.911
ln(Area)	1.362	0.328	1.058	0.379	0.832	0.413	4.831	0.093
ln(Volume)	1.336	0.332	1.974	0.255	0.880	0.401	5.255	0.084

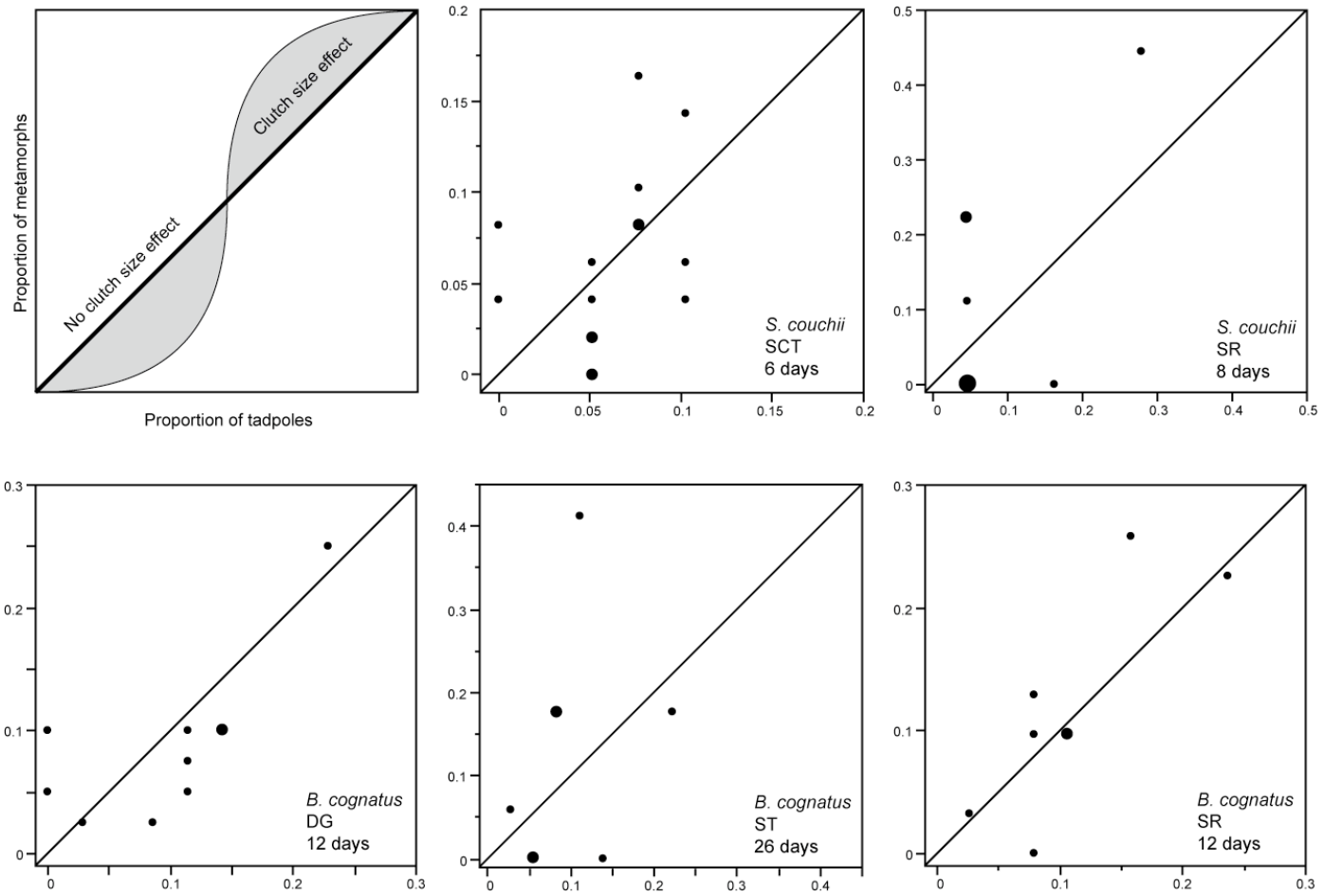

Figure 3.3. Relationships between the proportion of tadpole and metamorph samples that belong to the same kingroup. Upper left panel: diagram of the expected relationship under random mortality across all clutches (thick line) and an effect of clutch size on the probability of persistence (shaded area). Remaining five panels: results from two *S. couchii* and three *B. cognatus* ponds. Size of circle corresponds to one, two, or three kingroups with those proportions of tadpoles and metamorphs.

relative abundance of each kingroup did not vary predictably from tadpoles to metamorphs.

Effects of larval duration and pond size

The perimeter, area, and volume of ponds were not significantly correlated with changes in diversity indices for either species (Table 3.2). However, I found positive trends between the number of days between sampling events and the loss of AR and H_S between samples for *B. cognatus* although they were not significant (p_{AR} = 0.060, p_{Hs} = 0.078). The number of days between sampling events did not correlate with temporal changes in diversity for *S. couchii*, however, I did find a positive and significant correlation between the squared sampling interval and changes in genetic diversity in *B. cognatus* with proportionally greater diversity was lost at longer sampling intervals (Table 3.2, Figure 3.4).

DISCUSSION

The larval biology of pond breeding anuran communities has been well-studied (see Alford, 1999; Woodward and Mitchell, 1991), but we know little about the genetic consequences of the population and community dynamics that occur within ponds. High rates of larval mortality in amphibian species are common (e.g. Miller, 1909; Newman, 1987), however, previous studies have not quantified the reduction in genetic diversity attributable specifically to mortality at this life history stage. I find that within pond processes can contribute to the reduction of genetic diversity in larval populations of desert anurans, but that the loss of diversity is not equivalent for these two species.

As expected based on the larval duration of each species, the reduction in genetic diversity is more pronounced in *B. cognatus*. This result and the larger

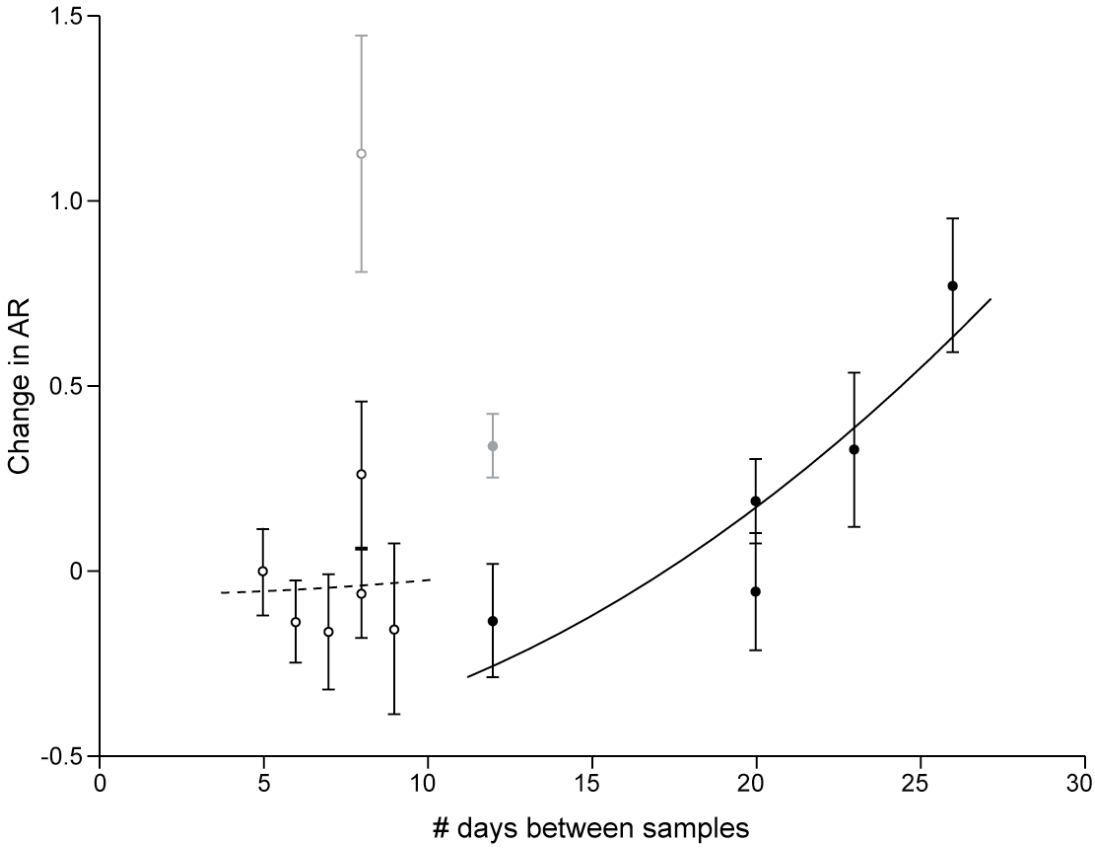

Figure 3.4. Relationship between the duration of the sampling interval and the mean change in allelic richness (AR) ± SE between tadpole and metamorph samples for *B. cognatus* (closed circles, solid line) and *S. couchii* (open circles, dashed line). Gray points indicate data for the diseased pond, SR, and are not included in the regression.

variance in changes in diversity across populations of *B. cognatus* relative to *S. couchii* (Figure 3.1) indicate that *B. cognatus* may be more sensitive to dynamics occurring within ponds and may more readily lose genetic diversity than *S. couchii.* At three ponds where both species co-occurred (EW, SCD, and ST), *B. cognatus* tended towards reduced genetic diversity over time whereas *S. couchii* remained stable in all populations. In identical ecological situations the larval environment has a more profound effect on levels of genetic diversity in *B. cognatus* and this may be attributed to unequal competitive abilities or predator avoidance behaviors between the species as well as to differences in the duration of larval development. All focal ponds included in this study contained both predators and competitors including aquatic invertebrates, carnivorous *Spea* tadpoles, and multiple species of omnivorous tadpoles (*S. couchii*, *Spea, B. cognatus*, and in pond DG, *B. alvarius*). In experimental manipulations, *S. couchii* larvae have greater competitive ability than *Bufo* species (*B. punctatus* and *B. speciosus*, Dayton and Fitzgerald, 2001; *B. woodhousei*, Woodward, 1982). Thus, *B. cognatus* may be more negatively affected by competition than *S. couchii* when these species co-occur. Likewise, studies of other *Bufo* and *Scaphiopus* species indicates that predation may have a more negative affect on *B. cognatus* and *S. couchii*. The ability to escape predators is positively correlated with tapdole burst speed and should be greater in *Scaphiopus* (Arendt, 2003; Dayton *et al.*, 2005) compared to *Bufo* (Wassersug and Hoff, 1985). In addition, activity rates are high in *Scaphiopus* compared to *Bufo* (Dayton and Fitzgerald, 2001) and though this could lead to higher rates of predation in the former, reduced activity by *B. cognatus* in the presence of predators decreases larval growth rate and can increase the time to metamorphosis (Lawler, 1989; Wilbur and Collins, 1973). In similar larval environments, *B. cognatus* will experience decreased growth rates from competition (Dayton and Fitzgerald, 2001) as well as from predation pressures (Lawler, 1989)

increasing the time that tadpoles are restricted to ponds where they are vulnerable to intense community dynamics and at risk of mortality due to pond desiccation.

The positive and exponential correlation between the length of the sampling interval and the amount of genetic diversity lost for *B. cognatus* (Figure 3.4) indicates that the rate at which diversity is lost increases with time spent in the larval environment. Adaptations of *S. couchii* for rapid development in ephemeral desert environments (Mayhew, 1965; Woodward and Mitchell, 1991) increase individual fitness. However, the ability for adults to utilize small ponds for reproduction and larvae to complete metamorphosis before ponds dry has additional positive consequences for the maintenance of genetic diversity at the population level. In larger ponds where community species richness is greater and interspecific interactions are more intense (MacKay *et al.*, 1990; Schneider and Frost, 1996; Spencer *et al.*, 1999; Woodward, 1983), the short larval duration of *S. couchii* prevents reduction in genetic diversity by minimizing the time spent with predators and competitors. This mechanism may apply to *B. cognatus* as well as I did not observe a reduction in genetic diversity in either species among samples collected less than 20 days apart. In the ponds considered here, both predation and competition pressures may increase with time as the number and activity of predators increases (Anholt and Werner, 1998; Schneider and Frost, 1996), as food resources are depleted (Alford, 1999; Newman, 1987), and as ponds begin to shrink. The additional time that *B. cognatus* tadpoles must spend in ephemeral ponds increases their risk of mortality from prolonged exposure to predators and competition in a closed, and likely resource limited environment (Low, 1976; Woodward and Mitchell, 1991). Therefore, the period of time that tadpoles are confined to these ephemeral environments may be the most important determinant of reductions to genetic diversity and may exacerbate the negative effects of poor competitive abilities and predator avoidance responses.

Loss of genetic variation at the population level may be unavoidable for *B. cognatus* populations; however, I do not find support that other factors besides larval period contribute to changes in diversity. Densities of predators and competitors increase with pond size in temporary ponds (Newman, 1987; Schneider and Frost, 1996; Woodward, 1983), nonetheless, pond size and reductions in genetic diversity are not positively correlated for either species. One possible explanation for this pattern is that community species richness and the overall density of competitors and predators may not correlate tightly with pond size across sites, and therefore, pond size may be a poor proxy for the intensity of interspecific interactions. A detailed study of the relationship between community structure and changes in genetic diversity within *B. cognatus* populations may elucidate the specific mechanisms underlying these patterns.

I do not find evidence for either family-correlated or clutch-size dependent mortality; these data instead indicate that larval mortality is random. The population specific relationships between the proportion of tadpoles and metamorphs belonging to kingroups (Figure 3.3) do not show any consistent pattern to changes in kingroup size between samples. Reductions in effective population size and allelic diversity can result from mild departures from random mortality (Waples 2002), but in *B. cognatus* reductions in diversity are correlated with the duration of the sampling interval indicating that mortality need not be non-random to affect levels of genetic variability.

It is important to note that this study focused on changes in genetic diversity within ponds where at least some larvae survived to metamorphosis. Thus, the changes I find report here are site specific and may reflect the genetic diversity that persists between adults and metamorphs across populations, but not necessarily the total magnitude of diversity that is lost within a breeding season. The loss of genetic variation within desert ponds due to larval mortality falls along a continuum; the

ponds included in this study represents one end of this spectrum where levels of predation and competition, likely to be typical of persistent ponds, results in the loss of diversity in some *B. cognatus* populations but not in *S. couchii* populations. However, complete reproductive failure can be common for desert anurans and such sites represent the other end of the continuum where no genetic diversity persists between adults and offspring. The maintenance of genetic diversity at the population level relies on those sites where there is some reproductive success. Evaporation of ponds prior to metamorphosis is frequent (> 50% of ponds surveyed) for both *S. couchii* (Morey, 1994; Newman, 1987) and *B. cognatus* (Bragg, 1940) and results in the elimination of entire larval cohorts. Across sites I surveyed from 2002 to 2005, I found failed recruitment in a number of ponds; at some sites mortality was clearly due to pond desiccation whereas at other sites, breeding aggregations occurred but no eggs or larvae developed for unknown reasons (Table 3.3). Species assemblages in ephemeral ponds can vary across sites due to stochastic colonization processes (MacKay *et al.*, 1990; Wilbur, 1980; Woodward and Mitchell, 1991) and the presence of particularly dominant competitors or predators may strongly influence larval survival. Large *Lethocerus* waterbugs and *Hydrophilus* beetles were present at some sites and likely responsible for mortality of eggs and larvae; *Lethocerus* adults and nymphs specialize on frogs and tadpoles and Bragg (1940) noted that *Hydrophilus* consumed *B. cognatus* tadpoles. Thus, the presence of a single predacious species can increase mortality rates such that diversity is completely lost within a pond.

I included samples of each species from SR even though tadpoles did not complete metamorphosis because tadpoles collected at late Gosner stages (Gosner, 1960) just prior to death provide an estimate of the genetic diversity that may be lost at mortality levels intermediate between those discussed above (e.g. Newman, 1987). Not surprisingly, the extremely high levels of mortality due to disease at SR correspond

Table 3.3. Evidence of failed reproduction at breeding sites of *B. cognatus* and *S. couchii* from 2002 to 2005. Potential sources of mortality in instances where ponds did not dry are given in parenthesis.

Year	Locality	Evidence of reproduction	Evidence of reproductive failure
2002	ER	*Bufo* tadpoles	Pond dried
	RV	*Bufo* tadpoles	Pond dried
	RR	*Bufo* breeding	Pond dried
	RT	*Bufo* eggs	No tadpoles
2003	RV	*Bufo* breeding	No eggs or tadpoles
	CW	*Scaphiopus* tadpoles	Pond dried
	EW	*Bufo* tadpoles	Pond dried
	SCD	*Bufo* breeding	Pond dried
2004	SR	*Bufo* and *Scaphiopus* tadpoles	Dead tadpoles (disease)
	DP	*Scaphiopus* tadpoles	Pond dried
	MR	*Bufo* and *Scaphiopus* breeding	No eggs or tadpoles (*Hydrophilus*)
	Sand	*Scaphiopus* tadpoles	No late stage tadpoles (carnivorous *Spea*)
2005	MR	*Bufo* and *Scaphiopus* breeding	No eggs or tadpoles (*Hydrophilus*)
	MQ	*Scaphiopus* tadpoles	Pond dried
	JV	*Bufo* tadpoles	Pond dried
	CR	*Scaphiopus* tadpoles	Pond dried
	TM	*Scaphiopus* eggs	No tadpoles (*Lethocerus*)
	W4	*Scaphiopus* breeding	No eggs or tadpoles
	DE	*Scaphiopus* tadpoles	Pond dried
	BR	*Scaphiopus* eggs	No tadpoles

with large reductions in genetic diversity in both species. *Scaphiopus couchii* did not show detectable reductions in diversity across sites where larvae persisted, but SR shows that low levels of diversity may still persist when mortality is severe.

Changes in genetic diversity during the larval period for *B. cognatus* support the hypothesis of Hedgecock (1994) that larval mortality can decrease genetic diversity, but I find little evidence for such a pattern in *S. couchii*. Both *B. cognatus* and *S. couchii* can lose genetic diversity between adults and metamorphs, but the proximate determinants of these changes are not the same for each species and are influenced by habitat selection and life history traits. Reproductive skew and larval mortality each contribute to the loss of genetic diversity in most *B. cognatus* ponds whereas only reproductive skew has an effect on levels of diversity in *S. couchii* and only at small ponds (Chan, Chapter 2). Loss of genetic diversity due to reproductive failure at some sites will occur for both species and is more likely to characterize smaller ponds with short hydroperiods (Newman, 1987). Thus, for *S. couchii* the chance that larval mortality will decrease genetic diversity is highest at ponds where reproductive skew is also the most pronounced. This reinforces the suggestion by Chan (Chapter 2) that large ponds are important to the persistence of genetic variation in *S. couchii*; reproduction at larger sites not only avoids the loss of diversity that can occur from reproductive skew, but also maintains diversity by limiting larval mortality.

Scaphiopus couchii is a desert adapted anuran and the persistence of genetic diversity is increased due to this species' adaptations to ephemeral and unpredictable environments. Rapid development in *S. couchii* maintains genetic diversity in a variety of ecological situations by limiting the time that tadpoles are vulnerable to negative intra- and interspecific interactions and by allowing breeding adults to exploit

a greater variety of breeding habitats with lower densities of competitors and predators. Ephemeral and unpredictable larval environments can result in high rates of mortality (Bragg, 1940; Newman, 1987), but in ponds where there is not reproductive failure, only *B. cognatus* experience a reduction in genetic diversity.

Determining the importance of specific aspects of the larval environment to patterns of tadpole mortality is difficult because of the multitude of factors in the aquatic community that may influence ecological processes. However, my data show that larval mortality can have a significant effect on levels of genetic diversity even in situations where tadpoles are able to complete metamorphosis. Recognizing that stage specific mortality may affect patterns of genetic diversity allows us to more fully understand how diversity is maintained within populations. By focusing on a particular window in the life history of these anurans, I am able to tease apart the effects of reproductive skew from the effects of larval mortality and address how the ecological context of breeding and larval development can independently influence changes in genetic diversity.

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CHAPTER FOUR

EFFECTIVE BREEDING AND POPULATION SIZES OF DESERT ANURANS

Abstract.— Effective breeding size (N_b) and effective population size (N_e) quantify the rate of loss of genetic diversity within groups due to genetic drift and are therefore central to conservation and evolutionary biology. Many amphibians have effective sizes much smaller than census sizes and understanding the underlying mechanisms that limit genetic diversity are particularly important given recent global declines in amphibian taxa. Genetic estimates of effective size differ in the temporal and spatial scale of processes that they describe and in whether they reflect historical or contemporary processes. Here, I use multiple estimates of N_b and N_e to examine the factors responsible for patterns of genetic diversity in two desert anurans. *Bufo cognatus* and *Scaphiopus couchii* are two frogs common to southwestern North America that breed explosively at ephemeral ponds. Reproductive skew, high larval mortality, and fluctuations in population sizes and recruitment across years due to environmental stochasticity may reduce N_b as well as long-term N_e. I find that reproductive skew can limit N_b within ponds, but that adult populations do not have small N_e. In contrast to expectations based on fluctuations in census sizes and reproductive success across years in these desert species, both *B. cognatus* and *S. couchii* may have temporally and spatially stable effective population sizes. Overlapping generations and metapopulation structure may contribute to the maintenance of genetic diversity in these two taxa and these results show that demographic and environmental stability may not be necessary for the temporal and spatial equilibrium of genetic diversity. In desert anurans, environmental stochasticity may promote connectivity among habitat patches and the persistence of high levels of genetic diversity. Future research examining the temporal and spatial aspects of

metapopulation dynamics will help to further elucidate the underlying mechanisms of within and among population genetic diversity in these desert species.

INTRODUCTION

Effective population size (N_e) is central to conservation and evolution because it determines the intensity of genetic drift and the rate at which genetic variation is lost within populations. By definition, N_e is equal to the size of an ideal population that experiences the same changes in gene frequencies and heterozygosity as the population of interest (Wright, 1931, 1938). Because the rate at which genetic diversity decreases within a population is inversely proportional to $2N_e$, N_e is an important indicator of population viability and risk of extinction. Quantifying N_e in natural populations allows us to connect ecological and organismal characteristics to changes in genetic diversity, thus furthering our understanding of the proximal determinants of population genetic structure.

Very few organisms in nature meet the assumptions of an ideal population and a number of models have examined the effects of these departures from the ideal state on estimates of N_e (e.g. Caballero and Hill, 1992; Chesser, 1991; Nunney, 1999; Wright, 1940). Organismal traits and population processes directly influence effective population size. Reproductive skew, variation in female fecundity, and fluctuating population sizes are a few examples of factors that will decrease N_e (see Frankham, 1995; Hedrick, 2000). In contrast, life history characteristics such as overlapping generations and long maturation time permit heterozygosity and allelic diversity to persist across generations and prevent a reduction in N_e (Nunney, 1993). Most taxa have traits that will both increase and decrease effective sizes and the relative contribution of these multiple factors to the maintenance of genetic diversity within and among populations is unclear. An understanding of the relationship between

species-typical phenotypes and effective population size allows us to assess population stability, predict future changes to genetic variability within and among populations (Chesser, 1991; Nunney and Elam, 1994), and determine historical levels of genetic diversity for use as conservation benchmarks (Crandall *et al.*, 1999).

The most commonly used indices of effective size belong to one of two categories: inbreeding effective size reflects changes in gene correlations and heterozygosity within individuals and variance effective size reflects changes in allele frequencies due to genetic drift (Crow and Denniston, 1988). These two effective sizes are the same when populations are at equilibrium (Wang, 2005), but inbreeding effective size often does not equal variance effective size because these metrics reflect the consequences of different microevolutionary processes (Chesser, 1991; Crandall *et al.*, 1999; Ryman *et al.*, 1995). Inbreeding effective sizes carry the genetic signature of multiple generations and therefore reflect historical processes and effective size over the long-term whereas variance effective sizes reflect contemporary processes and changes in genetic diversity due to recent demographic processes (Crandall *et al.*, 1999).

Methods to estimate effective size vary in the type of genetic changes they describe (inbreeding effective sizes versus variance effective size), the data required, the underlying assumptions, and the temporal and spatial windows at which they focus (Schwartz *et al.*, 1999; Wang, 2005). Some methods provide long-term average effective *population* size (N_e) (Beerli and Felsenstein, 2001; Nei, 1987) while other methods quantify the short-term sample specific effective *breeding* size (N_b) (Waples, 2005). A number of methods for estimating effective sizes from demographic and/or genetic data have been developed (Nunney and Elam, 1994; Schwartz *et al.*, 1998; Wang, 2005). For many taxa, genetic methods for estimating effective sizes may be preferable over demographic methods because they directly reflect gene dynamics and

can be more tractable, particularly when census sizes and life history parameters, such as age-specific fecundity and survivorship, are difficult to estimate. By utilizing multiple genetic methods to estimate effective breeding sizes and effective population sizes, we can infer the genetic consequences of multiple processes occurring at different spatial and temporal scales to better understand how within and among population processes each contribute to patterns of genetic structure and diversity

Among vertebrates, effective population sizes can be over an order of magnitude smaller than census population sizes (Frankham, 1995; Schmeller and Merilä, 2007; Turner *et al.*, 2002). Populations that appear to be healthy on the basis of censuses may still be vulnerable to the negative effects of inbreeding and local extinction that can accompany low effective population sizes (Frankham, 2005). Given recent global amphibian population declines (Lips *et al.*, 2005; Mendelson and *al.*, 2006; Stuart *et al.*, 2004), a more complete understanding of the factors limiting effective sizes and genetic diversity in amphibian taxa is particularly warranted (Beebee, 2005). Many amphibians are characterized by high census population sizes at breeding sites, but low effective sizes (Brede and Beebee, 2006; Jehle *et al.*, 2001; Rowe and Beebee, 2004; Schmeller and Merilä, 2007) due to mating system and life history traits as well as adult demographics (Frankham, 1995). The genetic consequences of processes occurring within populations are likely to be different from those occurring among populations (Chesser, 1991; Chesser *et al.*, 1993; Scribner and Chesser, 2001; Sugg *et al.*, 1996) and considering both levels can help to identify the factors that limit effective population sizes. Explosive breeding is characteristic of many amphibians that reproduce in temporary waters (Wells, 1977) and is likely to contribute to low effective sizes. Limited mating opportunities will reduce the effective breeding size within ponds (Ardren and Kapuscinski, 2003; Luikart and Cornuet, 1999; Scribner *et al*., 1997; Chan, Chapter 2) and can also reduce inbreeding

effective population sizes across sites when spatial population substructure exists (Chesser, 1991; Chesser *et al.*, 1993). However, the degree to which low N_b within a site influences long-term N_e and levels of genetic diversity across sites depends on levels of connectivity among independent breeding populations. For example, gene flow and/or overlapping generations may compensate for reduced effective breeding sizes (Chesser, 1991; Lippé *et al.*, 2006). Simultaneously examining effective size at multiple scales may elucidate the relative importance of within and among group dynamics to the persistence of genetic diversity and patterns of population genetic structure in amphibians.

In this study, I compare multiple estimates of effective breeding and effective population size in two anurans that are common in the deserts of southwestern North America (Table 4.1). I use two estimates of effective breeding size that reflect the change in genetic diversity due to inbreeding and variance in allele frequencies, respectively. In addition, I estimate inbreeding effective population sizes reflecting long term global and local average effective sizes. In comparison to other temperate amphibians, relatively little is known about effective sizes in desert taxa despite the potential for low effective sizes in this highly unpredictable environment. The GreatPlains Toad (*Bufo cognatus*) and Couch's Spadefoot Toad (*Scaphiopus couchii*) breed explosively in dense aggregations at ephemeral ponds when they fill with monsoon rains. These species co-occur at many breeding sites and are likely to have low N_b due to limited opportunities for mating and non-random larval mortality (Hedgecock, 1994; Waples, 2002; Chan, Chapter 3). In contrast, effective population sizes across breeding groups of both species may be high. N_e may be reduced by fluctuations in recruitment (Nunney, 1996; Waples, 2002b) across years due to high rates of larval mortality in unpredictable desert pond habitats (Bragg, 1940; Newman, 1987; Sullivan and Fernandez, 1999; Chan, Chapter 3). However, overlapping

Table 4.1. Abbreviations and descriptions for estimators of effective size. For each estimator used in this study, the method of estimation, the samples required of the method, a brief description of the type of effective size inferred, and reference are reported.

Abbreviation	Method	Required Sampling	Description	Ref.
N_e^{SMM}	H_e based	Adult samples	Long-term inbreeding effective population size based on the stepwise mutation model and expected heterozygosity.	1
N_e^{C}	Coalescent	Adult samples	Long-term population specific inbreeding effective population size estimated with Bayesian methods under a coalescent model.	2, 3
N_e^{LD}	Linkage disequilibrium	Tadpole sample	Population specific effective breeding size (an inbreeding effective size)	4, 5
N_b^{TM}	Moments-based temporal method	Adults and tadpoles	Population specific effective breeding size (a variance effective size)	6
N_e^{TM}	N_b^{TM} x t	Adults and tadpoles and generation time	Approximation of variance effective population size based on N_b^{TM} and generation time, t.	7

1. Nei, 1987; 2. Beerli and Felsenstein, 1999; 3. Beerli and Felsenstein, 2001; 4. Hill, 1981; 5. Bartley *et al.*, 1992; 6. Waples, 1989; 7. Waples, 1990

generations and opportunities for reproduction in future years may help maintain genetic diversity and N_e (Waples, 2002a). Additionally, while limited dispersal is common for pond breeding amphibians (e.g. Brede and Beebee, 2004; Newman and Squire, 2001; Zamudio and Wieczorek, 2007), populations of *B. cognatus* and *S. couchii* are well-connected by gene flow across broad geographic distances (Chan, Chapter 5) suggesting that inbreeding effective size in both species may be large.

Strongly male-biased sex ratios (Krupa, 1994; Sullivan, 1985) and long larval development times contribute to higher reproductive skew and larval mortality in *B. cognatus* in comparison to *S. couchii* (Chan, Chapter 2; Chapter 3) and suggest that N_b should greater in *S. couchii* (Wright, 1938). In addition, *Scaphiopus couchii* is adapted for rapid development in ephemeral environments and can breed in a greater range of aquatic sites with lower larval mortality rates (Chan, Chapter 3) and should therefore have a larger effective population size (Hedgecock, 1994) compared to *B. cognatus*.

Limited reproduction within breeding sites (Chan, Chapter 2), but high genetic diversity and low population genetic structure across ponds (Chan, Chapter 5) may mean that low reproductive success within pond may not reduce long-term N_e. However, populations may reflect equilibrium conditions and temporal stability in which case N_b and N_e will be very similar (Jehle *et al.*, 2001) and breeding sizes may be larger than expected and/or long term effective population sizes may be smaller than expected. By comparing N_b within breeding groups, long-term inbreeding N_e, and variance N_e, I examine whether dynamics occurring within or among breeding groups have a greater influence on patterns of genetic diversity and estimates of effective population size at broader spatial and temporal scales in these two frogs. The comparison of two co-occurring species and use of multiple measures of effective size to tease apart the importance of various species traits and ecological factors on genetic

diversity can provide a more complete picture of the processes contributing to genetic diversity.

METHODS

Tissue collection and laboratory protocols

I sampled individuals of both species from breeding ponds in the San Simon Valley and the San Bernardino Valley of Arizona and New Mexico during 2004 and 2005. Breeding in both species is explosive (Sullivan, 1989; Sullivan, 2005; Wells, 1977) and occurs over the course of one to three days after the summer monsoons. At five *B. cognatus* and four *S. couchii* breeding aggregations I marked adult individuals by toe clipping and preserved toe clips in 100% EtOH as a genetic sample of the adult generation. Following breeding, I monitored five ponds and nine ponds additional ponds known to be active breeding sites with tadpoles of each species for *B. cognatus* and *S. couchii* respectively. One locality, WL, consisted of three discrete ponds separated by less than 20 m; thus, I pooled adult samples, but treated tadpoles from each pond separately (NW, SW, and EW) because of the absence of larval movement among ponds. From a total of seven *B. cognatus* ponds and 14 *S. couchii* ponds I collected tadpoles tissue samples two to three days post hatching as a sample from offspring from each aggregation. For a single *S. couchii* pond (E4) tadpoles were uncommon and were sampled over a span of six days.

I isolated whole genomic DNA from tissues by incubating a small piece of toe or tail clip (approximately 1 mm^3) in 150 μL 5% Chelex solution (Chelex-100, BioRad) with 19 μg Proteinase K. The supernatant from extractions was used directly in amplification reactions via the polymerase chain reaction (PCR) and I genotyped all individuals of each species at twelve microsatellite loci. For *B. cognatus*, I used the primers and PCR conditions described for eleven loci in Chan (2007a) plus one locus

(ihhh) from Gonzales *et al.* (2004). For *S. couchii*, I used the primers and conditions described in Chan (2007b).

Previous studies have found that the loci and samples included in this study do not deviate from Hardy Weinberg equilibrium, supporting the absence of null alleles and of linkage to genes under selection (Chan, Chapter 2). In addition, the markers show no evidence of linkage disequilibrium indicating that these loci segregate independently (Chan, Chapter 2).

Estimates of effective sizes

Prior to estimating effective sizes, I omitted individuals with missing data at more than one locus because missing data can bias estimators of effective population size (Peel *et al.*, 2004). I used four genetic methods to estimate effective size for *B. cognatus* and *S. couchii* – N_e^{SMM}, N_e^{C}, N_b^{LD}, and N_b^{TM}. A fifth estimate of variance effective population size, N_e^{TM}, was calculated from N_b^{TM} and generation time (Table 4.1). Throughout this paper I note type of effective size with subscripts (N_e – effective population size , N_b – effective breeding size) and the method used to derive each estimate with superscripts.

I estimated long-term inbreeding effective population size of each species using two methods that assume a stepwise mutation model of microsatellite evolution (SMM). For these analyses, I omitted two loci from each species (BC52.12, bco04, scoD002, scoT142) because they showed high frequencies of single base pair mutations suggesting they did not conform to a SMM. I calculated point-estimates of N_e based on expected heterozygosity (H_e) under a stepwise mutation model (N_e^{SMM}) and also determined the Bayesian estimate of N_e under a coalescent model (N_e^{C}). At migration-drift equilibrium under a stepwise mutation model, N_e is related to H_e by $((1 - H_e)^{-2} - 1) / 8\mu$, where μ is the mutation rate (Nei, 1987). I calculated the mean H_e

across loci within and across adult populations in FSTAT 2.9.3 (Goudet, 1995) and used H_e to calculate N_e^{SMM} for each population and globally assuming a mean mutation rate of 10^{-4} (Lehmann *et al.*, 1998; Thuillet *et al.*, 2005).

I estimated long-term N_e using a coalescent model (N_e^C) inferred via Bayesian analysis implemented in MIGRATE (Beerli, 2006; Beerli and Felsenstein, 1999). Estimates of effective population size in MIGRATE are sensitive to sample sizes, therefore, I made population sample sizes equal by including only 17 randomly selected adult genotypes from each of five *B. cognatus* populations and three *S. couchii* populations; the fourth population of *S. couchii* (E4) had only 12 individuals, thus, I included all samples. For each species, I estimated mutation-scaled, sample-specific effective population sizes ($\Theta = 4N_e\mu$) and mutation-scaled migration rates, ($M = m / \mu$) where N_e is the effective size and μ is the mutation rate. I conducted the initial search with start parameters for Θ and M estimated from F_{ST} and an exponential prior on Θ and a uniform prior for M. I used the mode of the posterior distribution for each parameter from the initial run as the start parameter for subsequent analyses and conducted multiple runs that used the same mutation model and priors for Θ and M. I varied the number of long chains and the number and temperature of heated chains to assure that chains were mixing adequately and converging on the same posterior distribution. The final run for each species consisted of four long chains each with four heated chains. I sampled every 20 steps for 200,000 steps and discarded 20,000 trees per chain. Samples for the posterior distribution were recorded from the final chain. Population specific estimates of coalescent-based effective size, N_e^C were calculated by dividing Θ by 4μ. I calculated the global coalescent-based effective population size as the sum of population specific estimates of N_e^C.

Estimates of inbreeding effective size with the linkage disequilibrium method reflect non-random associations of alleles at pairs of loci within a single sample and

are based on the expectation of increased disequilibrium in small populations (Bartley *et al.*, 1992; Hill, 1981). When applied to a single cohort, the effective size estimated by the linkage disequilibrium method is equivalent to the short-term effective breeding size, N_b^{LD}, reflecting current processes (Wang, 2005; Waples, 2005). I used the linkage disequilibrium method of Bartley *et al.* (1992) implemented in NEESTIMATOR v1.3 (Peel *et al.*, 2004) to estimate N_b^{LD} for all offspring samples.

Finally, I used a moments-based temporal method to estimate the variance effective size of all groups for which I had both adult and tadpole samples. This method assumes that the variance in allele frequencies between samples due to genetic drift is greater in small populations (Waples, 1989). The temporal sampling I used here follows Plan I of Waples (1989) and because sampled tadpoles are the offspring of the sampled adults, the estimate of effective size is equivalent to the effective breeding size, N_b^{TM} (Waples, 2005). I used NEESTIMATOR to calculate N_b^{TM} for six *B. cognatus* and five *S. couchii* ponds.

Transforming contemporary temporal estimates of variance effective breeding size (N_b^{TM}) to variance effective population size (N_e^{TM}) to account for overlapping generations requires detailed demographic data (Jorde and Ryman, 1995) that are not available for either species in this study. Thus, to approximate N_e^{TM}, I multiplied N_b^{TM} by an estimate of generation time (t) (Waples, 1990) to approximate N_e^{TM}. While this approximation was developed for semelparous salmon, it may be appropriate for iteroparous frogs that have a low chance of reproductive success over multiple years (Waples, 1990). This is a reasonable assumption for *B. cognatus* and *S. couchii* because they have high reproductive skew (Chan, Chapter 2) and high rates of reproductive failure (Chan, Chapter 3). The age at first reproduction in both *B. cognatus* and *S. couchii* is estimated to be 2 years (Sullivan and Fernandez, 1999) but variation in age-specific reproductive success has not been documented. Thus, I

estimated *t* from the age distribution of individuals of *B. cognatus* and *S. couchii* determined by skeletochronology (Sullivan and Fernandez, 1999). For each species, I computed *t* as the weighted average of individuals two years of age or older species. This estimate of *t* is likely to be conservative because bone-remodeling can lead to underestimates of individual age in skeletochronology studies (Bastien and Leclair, 1992; Hemelaar, 1985).

A previous study of these same breeding aggregations implemented Bayesian clustering methods to infer the number of parents contributing to tadpole samples (Chan, Chapter 2). Thus, for breeding ponds included in both the original parentage study (Chan, Chapter 2) and this study, I estimated the correlation between estimates of the number of inferred parents from Bayesian clustering analyses (N^P) to the effective number of breeders estimated by linkage disequilibrium method (N_b^{LD}) using linear regression. If the number of breeding parents is a good predictor of gene dynamics, then I expect the relationship between N^P and N_b^{LD} to pass through the origin and have a slope of one.

RESULTS

Estimates of long term effective population size based on H_e (N_e^{SMM}) for *B. cognatus* were approximately four times that of *S. couchii* and varied from 36362.9 to 61268.9 and 10888.7 to 12911.4, respectively (Table 4.2). In comparison, estimates of population specific N_e^C based on the coalescent were over an order of magnitude smaller than N_e^{SMM} estimates but similar between the species ranging from 196.88 to 278.13 in *B. cognatus* and 196.88 to 334.38 in *S. couchii*. Although combined estimates of N_e^C across populations were greater than 1,000 for both species, these estimates were still an order of magnitude less than estimates of N_e^{SMM} based on overall H_e of each species.

Table 4.2. Long-term inbreeding effective population sizes: N_e^{SMM} is based on expected heterozygosity under migration-drift equilibrium and stepwise mutation model. Θ is the mutation-scaled effective population size ($4N_e^C\mu$) and N_e^C is the effective population size from Bayesian inference under a coalescent model assuming a mutation rate of 10^{-4}. Estimates of Θ and N_e^C include 95% credible intervals.

	N_e^{SMM}	Θ	N_e^C
B. cognatus			
DG	47823.2	0.0788 (0.0638 - 0.0913)	196.88 (159.38 - 228.13)
JV	58781.2	0.0913 (0.0763 - 0.1063)	228.13 (190.63 - 265.63)
SCD	61268.9	0.1113 (0.0963 - 0.1338)	278.13 (240.63 - 334.38)
SR	46379.9	0.0843 (0.0692 - 0.0969)	210.65 (172.88 - 242.12)
WL	36362.9	0.0988 (0.0838 - 0.1113)	246.88 (209.38 - 278.12)
overall	51735.5	0.4643 (0.3892 - 0.5394)	1160.65 (972.88 - 1348.38)
S. couchii			
DG	12911.4	0.1013 (0.0838 - 0.1388)	253.13 (209.38 - 346.88)
CR	10888.7	0.1338 (0.1063 - 0.1713)	334.38 (265.63 - 428.13)
E4	12694.6	0.0788 (0.0613 - 0.1163)	196.88 (153.13 - 290.63)
WL	11867.1	0.0913 (0.0713 - 0.1213)	228.13 (178.13 - 303.13)
overall	12038.4	0.4050 (0.3225 - 0.5475)	1012.50 (806.25 - 1368.75)

Effective breeding size was smaller than effective population size for most populations. N_b^{LD} varied across populations and ranged from 10.5 to 242.9 individuals in *B. cognatus* and 5.6 to 163.4 individuals in *S. couchii* (Table 4.3). N_b^{TM} estimates were low as well and similar to N_b^{LD} in general, although the 95% confidence intervals (CI) were considerably wider for some populations (e.g. EW, SR for *B. cognatus* and DG, EW, and SW for *S. couchii*). N_b^{TM} ranged from 16.1 to 97.8 in *B. cognatus* and from 8.1 to 139.6 in *S. couchii*; in one *S. couchii* population (E4) N_b^{TM} was equal to infinity possibly due to small sample sizes for both adults and tadpoles (Tables 4.2; Table 4.3). Based on the age distributions reported by Sullivan and Fernandez (1999) I estimated that the generation time, *t*, was 3.625 years in *B. cognatus* and 2.829 years in *S. couchii*.

N^P was positively correlated with N_b^{LD} (Figure 4.1), however, the slope was much greater than one. Tadpole sample size imposes an upper limit on estimates of N^P in Bayesian clustering methods where the maximum N^P is twice the tadpole sample size. If we consider only samples with $N^P < 30$ corresponding to no shared kinship among offspring (Chan, Chapter 2), the relationship between N^P and N_b^{LD} remains significantly positive ($p = 0.0134$; Figure 4.1) but is only slightly greater than one. Given sufficient sample size, the number of inferred parents in Bayesian analyses predicts effective breeding size estimated from correlations of alleles across loci.

DISCUSSION

Small effective breeding sizes within ponds (N_b^{LD} and N_b^{TM}) in comparison to moderate to large long-term inbreeding effective population sizes (N_e^{SMM} and N_e^{C}) suggests that contemporary demographics do not drive patterns of genetic diversity observed at larger spatial and temporal scales for either species. In general, I find similar ranges of effective breeding and effective population sizes for both species.

Table 4.3. Effective breeding size estimates with 95% confidence intervals estimated by the linkage disequilibrium method (N_b^{LD}) and the moments-based temporal method (N_b^{TM}). Sample sizes for adults (N_a) and tadpoles (N_t) are reported. Multiplying N_b^{TM} by generation time, *t*, yields N_e^{TM}, the variance effective population size.

Locality	N_a	N_t	N_b^{LD}	N_b^{TM}	N_e^{TM}
B. cognatus					
DG	23	24	11.4 (10.4 - 12.6)	16.1 (9.7 - 30.1)	58.36
JV	17	50	14.9 (14.1 - 15.8)	23.7 (13.6 - 50.8)	85.91
SCD	52	51	128.3 (107.6 - 157.7)	97.8 (46.4 – 552.0)	354.53
SR	18	37	10.5 (9.8 - 11.3)	51 (20.1 – ∞)	184.88
EW	43	34	242.9 (153.1 - 556.9)	85.9 (36.6 - 5566.5)	311.39
NW	43	29	67.2 (54.3 – 87.0)	72.8 (31.7 - 1285.6)	263.90
ST	-	33	26 (23.5 – 29.0)	- -	-
S. couchii					
DG	25	33	93.4 (66.0 - 153.0)	139.6 (29.8 - ∞)	394.93
CR	23	15	5.6 (4.9 - 6.6)	8.1 (4.8 - 14.7)	22.91
E4	12	18	13.6 (11.6 - 16.3)	∞ -	-
EW	19	33	87.1 (63.9 - 132.7)	135.5 (27.1 - ∞)	383.33
SW	19	35	90.6 (67.8 - 132.7)	122.1 (26.9 - ∞)	345.42
SCD	-	38	163.4 (107.6 - 321.6)	- -	-
SR	-	42	22.8 (20.6 - 25.4)	- -	-
BR	-	23	15.6 (13.5 - 18.3)	- -	-
CW	-	39	14.4 (13.1 - 15.9)	- -	-
DE	-	42	43.8 (37.7 - 51.5)	- -	-
LV	-	19	25.3 (20.2 - 33.2)	- -	-
SCD	-	20	14.6 (12.4 - 17.5)	- -	-
SCT	-	39	35 (30.5 - 40.7)	- -	-
ST	-	36	121.6 (86.7 - 196.8)	- -	-

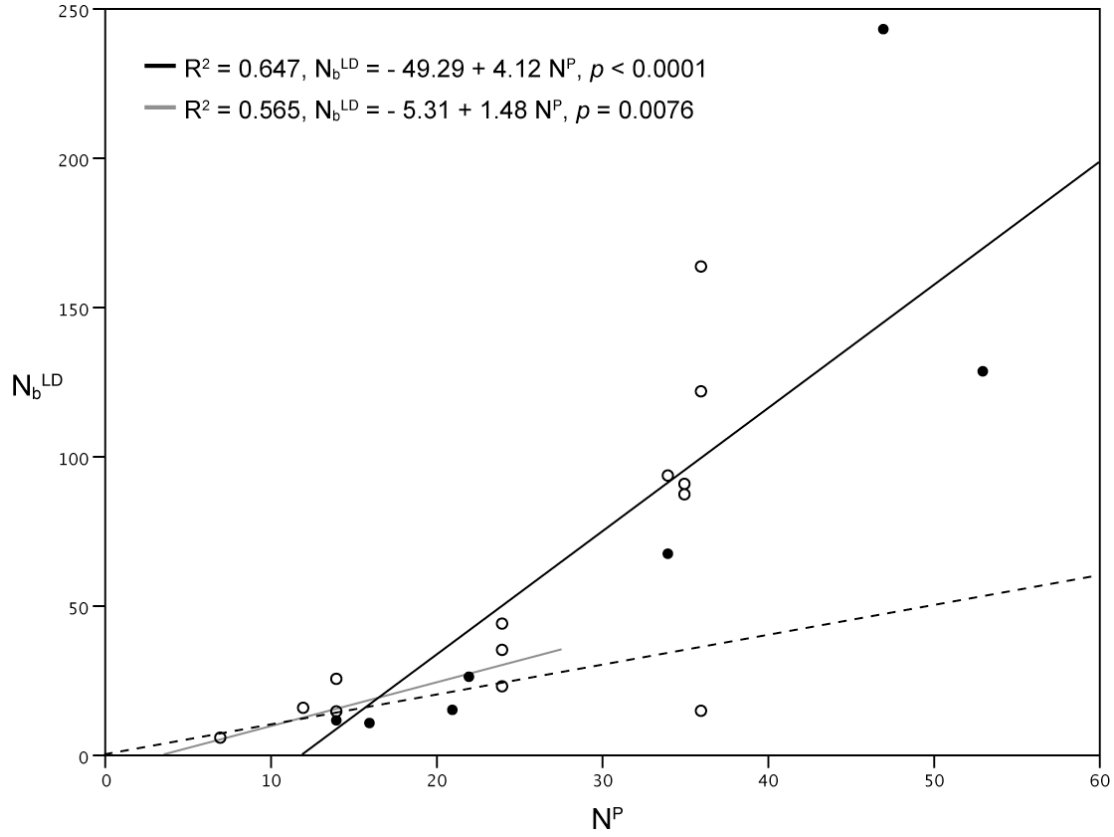

Figure 4.1. Linear regression of the number of inferred parents (N^P) from Bayesian analyses (Chan, Chapter 2) and the linkage disequilibrium estimate of the effective breeding size (N_b^{LD}) within ponds for *B. cognatus* (solid circles) and *S. couchii* (open circles) combined. Black line is the regression through all samples. The gray line is for all samples where N^P is less than 30; this corresponds to populations where estimates of N^P were not bounded by the sample size. The dashed line indicates the relationship under the hypothesis of $N^P = N_b^{LD}$.

Variation in effective breeding size across ponds suggests that metapopulation structure may be important to the maintenance of genetic diversity and large effective population sizes. However, the similarity of N_e across sites and between species indicates that a common set of processes may govern the maintenance of genetic diversity in both anurans. For these desert species, multiple breeding opportunities within a lifetime may compensate for low reproductive success within a single breeding season and prevent the loss of genetic diversity that might otherwise occur with low effective breeding sizes and temporal and spatial fluctuations in recruitment.

Estimates of effective breeding size (N_b^{LD} and N_b^{TM}) are low and within the range of values found in other pond breeding anurans (Brede and Beebee, 2006; Schmeller and Merilä, 2007; Scribner *et al.*, 1997), but vary widely across breeding ponds for both species. Unequal sex ratios (Wright, 1938), variance in reproductive success (Caballero and Hill, 1992; Nunney, 1991), and high larval mortality (Hedgecock, 1994; Waples, 2002a) are each expected to reduce effective size in these species. The number of breeding adults (N^P) previously inferred from Bayesian clustering techniques (Chan, Chapter 2) corresponds closely with estimates of N_b^{LD} from this study for lower estimates of N^P and provides evidence that variance in reproductive success among adults is responsible for this pattern. High larval mortality was considered the predominant source of reduced effective population size in *Rana temporaria* (Schmeller and Merilä, 2007), but because the samples I used in this study bracketed only the early portion of the larval period, my data support a primary role for reproductive skew directly reduces N_b. While a loss of genetic diversity during the larval period could further decrease the effective breeding size (Hedgecock, 1994; Waples, 2002a), other research with *B. cognatus* and *S. couchii* has shown that reproductive skew has a larger effect on changes in genetic diversity than

does larval mortality (Chan, Chapter 2, Chapter 3). Therefore, N_b is most likely primarily determined by reproductive skew and not larval dynamics.

Variance in estimates of N_b^{LD} and N_b^{TM} both within and between species supports previous findings that context and species dependent breeding dynamics within ponds have consequences for the maintenance of genetic diversity (Chan, Chapter 2). Breeding opportunities in desert anurans are limited by the short duration of reproductive activity and can vary stochastically across sites due to breeding densities and sex-ratios as well as pond characteristics. The range of N_b^{LD} and N_b^{TM} across sites are similar for *B. cognatus* and *S. couchii* and effective breeding sizes are low at some sites (e.g. *B. cognatus* SR and ST, *S. couchii* CR and CW) but not all. At sites where both species reproduced, *B. cognatus* generally had lower effective breeding sizes than *S. couchii* (Table 4.3). Highly male-biased sex-ratios can result in fewer breeding opportunities for *B. cognatus* and these data corroborate conclusions from previous studies which found that changes in genetic diversity due to reproductive skew were more prevalent in *B. cognatus* populations (Chan, Chapter 2)..

Levels of heterozygosity in both *B. cognatus* and *S. couchii* are high relative to other amphibian species (Monsen and Blouin, 2004; Newman and Squire, 2001) resulting in large estimates of N_e^{SMM} that are over an order of magnitude greater than local effective population size estimates from the Bayesian approach (N_e^{C}). Population specific estimates of N_e^{SMM} most likely violate the assumption of no migration; thus, N_e^{SMM} based on overall H_e may represent the maximum effective population sizes of *B. cognatus* and *S. couchii* at broader spatial scales than those sampled and at which gene flow occurs (Schmeller and Merilä, 2007). Overall N_e^{SMM} is close to four times greater in *B. cognatus* and *S. couchii*, but given higher rates of gene flow in *S. couchii* (Chan, Chapter 5) this result may simply reflect differences in heterozygosity among the microsatellite markers used.

The coalescent-based estimate of inbreeding effective population size, N_e^C, may be more appropriate for comparisons among these species because this method is not sensitive to H_e and is a local, sample-specific estimate of N_e (Beerli and Felsenstein, 1999, 2001). Estimates of N_e^C are more likely than N_e^{SMM} to reflect the actual long-term effective size for each of these populations because this model incorporates population structure and migration (Beerli and Felsenstein, 2001). Both conditions can bias estimates of effective population size (Chesser *et al.*, 1993; Nunney, 1999; Vucetich *et al.*, 1997) and are present among these two anurans (Chan, Chapter 5). In contrast to N_e^{SMM}, N_e^C estimates are smaller and similar across breeding sites and between species included here (Table 4.2). The stability of these local, long-term estimates of N_e^C may reflect population equilibrium among adult individuals in contrast to expectations based on temporal and spatial variability in census sizes (L. Chan, unpublished) and in recruitment due to high rates of larval mortality in desert environments (Newman, 1987; Woodward and Mitchell, 1991).

N_e^C within populations can overestimate true N_e if populations exchange a high number of migrants with unsampled populations (Beerli, 2004), however, the potential bias due to within pond estimates of effective population size caused by unsampled populations may be minimized by source-sink dynamics (Beerli, 2004). Within the San Simon and San Bernardino Valleys, there are multiple breeding ponds that may exchange migrants with the focal ponds for which I estimated N_e^C given the geographic distance between samples and the low levels of genetic differentiation at this scale (Chan, Chapter 5). However, adult breeding ponds here do not fill predictably every year and migration among sites is likely highly variable and asymmetrical due to variable recruitment across ponds in any given year (Chan, Chapter 2, Chapter 3). Ponds that serve as source populations in some years are likely to be breeding sinks in other years. Thus, these estimates of N_e^C should be minimally

biased and reflect the relative size of local adult effective population sizes. Genetic connectivity through migration among sites and gene flow across overlapping generations can help populations maintain large inbreeding effective population sizes (Chesser, 1991; Lippé *et al.*, 2006). The similarity in the magnitude of effective population sizes between *B. cognatus* and *S. couchii* indicates that both species experience the same level of genetic drift and that a common set of mechanisms may underlie the persistence of genetic diversity at broader geographic scales in these species.

Effective breeding sizes estimated by the linkage disequilibrium method (N_b^{LD}) are lower than local inbreeding effective population sizes (N_e^{C}) for all populations for which I had both estimates with examined with the exception of *B. cognatus* at EW. Not surprisingly, the gene correlations among offspring that are generated by one episode of limited reproduction do not result in increased gene correlations among adults, reflecting overlapping generations and indicating potential gene flow among adult populations. Variance effective breeding sizes (N_b^{TM}) are also lower than corresponding N_e^{C}, but the 95% confidence intervals (CI) around each mean were broad for larger estimates of N_b^{TM}. Changes in gene frequencies between parent and offspring generations, particularly when reproductive success is high are likely too small to provide a precise estimate of effective size due to drift (Wang, 2005; Waples, 2005).

The variance effective population sizes (N_e^{TM}) calculated from the variance effective breeding sizes (N_b^{TM}) are approximations based on generation time, *t*, and assuming that most adults only reproduce successfully once in their lifetime despite being iteroparous (Waples, 2002b). N_e^{TM} is low relative to N_e^{C} in two *B. cognatus* ponds and one *S. couchii* pond, but not at others indicating that despite restricted

breeding at some sites (low N_b), that overlapping generations help to increase variance effective sizes in both species. The greater loss of genetic diversity due to reproductive skew and larval mortality within some *B. cognatus* breeding sites in comparison to *S. couchii* (Chan, Chapter 2; Chapter 3) does not have a larger influence on effective population size on *B. cognatus*. Because N_e^{TM} is a variance effective size based on changes in allele frequencies, this estimator is the contemporary effective size and reflects recent demographic history. In contrast, N_e^{C} is an inbreeding effective size that carries the signature of evolutionary gene correlations and is therefore reflects the long-term effective size. As Crandall *et al*. (1999) suggested, comparison of variance and inbreeding effective sizes can help us to understand the relative impact of contemporary versus historical processes. Though the precise values of N_e^{TM} and N_e^{C} rest on assumptions about generation time and mutation rate, respectively, the similarity of these estimates at some ponds suggests that these toads may be at equilibrium despite seemingly pronounced temporal and spatial fluctuations in abundance and reproductive success.

In contrast to the expectations for desert anurans and two species with different life histories, these results indicate that long term effective size may be maintained as long as some proportion of ponds are reproductively successful. The network of breeding sites may constitute a metapopulation with spatially and temporally fluctuating source-sink dynamics. In structured populations, N_e should reflect the contribution of source populations to overall levels of genetic diversity through gene flow (Whitlock, 2004). Ponds with high recruitment in one year (e.g. SCD, EW, NW of *B. cognatus*) will serve as important sources of migrants to breeding aggregations at other ponds in following years. Thus, gene flow among ponds and overlapping generations, particularly in *B. cognatus*, can help to maintain inbreeding effective size despite variation across ponds in effective breeding size. The magnitude of N_e^{TM}

relative to N_e^C suggests that reproductive success was high in all the sites sampled in 2004 (ponds DG, EW, and SW) and that variance effective size does not vary widely across ponds in *S. couchii* as would be expected based on generally lower levels of reproductive skew (Chan, Chapter 2). In the time period that this study was conducted, the influence of contemporary processes on effective breeding sizes within ponds may not require source-sink dynamics and high rates of gene flow to maintain inbreeding effective size. For both of these desert species, reproductive success across some years can be close to zero (Bragg, 1940; Newman, 1987; Sullivan and Fernandez, 1999), thus, the importance of metapopulation dynamics in maintaining genetic diversity and effective population sizes may vary temporally.

Measurements of effective size can provide insight into population viability and help us to identify populations at risk. Understanding how within and among population processes influence effective sizes allows us to link patterns of genetic diversity to specific organismal traits. *Bufo cognatus* and *S. couchii* are both common anurans that do not appear to be in decline, thus, these data and those of similar studies (Chan, Chapter 2, Chapter 3) are a baseline for the relationships among genetic diversity, reproductive success, and effective population size. My data reveal that connectivity among breeding populations and variability in reproductive success may be two important components to maintaining effective population sizes in desert species. These results are in sharp contrast to other anurans which often have both low effective population sizes (see review in Frankham, 1996; Schmeller and Merilä, 2007) and pronounced population genetic structure (see Chan, Chapter 5). By combining information from several different estimates of effective size, the consequences of within population changes to genetic diversity for overall population viability and genetic structure are becoming more clear for these desert species.

Both *B. cognatus* and *S. couchii* maintain large population specific effective population sizes, despite high temporal and spatial variance in reproductive success. The unpredictable nature of desert habitats may promote population persistence through metapopulation dynamics by selecting for behaviors which allow individuals to exploit a wide diversity of breeding habitats. Some consequences of adaptations to ephemeral ponds in an unpredictable and arid landscape may be reduced site fidelity and increased dispersal abilities and tendencies relative to other pond-breeding anurans. Very little is known about the population dynamics and population genetic structure of desert anurans and examining the spatial and temporal metapopulation structure of these species would be an interesting avenue for future research.

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CHAPTER FIVE

THE GEOGRAPHIC SCALE OF POPULATION GENETIC DIVERSITY IN DESERT ANURANS

Abstract.— Many amphibians show high levels of genetic differentiation across short geographic distances due to low vagility, high site fidelity, and reliance on patchily distributed moist microhabitats to prevent desiccation. Although data exist for a number of temperate amphibians, patterns of population genetic structure in desert amphibians have not been well-studied. I examined fine-scale genetic structure in two anurans that co-occur in the southwestern deserts of North America. *Bufo cognatus* and *Scaphiopus couchii* breed explosively at ephemeral ponds that fill with summer rains and are expected to have particularly pronounced population structure at fine-scales due to the arid landscape and their physiological requirements, mating system characteristics, and life history traits. I used microsatellite markers to characterize genetic structure among populations of each species in three valleys in southeastern Arizona and southwestern New Mexico. I found high genetic diversity and low levels of genetic structure at broad geographic scales with greater genetic differentiation in *B. cognatus* compared to *S. couchii* suggesting a role for species traits in determining the patterns of population genetic structure. I compared these results to fine-scale studies of population genetic structure in other temperate amphibians using a standardized metric of differentiation that corrects for variation in overall genetic diversity and found that levels of differentiation in *B. cognatus* and *S. couchii* were lower than those of other temperate amphibians studied. These results stand in contrast to the expectation of high genetic structure for amphibians in desert environments and indicate that populations are well-connected by gene flow across large geographic distances despite inhospitable habitats. This study adds to the

growing database for fine-scale population genetic structure and extend our understanding of temperate amphibians by contributing information for anurans in desert environments. Data for species occupying very different landscapes allows us to examine the role of broad ecological differences on patterns of population genetic structure.

INTRODUCTION

Local patterns of population genetic structure reflect contemporary processes that influence the distribution and persistence of genetic variation (Slatkin, 1987) across landscapes. Patterns of differentiation are therefore affected not only by landscape features (Manel *et al.*, 2003; Storfer *et al.*, 2006; Wiens, 2001), but also by organismal traits such as effective population sizes, habitat requirements, physiological tolerances, dispersal capacities, philopatry, and site fidelity (e.g. Chesser, 1991; Matocq and Lacey, 2004; Scribner and Chesser, 2001; Sugg *et al.*, 1996). Identifying the spatial scale and context in which population genetic structure exists is an important step in linking landscape and taxon-specific characteristics to patterns of differentiation; such data have important implications for evolution and conservation.

Amphibians are generally considered to have low vagility (Beebee, 2005), small effective population sizes (Beebee and Griffiths, 2005; Schmeller and Merilä, 2007), and high levels of site fidelity (Smith and Green, 2005); all characteristics that should result in high degrees of population differentiation at relatively small spatial scales. Indeed, significant genetic differentiation at the scale of a few kilometers has been found for a number of amphibian species from a variety of habitat types including agricultural areas (Brede and Beebee, 2004), montane zones (Funk *et al.*, 2005), woodlands (Squire and Newman, 2002), deciduous forests (Zamudio and

Wieczorek, 2007), and prairie wetlands (Newman and Squire, 2001). However, despite a significant focus on patterns of differentiation in temperate amphibian species, very little is known about population genetic structure in desert taxa. Reliance on moist habitats to prevent desiccation and ephemeral ponds for larval development combined with arid environmental conditions and limited opportunities for successful reproduction, suggests that desert anurans might have substantial population genetic structure at fine spatial scales.

Several families of amphibians occur in the xeric regions of North America (Duellman and Sweet, 1999) and most breed in ephemeral ponds that fill with summer rains (Sullivan, 2005). Some true toads (Family Bufonidae) and spadefoot toads (Family Scaphiopodidae) occupying desert environments in North America are explosive breeders (Sullivan, 1989; Wells, 1977) aggregating at ponds for only one to three nights following heavy summer rains (Bragg, 1941; Sullivan, 1985). Most species are active above ground for only several summer months (Bragg, 1965; Woodward and Mitchell, 1991) and individuals aggregate at patchily distributed ponds where breeding and larval development occurs. Desert summers are characterized by high air temperatures and localized and unpredictable rainfall events (Whitford, 2002; Woodward and Mitchell, 1991) thus the opportunity for dispersal is likely to be limited. Long distance dispersal is considered to be infrequent in amphibians due to site fidelity and risk of desiccation away from moist habitats (see review in Smith and Green, 2005); dispersal among ponds in deserts is expected to be particularly rare because of high temperatures and the aridity of intervening habitats resulting in population divergence. Furthermore, reproductive skew due to male biased-sex ratios and mating system characteristics (Sullivan, 1985; Chan, Chapter 2) and high larval mortality rates (Bragg, 1940a; Newman, 1987; Chan, Chapter 3) can reduce genetic diversity and the effective breeding size (Chan, Chapter 4). Given the unique

constraints imposed by desert landscapes resulting in potentially high levels of genetic drift within breeding groups and limited gene flow among groups, I expect genetic population structure to be substantial in desert amphibians.

Here, I examine population genetic structure in two co-occurring anurans common to the deserts of the southwestern United States. The Great Plains Toad (*Bufo cognatus*) and Couch's Spadefoot Toad (*Scaphiopus couchii*) are both medium-sized frogs that breed explosively at ephemeral ponds at the onset of summer monsoons (Sullivan, 1985). *Bufo cognatus* occurs from southern Saskatchewan to central Mexico (Graves and Krupa, 2005; Stebbins, 2004), whereas *S. couchii* is truly a desert adapted species (Mayhew, 1965; Woodward and Mitchell, 1991; Chan, Chapter 3) occurring only in southwestern United States to central Mexico (Stebbins, 2004).

Many studies on the population biology of desert anurans have focused on adult breeding behaviors or larval ecology (e.g. Bragg, 1936; Newman, 1998; Sullivan, 1983; Woodward, 1987). In contrast, little is known about the terrestrial ecology of juveniles and adults away from breeding aggregations (but see, Bragg, 1937; Graves *et al.*, 1993; Ruibal *et al.*, 1969). Adults of *B. cognatus* and *S. couchii* can spend several weeks above ground before and after breeding to forage (Bragg, 1940b; Dimmitt and Ruibal, 1980; Ruibal *et al.*, 1969; Smith and Bragg, 1949) and burrows of *S. couchii* have been found close to sites of ephemeral water-bodies (Ruibal *et al.*, 1969) suggesting site fidelity across years. The few direct measures of dispersal that exist for these two species indicate that long distance dispersal is at least possible; adult *B. cognatus* and *S. couchii* can move at least one km and 400 m, respectively (Ewert, 1969; Mayhew, 1965) and dense migrations of juvenile *B. cognatus* have been observed in the more mesic grasslands of Oklahoma (Bragg and Brooks, 1958; Smith and Bragg, 1949).

The true frequency and distance of dispersal is still unknown for these anurans, but even if movement among ponds is moderate, dispersal events may rarely result in gene flow. Studies focusing on reproductive success and larval survivorship within breeding sites for *B. cognatus* and *S. couchii* found that both reproductive skew and larval mortality can be high (Chan, Chapter 2, 3) with correspondingly low effective breeding sizes (Chan, Chapter 4). Thus, the chance of a dispersing individual reproducing successfully post-dispersal is likely to be very low such that levels of gene flow may be slight even if rates of movement are high. Characterization of population genetic structure in these two desert species increase our overall understanding of temperate amphibians and by allowing us to consider, more broadly, how ecological conditions and species characteristics influence patterns of genetic structure.

METHODS

Tissue sampling and laboratory protocols

During the summers of 2002 through 2005, I collected tissue samples from *B. cognatus* and *S. couchii* in southeastern Arizona and southwestern New Mexico from four lowland valleys which run north to south. The south end of the San Simon Valley adjoins the northern San Bernardino Valley and these together are separated from Sulphur Springs Valley to the west by the Chiricahua Mountains and from Animas Valley to the east by the Peloncillo Mountains (Figure 5.1). I sampled tissues from adults at eleven and ten breeding aggregations of *B. cognatus* and *S. couchii*, respectively (Table 5.1; Figure 5.1). Toe clips were collected and preserved in 100% EtOH for subsequent genetic analysis. Five additional collection localities do not represent adult breeding aggregations: I collected tissues from both species captured along a road at one locality at the northern tip of the San Simon Valley (BA) and from

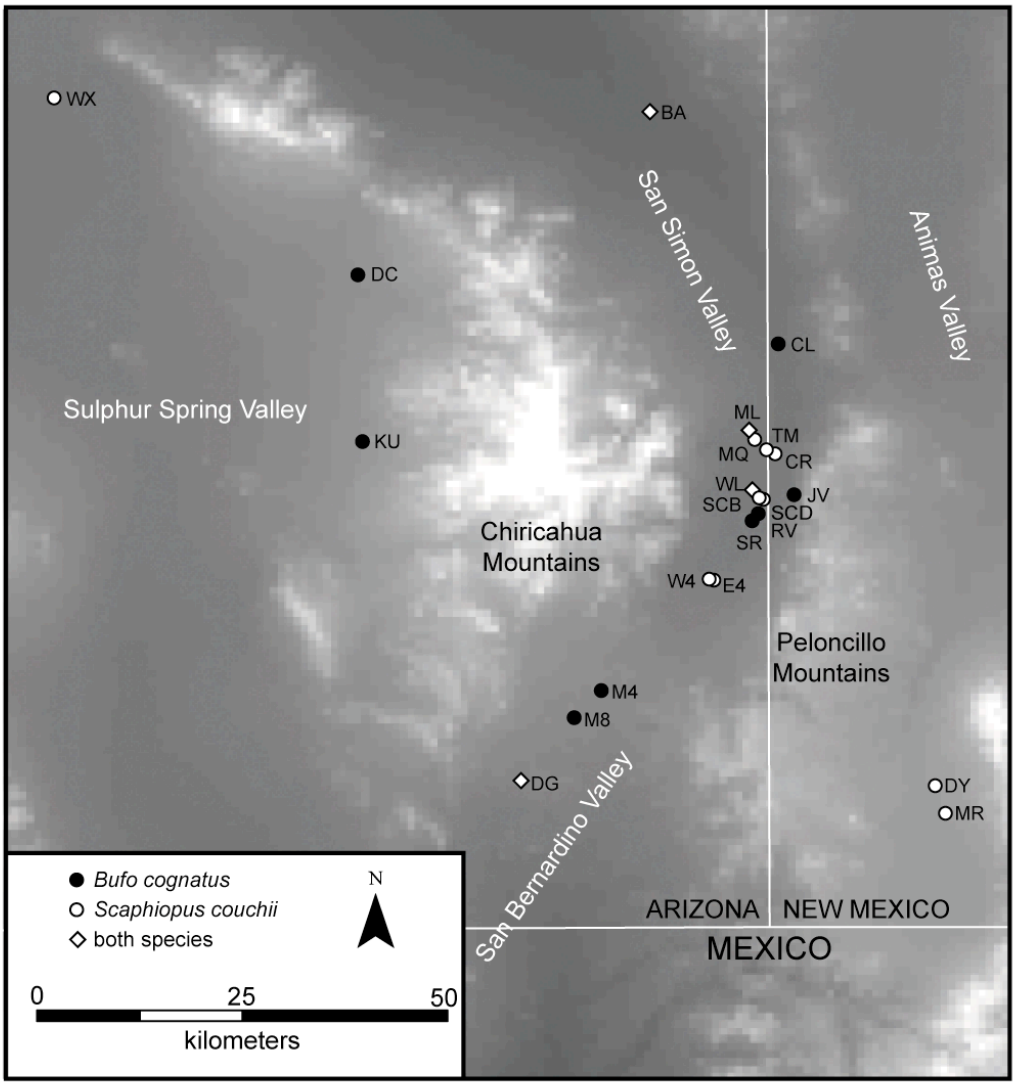

Figure 5.1. Map of tissue collection localities in the southwestern corner of Arizona at the border with New Mexico and Mexico. Dark shades indicate low elevations and light shades indicate high elevations.

Table 5.1. Collection locality names, abbreviation, latitude, and longitude. For each locality, the sample sizes of *Bufo cognatus* (N_B) and *Scaphiopus couchii* (N_S) are given as well as the group membership (B – *B. cognatus*; S – *S. couchii*) of that locality in the pairwise comparison of genetic differentiation between geographic distance based groups of localities.

Locality Name	Abbreviation	Latitude	Longitude	N_B / N_S	Group
Barnes Road	BA	32.23626758	-109.1761260	21 / 20	
Corner Pond II	CR	31.86209681	-109.0390503	0 / 24	S1
Culvert 12	CL	31.98250280	-109.0357943	21 / 0	
Dangerous Ditch	DG	31.50529942	-109.3174678	23 / 32	
Day Pond	DY	31.49881517	-108.8638295	0 / 13	S3
Dos Cabezas	DC	32.05821499	-109.4964261	17 / 0	
E409 Pond	E4	31.72378894	-109.1063836	0 / 12	S2
Javelina Pond	JV	31.81714348	-109.0187584	17 / 0	B1
Kuykendall Road	KU	31.87526800	-109.4913286	25 / 0	
Marsh Pond	MR	31.46811251	-108.8525518	0 / 6	S3
Mesquite Pond	MQ	31.89328926	-109.0715750	0 / 12	S1
Mile 394 Pond	M4	31.57302125	-109.2592193	11 / 0	B2
Mile 398 Pond	M8	31.60272379	-109.2295881	8 / 0	B2
Miller Pond	ML	31.87766309	-109.0618377	26 / 71	B1, S1
River Pond	RV	31.79638000	-109.0580300	53 / 0	B1
Sky Ranch 80 Pond	SR	31.78843808	-109.0646844	18 / 0	B1
Sulphur Canyon Bufo Pond	SCB	31.81238896	-109.0519215	20 / 0	B1
Sulphur Canyon Ditch	SCD	31.81394728	-109.0541386	74 / 0	B1
Tomberlin Pond	TM	31.86631838	-109.0490225	0 / 9	S1
W409 Pond	W4	31.72481399	-109.1116287	0 / 20	S2
Willcox Ditch	WX	32.25192419	-109.8289155	0 / 21	
Willow Ponds	WL	31.81386963	-109.0596753	47 / 19	B1

B. cognatus at two sites in Sulphur Springs Valley (KU and DC). I also included two population samples of *S. couchii* collected as tadpoles from ephemeral ponds in the Animas Valley (DY and MR). Samples within the San Simon/San Bernardino Valleys spanned a distance of 0.5 to 82.5 km for both species; across valley distances ranged from 20.4 to 63.8 for *B. cognatus* and 34.0 – 127.0 km in *S. couchii*.

Whole genomic DNA was isolated from tissue samples with a standard Chelex extraction consisting of approximately 1 mm^3 of tissue in 150 µL of a 5% Chelex solution (Chelex-100, BioRad) incubated with19 µg ProteinaseK at 55 °C for 180 min. and 99 °C for 10 min. The supernatant from extractions was used directly as template for amplification by polymerase chain reactions (PCR). I genotyped all individuals of *B. cognatus* and *S. couchii* at twelve species-specific microsatellite loci. PCRs and genotyping of *B. cognatus* followed the conditions for eleven loci described in Chan (2007a) and one locus (ihhh) described in Gonzales *et al.* (2004). All individuals of *S. couchii* were genotyped at twelve loci following the conditions in Chan (2007b).

Microsatellite data

For each species, I grouped samples across years according to collection locality. I tested for departures from Hardy Weinberg equilibrium (HWE) and for evidence of linkage disequilibrium within populations in GENEPOP V3.4 (Raymond and Rousset, 1995). A Markov chain method (Guo and Thompson, 1992) with 5,000 dememorization steps and 100 batches of 5,000 iterations per batch was used to determine and the significance of all tests. I calculated average expected heterozygosity (H_e) across all loci for each species using GENALEX V6 (Peakall and Smouse, 2006). In addition, I used HP-RARE (Kalinowski, 2005) to determine allelic richness and private allelic richness at each locus while accounting for population variation in sample sizes (Kalinowski, 2004).

Larval samples are a particular challenge in studies of population structure because sampled individuals may represent family groups due to reproductive skew and/or a limited number of breeding adults. In these cases, larvae will be closely related and therefore will not reflect population level genetic diversity. To determine whether the two *S. couchii* tadpole samples (DY and MR) consisted of closely related individuals I compared relatedness within each larval sample to relatedness among adults from all other collection localities. I computed Queller and Goodnight's estimate of relatedness (Queller and Goodnight, 1989) across all individuals sampled in GENALEX (Peakall and Smouse, 2006). I used 999 bootstrap replicates to generate 95% confidence intervals (CI) around mean relatedness within populations and 999 permutations across populations to determine the distribution of relatedness estimates under the null hypothesis of equal relatedness among populations.

Population-based genetic structure

I used FSTAT 2.9.3 (Goudet, 1995) to estimate *F*-statistics (F_{IS}, F_{ST}, and F_{IT}; Weir and Cockerham 1984) among population pairs and over all loci and samples. Mean F_{IS}, F_{ST}, and F_{IT} and associated standard errors (SE) were determined by jackknifing over loci (Goudet, 1995). Exact *p*-values for each pairwise F_{ST} estimate were determined by randomizing multilocus genotypes between the two populations. For each species, I determined table wide significance at α of 0.05 after sequential Bonferroni correction for multiple comparisons. Because demes may consist of more than a single pond (Funk *et al.*, 2005; Jehle *et al.*, 2005; Rowe and Beebee, 2007), I also estimated population differentiation for samples pooled by geographic proximity. Sampled localities were not evenly distributed throughout the valleys, thus I was able to pool sampled ponds at three spatial scales based on the distribution of pairwise geographic distances among ponds. I pooled localities within five or six km (*B.*

cognatus and *S. couchii,* respectively), ten km, and 22 km (see Table 5.1 for groupings of sampling localities). For each of these pooled datasets, I estimated overall F_{IS}, F_{ST}, and F_{IT} and pairwise F_{ST} among groups of ponds using FSTAT.

If sample localities are in migration-drift equilibrium, we expect pairwise F_{ST} values to reflect isolation by distance (Wright, 1943), with more distant population pairs having greater differentiation than neighboring populations. For each species, I examined the relationship among pairwise values of the natural logarithm of geographic distance and genetic distance, as $F_{ST} / (1 - F_{ST})$ (Rousset, 1997). I conducted Mantel tests (Mantel, 1967) with 10,000 permutations on the geographic and genetic distance matrices using the Isolde option in GENEPOP to determine whether the slope of the regression was significantly greater than zero ($p < 0.05$).

Individual-based analyses

Analyses based on predefined delineations of populations can obscure patterns of differentiation (Jehle *et al.*, 2005); individual-based approaches are an alternative method that may better identify spatial genetic structure and barriers to gene flow (Manel *et al.*, 2005; Rowe and Beebee, 2007). I examined population genetic structure without relying on *a priori* delineation of populations using two individual-based approaches: 1) Bayesian assignment methods and 2) genetic autocorrelation analyses in a spatially explicit framework.

I used Bayesian clustering methods to infer patterns of genetic differentiation for both *B. cognatus* and *S. couchii* in the program STRUCTURE 2.1 (Falush *et al.*, 2003; Pritchard *et al.*, 2000). STRUCTURE uses Markov chain Monte Carlo (MCMC) algorithms to determine the most probable number of genetic clusters in a sample, *K*, and to determine the posterior probability that an individual belongs to each of *K* clusters under a model of within population Hardy-Weinberg equilibrium and linkage

equilibrium. For each species, I conducted ten replicate runs at each value of *K* possible clusters (*B. cognatus*: possible *K* ranged from 1 to 14; *S. couchii*: possible *K* ranged from 1 to 12). Each run consisted of 1,200,000 steps with the first 200,000 steps discarded as burn in. Because the genetic composition of individuals could reflect recent gene flow, I used the admixture model where individuals can have a genetic signature from other demes with a uniform prior on the admixture parameter, α. I used the *F* model (Falush *et al.*, 2003) assuming correlated allele frequencies across clusters and did not include collection locality as prior information. I calculated the average natural logarithm of the probability of the data at each *K* across replicates and from these averages used Bayes' Rule to calculate posterior probability of each *K* (Pritchard and Wen, 2004). Bayes' Rule may overestimate the number of clusters in a sample, therefore I also computed the most probable *K* using the method described by Evanno *et al*. (2005) which examines the rate of change in the natural logarithm of the probability of the data between successive *Ks*.

I also investigated patterns of genetic structure in these two species with individual-based spatially explicit analyses. Spatial autocorrelation methods (Peakall *et al.*, 2003) quantify the degree of genetic non-independence among individuals within pre-defined classes of geographic distance. To determine whether evidence of autocorrelation existed for either species at fine scales, I conducted two separate analyses examining spatial autocorrelation (r_c) at two and five km distance classes. I tested the significance of r_c at each distance class interval in GENALEX using 999 bootstrap replicates to generate 95% CI about the mean r_c and 999 permutations of the data across distance classes to generate the null distribution of r_c under a hypothesis of no autocorrelation. Following Peakall *et al.* (2003), I rejected the null hypothesis when the average r_c fell outside the 95% CI of the null distribution and the 95% CI about the mean did not include $r_c = 0$.

Standardized genetic differentiation

One of the challenges to comparative studies of genetic differentiation is understanding how geographic genetic structure varies across taxa and across environments. Comparisons across groups are complicated by differences in the geographic scale of studies and the uniqueness of the genetic markers to each study. I compared the pairwise levels of differentiation for *B. cognatus* and *S. couchii* to data for two other temperate amphibians from the northeastern United States using a standardized measure of differentiation (Meirmans, 2006). I used genotypic data for 29 populations of the spotted salamander (*Ambystoma maculatum*) in Tompkins County, New York, USA spanning distances of 1.6 to 47.1 km (Zamudio and Wieczorek, 2007) and for 11 populations of the bullfrog (*Rana catesbeiana*) in Ontario, Canada spanning 0.7 to 50.9 km (Austin *et al.*, 2004). The maximum value of genetic differentiation metrics such as F_{ST} and G_{ST} are constrained by overall levels of heterozygosity at the genetic markers used (Hedrick, 1999, 2005) with a lower maximum level of differentiation with greater heterozygosity. Thus, I computed a standardized measure of genetic differentiation (Hedrick, 2005; Meirmans, 2006) to compare estimates of differentiation across *B. cognatus*, *S. couchii*, *A. maculatum*, and *R. catesbeiana*. I used the program RECODE to alter each of the four datasets such that every population had a unique set of alleles (Meirmans, 2006) and used FSTAT to calculate the maximum genetic differentiation ($F_{ST\mathit{max}}$) over all populations as well as the maximum pairwise F_{ST} values for all population pairs. Standardized measures of genetic differentiation (F_{ST}') were computed by dividing the true pairwise values of F_{ST} by $F_{ST\mathit{max}}$. Negative values of standardized differentiation in pairwise comparisons were set to zero (Meirmans, 2006). For each species, I used linear regression to examine the relationship between pairwise natural logarithm of geographic distance and the standardized F_{ST}'. I used 10,000 permutations of the Mantel test (Mantel,

1967) implemented in GENEPOP to determine whether the slope of each regression was significantly greater than zero.

Finally, I compared the geographic scales of genetic differentiation inferred for the species in this study to those reported in other temperate amphibians. I surveyed the literature for studies of temperature pond-breeding amphibians which used microsatellite markers to examine fine-scale population genetic structure and that included comparisons of sites with a pairwise geographic distance of 10 km or less. For each species in the literature, I recorded overall F_{ST}, H_e, and the range of geographic distances between sampled population pairs. When overall genetic differentiation estimates were unavailable, I computed mean F_{ST} and the mean H_e (weighted by population sample sizes) over all populations; for studies where population specific values were not included, I used the range values reported rather than a single value. The range of geographic distances among population pairs were taken directly from published studies or estimated using coordinates published therein.

To estimate standardized F_{ST}', F_{ST} values must be divided by F_{STmax} estimated from the original data set. Thus, I examined whether H_e was a good predictor of F_{STmax} for the four species for which I had direct estimates of H_e and F_{STmax} from genetic data (*B. cognatus*, *S. couchii*, *A. maculatum*, and *R. catesbeiana*) using linear regression. I used this linear model to extrapolate F_{STmax} for each study from my comparative survey using the published values of H_e. From these estimates of F_{STmax}, I calculated F_{ST}' for each species; in instances where a range of H_e and/or F_{ST} were available rather than a single estimate, I calculated the range of possible values F_{ST}'.

RESULTS

Microsatellite data

Genotypic frequencies within *B. cognatus* populations differed significantly from expectations under HWE in only three out of 183 estimates after sequential Bonferroni correction for multiple comparisons ($p < 0.00029$ at α of 0.05). For *S. couchii* only one locus in one population (sco126 in WX) had a significant deficiency in heterozygotes after Bonferroni correction ($p < 0.000379$ at $\alpha = 0.05$) suggesting that all twelve loci for *B. cognatus* and *S. couchii* were at equilibrium and were not biased by null alleles. Only a single pair of loci (BC52.12 and BC60.20) showed evidence of LD after Bonferroni corrections for *B. cognatus*. However, these loci showed evidence of disequilibrium at only one site (WL) suggesting that factors other than linkage might explain this pattern. I did not find evidence of LD in pairwise comparisons of loci within populations for *S. couchii* indicating that these markers were segregating independently.

Estimates of locus specific allelic richness and private allelic richness were standardized for variation in sample size across ponds by rarefaction, using a balanced sample size of twelve genes in HP-RARE (Kalinowski, 2005). Mean allelic richness was similar across all ponds for each species (Figure 5.2). Allelic richness ranged from 3.55 to 9.19 alleles/locus (mean = 6.54) for *B. cognatus* and from 2.04 to 9.32 alleles/locus (mean = 5.19) for *S. couchii.* Average private allelic richness within populations was more variable across populations of *B. cognatus* than *S. couchii* (Figure 5.2) and ranged from 0.069 to 0.546 alleles/locus (mean = 0.247) for *B. cognatus* and from 0.141 to 0.302 alleles/locus (mean = 0.209) for *S. couchii*. Similarly, average H_e was greater for populations of *B. cognatus* (weighted average = 0.819, range = 0.774 – 0.843) compared to *S. couchii* (weighted average = 0.709, range = 0.667 – 0.729).

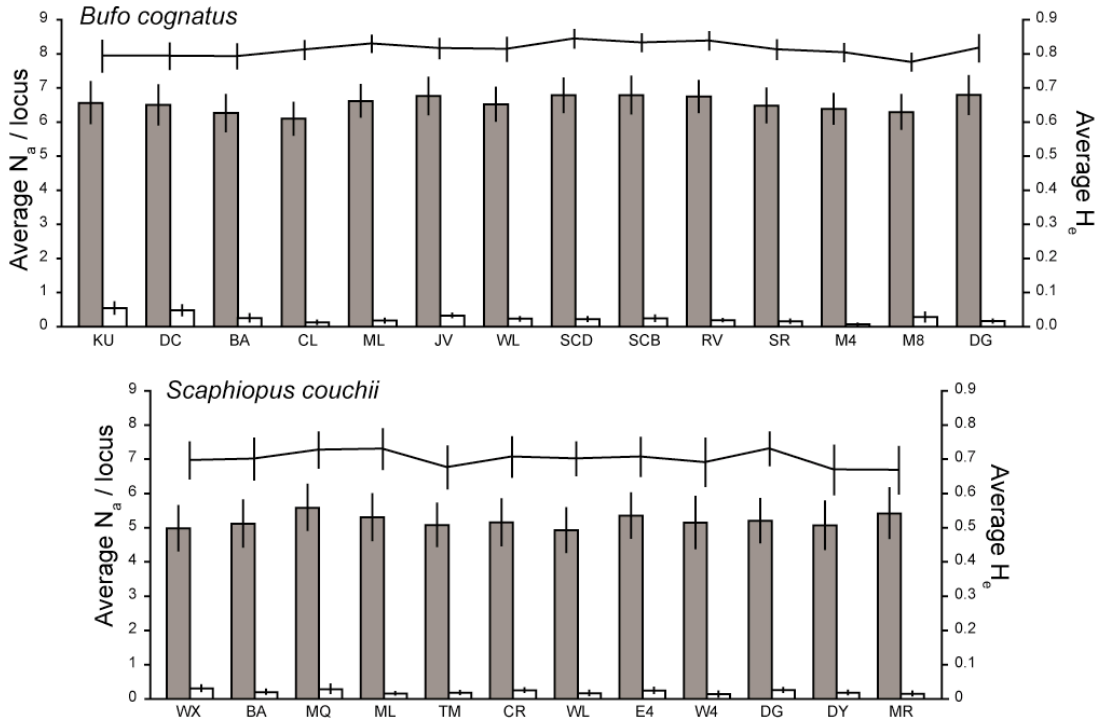

Figure 5.2. Average allelic richness (gray bars) and private allelic richness (white bars) ± SE for each population standardized for a sample size of seven individuals for *B. cognatus* and six individuals for *S. couchii*. Average expected heterozygosity (H_e) ± SE for each population indicated by the black line.

Mean pairwise relatedness estimates for the two tadpole sample of *S. couchii* (DY and MR) were not significantly greater than expected under a null hypothesis of equal relatedness among all individuals from all populations sampled ($p_{DY} = 0.052$; $p_{MR} = 0.454$). Given the weak evidence for kinship within these larval samples, I assumed that they reflected adult genotype frequencies at DY and MR and included them in subsequent analyses.

Population based estimates of diversity and differentiation

Overall levels of genetic differentiation among ponds were generally low for both *B. cognatus* and *S. couchii* and only slightly higher for the former in overall tests (*B. cognatus*: $F_{ST} = 0.006 \pm 0.001$; *S. couchii*: $F_{ST} = 0.003 \pm 0.001$). Similarly, H_S was also slightly higher for *B. cognatus* (*B. cognatus*: $H_S = 0.834$; *S. couchii*: $H_S = 0.724$; Table 5.2). Tests of pairwise differentiation among sampling localities were significant for 22 of 91 comparisons for *B. cognatus* and two of 66 comparisons for *S. couchii* after Bonferroni correction (Table 5.3). For *B. cognatus*, significant estimates of F_{ST} between sampling localities within the San Simon/San Bernardino Valleys ranged from 0.006 to 0.017 (mean = 0.0097), whereas pairwise estimates for sampling localities from different valleys were 0.007 to 0.033 (mean = 0.0155). In contrast, for *S. couchii*, I only found significant differentiation between two pairs of populations from different valleys (WX & ML - $F_{ST} = 0.012$; WX & DY $F_{ST} = 0.023$; Table 5.3). Patterns of genetic differentiation with populations pooled according to geographic distance were similar for *B. cognatus*: I again find weak genetic differentiation both within and among valleys, but at levels similar to that in sampling locality-based analyses (Tables 5.4). For *S. couchii*, pairwise F_{ST} values at each of the three geographic scales were significant only across valleys and not within valleys further supporting no population structure within the San Simon/San Bernardino

Table 5.2. *F*-statistics ± SE after jackknifing across loci for all sampling localities and pooled according to one of three distance criteria.

	F_{IS}	F_{ST}	F_{IT}
Bufo cognatus			
All localities	0.057 ± 0.024	0.006 ± 0.001	0.062 ± 0.024
5 km	0.058 ± 0.024	0.007 ± 0.001	0.064 ± 0.024
10 km	0.058 ± 0.024	0.008 ± 0.001	0.065 ± 0.024
22 km	0.059 ± 0.024	0.007 ± 0.001	0.065 ± 0.024
Scaphiopus couchii			
All localites	0.033 ± 0.019	0.003 ± 0.001	0.036 ± 0.018
6 km	0.032 ± 0.019	0.004 ± 0.002	0.036 ± 0.018
10 km	0.032 ± 0.019	0.005 ± 0.001	0.037 ± 0.018
22 km	0.032 ± 0.018	0.006 ± 0.002	0.038 ± 0.018

Table 5.3. Pairwise estimates of F_{ST} for *Bufo cognatus* and *Scaphiopus couchii* across sampling localities above the diagonal and pairwise geographic distances (km) among populations below the diagonal. Pairwise estimates of F_{ST} that are significantly different from zero after Bonferroni correction at $\alpha = 0.05$ are indicated in bold. Locality abbreviations are listed in Appendix 2.

Bufo cognatus

	KU	DC	BA	CL	ML	JV	WL	SCD	SCB	RV	SR	M4	M8	DG
KU	-	0.006	**0.011**	**0.033**	**0.013**	0.006	**0.007**	**0.010**	**0.021**	**0.012**	0.007	0.014	0.005	0.002
DC	20.4	-	**0.014**	**0.029**	**0.016**	0.008	**0.014**	**0.010**	0.017	**0.010**	0.004	0.016	0.008	0.006
BA	50.0	36.1	-	0.013	0.007	0.008	**0.006**	**0.009**	0.007	**0.007**	0.003	**0.017**	0.003	0.005
CL	44.7	44.3	31.2	-	0.008	0.014	**0.017**	**0.010**	0.007	0.007	**0.009**	**0.016**	0.010	**0.016**
ML	40.6	45.7	41.4	11.9	-	-0.003	0.003	-0.002	0.010	0.000	-0.002	0.008	-0.001	0.003
JV	45.2	52.5	49.0	18.5	7.9	-	0.004	-0.001	0.013	0.001	0.001	0.007	-0.004	0.006
WL	41.4	49.4	48.3	18.9	7.1	3.9	-	0.001	0.012	0.002	-0.001	0.011	-0.002	0.005
SCD	41.9	49.9	48.4	18.8	7.1	3.4	0.5	-	0.009	0.000	0.000	0.003	-0.001	0.002
SCB	42.1	50.1	48.6	19.0	7.3	3.2	0.8	0.3	-	0.001	0.002	0.011	0.012	0.005
RV	41.9	50.7	50.2	20.8	9.1	4.4	2.0	2.0	1.9	-	-0.004	0.004	0.000	0.003
SR	41.5	50.7	51.0	21.8	9.9	5.4	2.9	3.0	2.9	1.1	-	0.000	0.000	-0.004
M4	40.2	58.5	74.3	50.3	38.7	35.5	32.8	33.1	33.1	31.3	30.2	-	0.002	0.001
M8	39.2	56.6	70.7	46.1	34.5	31.1	28.5	28.8	28.8	27.0	25.9	4.3	-	-0.006
DG	44.4	63.8	82.5	59.4	48.0	44.8	42.2	42.5	42.5	40.7	39.6	9.4	13.7	-

Scaphiopus couchii

	WX	BA	MQ	ML	TM	CR	WL	E4	W4	DG	DY	MR
WX	-	0.014	0.008	**0.012**	0.018	0.008	0.007	0.008	0.005	0.005	**0.023**	0.011
BA	61.5	-	0.009	0.003	0.015	0.007	0.003	0.002	0.010	0.002	0.002	0.015
MQ	81.8	39.4	-	-0.005	-0.015	-0.006	0.001	-0.001	-0.005	0.003	0.011	-0.011
ML	83.5	41.4	2.0	-	-0.005	-0.002	-0.002	-0.004	-0.002	0.004	0.013	0.003
TM	85.2	42.9	3.7	1.8	-	0.005	-0.006	0.004	-0.004	0.001	0.020	-0.001
CR	86.2	43.6	4.6	2.8	1.1	-	0.000	-0.008	0.000	0.004	0.014	-0.004
WL	87.5	48.3	8.9	7.1	5.9	5.7	-	-0.004	-0.002	-0.004	0.016	0.011
E4	90.1	57.4	19.2	17.6	16.8	16.7	11.0	-	-0.005	0.000	0.011	0.009
W4	89.6	57.3	19.1	17.7	16.8	16.8	11.1	0.5	-	0.003	0.016	0.004
DG	96.2	82.5	49.1	48.0	47.6	47.7	42.2	31.5	31.3	-	0.013	0.008
DY	123.9	87.2	48.1	46.2	44.5	43.7	39.7	34.0	34.4	43.1	-	0.026
MR	127.0	90.8	51.7	49.7	48.1	47.3	43.2	37.3	37.7	44.3	3.6	-

Table 5.4. Pairwise F_{ST} values for pooled samples of *B. cognatus* at 5 km (a), 10 km (b), 22 km (c), and of *S. couchii* at 6 km (d), 10 km (e), and 22 km (f). Significant values at $\alpha = 0.05$ after Bonferroni correction indicated in bold.

a) *Bufo* 5 km	KU	DC	BA	CL	ML	B1	B2	DG
KU	-							
DC	0.006	-						
BA	**0.011**	**0.014**	-					
CL	**0.033**	**0.029**	**0.013**	-				
ML	**0.013**	**0.016**	0.007	0.008	-			
B1	**0.010**	**0.010**	**0.006**	**0.010**	0.000	-		
B2	**0.010**	**0.012**	**0.010**	**0.014**	0.004	0.002	-	
DG	0.002	0.006	0.005	**0.016**	0.003	0.002	-0.002	-

b) *Bufo* 10 km	KU	DC	BA	CL	ML & B1	B2	DG
KU	-						
DC	0.006	-					
BA	**0.011**	**0.014**	-				
CL	**0.033**	**0.029**	**0.013**	-			
ML & B1	**0.010**	**0.011**	**0.006**	**0.010**	-		
B2	**0.010**	**0.012**	**0.010**	**0.014**	0.002	-	
DG	0.002	0.006	0.005	**0.016**	0.002	-0.002	-

c) *Bufo* 22 km	KU	DC	BA	CL, ML, & B1	B2 + DG
KU	-				
DC	**0.006**	-			
BA	**0.011**	**0.014**	-		
CL, ML, & B1	**0.011**	**0.011**	**0.006**	-	
B2 & DG	**0.007**	**0.009**	**0.008**	**0.003**	-

d) *Scaphiopus* 6 km	WX	BA	S1	WL	S2	DG	S3
WX	-						
BA	0.014	-					
S1	**0.012**	0.006	-				
WL	0.007	0.003	-0.001	-			
S2	0.008	0.009	-0.001	-0.001	-		
DG	0.005	0.002	0.005	-0.004	0.004	-	
S3	**0.013**	0.002	0.004	0.009	0.006	**0.006**	-

e) *Scaphiopus* 10 km	WX	BA	S1 & WL	S2	DG	S3
WX	-					
BA	0.014	-				
S1 & WL	**0.011**	0.006	-			
S2	**0.008**	0.009	-0.001	-		
DG	0.005	0.002	0.004	0.004	-	
S3	**0.013**	0.002	0.005	0.006	**0.006**	-

f) *Scaphiopus* 22 km	WX	BA	S1, S2, & WL	DG	S3
WX	-				
BA	0.014	-			
S1, S2, & WL	**0.011**	0.007	-		
DG	**0.005**	0.002	0.004	-	
S3	**0.013**	0.002	0.005	**0.006**	-

Valleys (Table 5.4). Overall F-statistics were comparable across the four geographic scales although F_{ST} increased slightly from the population based dataset to the 22 km pooled dataset (Table 5.2).

These spatial patterns are corroborated by statistical support for a pattern of isolation by distance in both *B. cognatus* and *S. couchii*. The natural logarithm of pairwise geographic distance was correlated with genetic differentiation ($F_{ST} / (1 - F_{ST})$) in both species and slopes were significantly greater than zero after permutations in Mantel tests (Figure 5.3).

Individual based estimates of genetic differentiation

Bayesian assignment methods in STRUCTURE (Falush *et al.*, 2003; Pritchard *et al.*, 2000) did not reveal finer population structure than did pairwise population estimates of genetic structure using F_{ST}. The posterior probability distribution and the rate of change in the natural logarithm of the probability of the data at each *K* (Evanno *et al.*, 2005) showed greatest support for three genetic clusters among *B. cognatus* samples. However, the 90% probability intervals around the membership coefficients for each individuals to each inferred cluster at $K = 2$ and $K = 3$ were broadly overlapping suggesting that these are overestimates of the numbers of genetic clusters (Figure 5.4a). For the *S. couchii* dataset, I did not find any evidence of population genetic structure; the posterior probability distribution supported $K = 1$ and the method of Evanno et al. (2005) found greatest support for the minimal *K*, $K = 2$ (Figure 5.4b).

Tests of spatial autocorrelation at even distance class intervals of two and five km did not support spatial autocorrelation in either species. Mean r_c were not significantly different than permuted values under the null hypothesis of no spatial autocorrelation and the 95% CI for each estimate included $r_c = 0$.

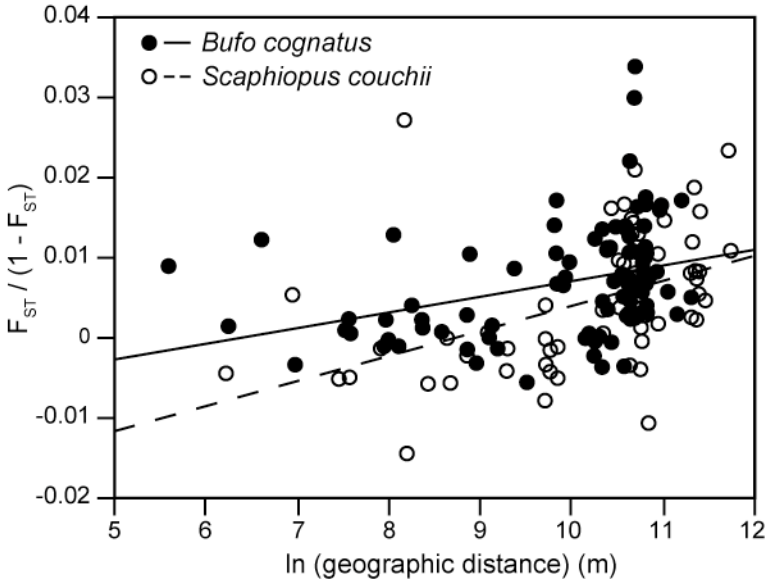

Figure 5.3. Linear regression of pairwise natural logarithm of distance and $F_{ST} / (1 - F_{ST})$ from Mantel tests. *Bufo cognatus*: $F_{ST} / (1 - F_{ST}) = - 0.0126 + 0.00196$ (ln(distance)); $p = 0.00970$. *Scaphiopus couchii*: $F_{ST} / (1 - F_{ST}) = - 0.0274 + 0.00313$ (ln(distance)); $p = 0.01110$.

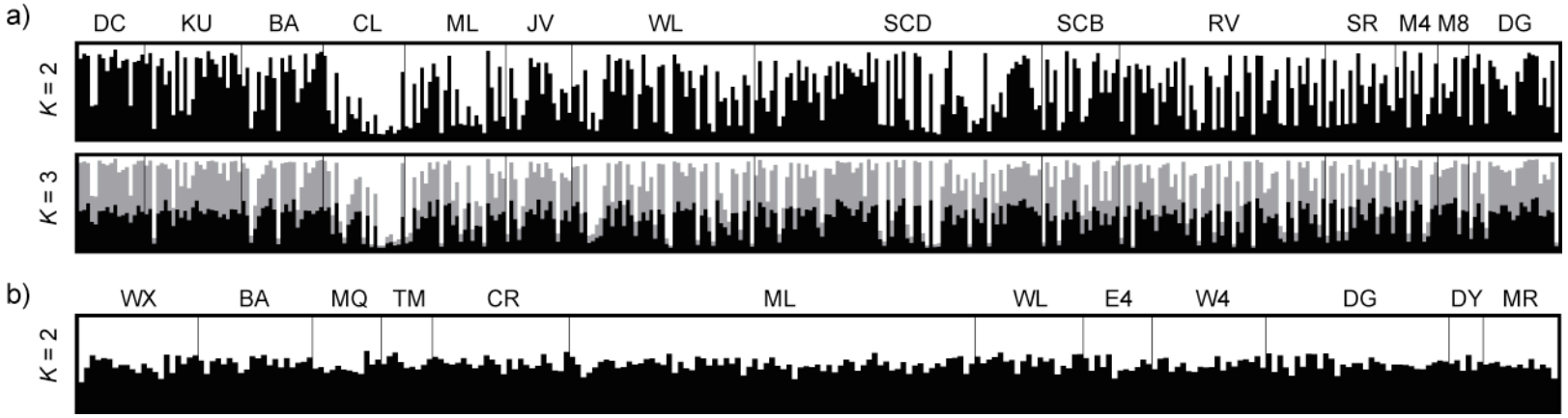

Figure 5.4. Genetic membership from Bayesian assignment tests in STRUCTURE. Individual membership of *B. cognatus* to each cluster at $K = 2$ and $K = 3$ (a) and of *S. couchii* to each cluster at $K = 2$ (b). Collection locality of individuals are delineated by thin black lines and the names are indicated above membership plots.

Comparisons among temperate amphibians

Bufo cognatus populations showed greater overall standardized differentiation (F_{ST}') than *S. couchii*, corroborating differences detected in the unstandardized estimation of population differentiation (Table 5.5). Both *A. maculatum* and *R. catesbeiana* had much higher levels of standardized F_{ST}' (Table 5.5). F_{ST}' for *A. maculatum* and *R. catesbeiana* were approximately an order of magnitude greater than those for *S. couchii* and *B. cognatus*. Both *B. cognatus* and *S. couchii* continued to show a pattern of isolation by distance for the natural logarithm of distance (*d*) against standardized F_{ST}' instead of $F_{ST} / (1 - F_{ST})$ (*B. cognatus*: $F_{ST}' = - 0.057 + 0.010\ d$, $p = 0.017$; *S. couchii* $F_{ST}' = - 0.053 + 0.007\ d$, $p = 0.014$). Standardized genetic differentiation was positively correlated with the natural logarithm of distance for *A. maculatum* ($F_{ST}' = - 0.445 + 0.070\ d$, $p < 0.001$), but only approached significance for *R. catesbeiana* ($F_{ST}' = - 0.156 + 0.029\ d$, $p = 0.057$). Pairwise values of F_{ST}' were greatest overall for *A. maculatum* followed by *R. catesbeiana*, *B. cognatus*, and finally *S. couchii*. Therefore, at any given geographic distances *A. maculatum* is more differentiated than the other three species (Figure 5.5).

I found a tight correlation between H_e and $F_{ST\mathit{max}}$ for the four species datasets in this study ($R^2 = 0.99$; $p = 0.0024$; $F_{ST\mathit{max}} = 1.016 - 1.042\ H_e$) justifying the use of this relationship to estimate $F_{ST\mathit{max}}$ for other amphibian species. Approximations of F_{ST}' for previously conducted studies of population genetic differentiation in amphibians ranged from 0.018 to 0.667 (Table 5.6). The lowest and highest values in this range were measured for populations of *R. cascadae* studied at fine scales (1 – 23 km) and at broader geographic scales (26 – 670 km) using a range of population specific expected heterozygosities (Table 5.6, Monsen and Blouin 2004). Among the remaining studies, F_{ST}' ranged from 0.025 in *R. sylvatica* at fine scales (< 0.5 – 20 km) to 0.537 in *B. bufo*, also at fine scales (5.5 – 14.4 km). Overall, levels of genetic

Table 5.5. Expected heterozygosity (H_e) and mean F_{ST} and F_{STmax} ± SE after jackknifing over samples and loci within each species. Standardized genetic differentiation (F_{ST}') is calculated as F_{ST} / F_{STmax}.

Species	H_e	F_{ST}	F_{STmax}	F_{ST}'
Bufo cognatus	0.819	0.006 ± 0.001	0.165 ± 0.032	0.036
Scaphiopus couchii	0.709	0.003 ± 0.001	0.274 ± 0.061	0.011
Ambystoma maculatum[1]	0.657	0.073 ± 0.010	0.339 ± 0.020	0.215
Rana catesbeiana[2]	0.670	0.041 ± 0.009	0.314 ± 0.070	0.131

[1] Zamudio and Wieczorek, 2007, [2] Austin *et al*., 2004

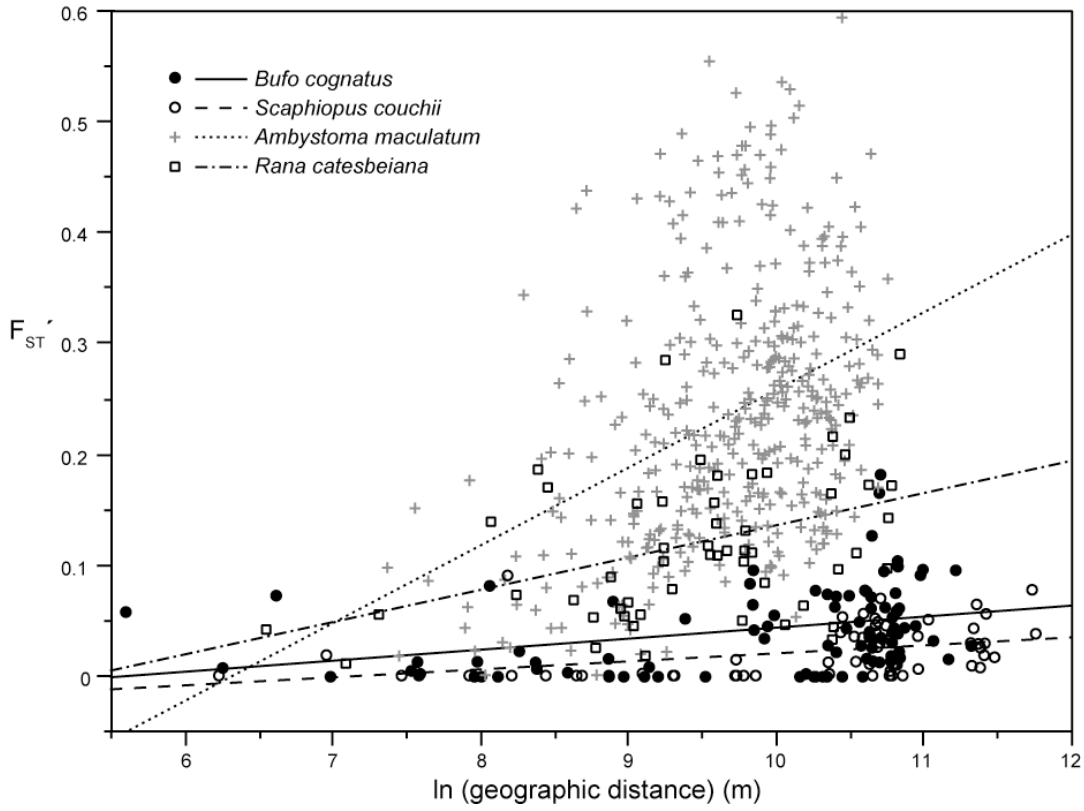

Figure 5.5. Linear regression between natural logarithm of geographic distance (m) and standardized genetic differentiation (F_{ST}') for four species of amphibians.

differentiation in the taxa surveyed were comparable to *A. maculatum* and *R. catesbeiana* and high in comparison to *B. cognatus* and *S. couchii* (Table 5.6). Only three species showed low levels of F_{ST}' approaching those observed in the two focal species of this study: *B. bufo* (Scribner *et al.*, 1994), *R. cascadae* (Monsen and Blouin, 2004), and *R. sylvatica* (Newman and Squire, 2001). These taxa showed F_{ST}' values less than or equal to 0.05; however, these studies focused primarily at spatial scales of less than 20 km.

DISCUSSION

This is one of the few studies of fine-scale genetic structure among populations of North American desert amphibians and these results are a surprising contrast to the patterns of high genetic differentiation generally found for temperate amphibians (Table 5.6). I expected to find substantial population structure among populations with particularly high levels of differentiation among valleys because mountains can constitute barriers to gene flow for amphibians (Funk *et al.*, 2005; Spear *et al.*, 2005). However, these data show that there is no population genetic structure within valleys for *S. couchii* and only weak genetic differentiation across valleys for both species. Habitats in the deserts of North America are more conducive to gene flow than previously assumed and adaptations of these species to desert environments may additionally promote connectivity among breeding ponds.

Differences between B. cognatus *and* S. couchii

Despite low genetic divergence in both *B. cognatus* and *S. couchii*, the patterns of genetic differentiation in these species are not identical suggesting that species-specific characteristics contribute to patterns of gene flow and the maintenance of genetic diversity. Neither Bayesian clustering methods nor spatial autocorrelation

Table 5.6. Summary of studies of population differentiation in temperate amphibians. Distances marked with [§] were calculated from published UTM coordinates, [*] denotes F_{ST} calculated as the average of all pairwise values, and [†] denotes H_e calculated as the weighted average across populations.

Species	Scale (km)	F_{ST}	H_e	F_{ST}'	Ref.
Bufo bufo	5.5 - 14.4	0.016	0.683	0.053	1
	5.5 - 14.4	0.032	0.527	0.068	1
	2.5 - 12	0.222[*]	0.579[†]	0.537	2
Bufo calamita	2.3	0.06	0.242 - 0.376	0.079 - 0.096	3
	2-16	0.224	0.242 - 0.376	0.293 - 0.359	3
	3.3 - 9	0.111	0.242 - 0.376	0.145 - 0.178	3
Bufo cognatus	0.3 - 82.5	0.006	0.819	0.036	4
Rana arvalis	0.3 - 7.6	0.052	0.378	0.084	5
	0.3 - 150	0.065	0.384	0.105	5
Rana cascadae	1 - 23	0.010 – 0.260	0.450 - 0.730	0.018 – 0.356	6
	26 - 670	0.040 - 0.520	0.450 - 0.780	0.073 – 0.667	6
Rana catesbeiana	0.7 - 50.9	0.041	0.67	0.131	4, 7
Rana lutieventris	0.4 - 10.3[§]	0.115[*]	0.540[†]	0.253	8
	0.4 - 6.2[§]	0.460[*]	0.653[†]	0.137	8
	0.1 – 15.0[§]	0.115[*]	0.444[†]	0.208	8
	1.2 - 17[§]	0.223[*]	0.374[†]	0.356	8
	1.3 - 5.6[§]	0.149[*]	0.449[†]	0.272	8
Rana sylvatica	< 0.5 - 20	0.014	0.44 - 0.50	0.025 - 0.028	9
Rana temporaria	2.5 - 12	0.050[*]	0.669[†]	0.156	2
Scaphiopus couchii	0.5 - 127	0.003	0.709	0.011	4
Ambystoma maculatum	1.6 - 47.1	0.073	0.657	0.215	4, 10
Ambystoma tigrinum melanostictum	0.5 - 50	0.240	0.320[†]	0.255	11, 12
Triturus cristatus	0.4 - 5.9	0.07	0.611[†]	0.184	13
Triturus marmoratus	0.2 - 6.4	0.11	0.448[†]	0.200	13

1. Scribner *et al.,* 1994; 2. Brede and Beebee, 2004; 3. Rowe *et al.,* 2000; 4. This study; 5. Vos *et al.,* 2001; 6. Monsen and Blouin, 2004; 7. Austin *et al.,* 2004; 8. Funk *et al.,* 2005; 9. Newman and Squire, 2001; 10. Zamudio and Wieczorek, 2007; 11. Spear *et al.,* 2005; 12. Personal communication; 13. Jehle *et al.,* 2005b.

analyses detected finer geographic population differentiation in these species than did pairwise population comparisons with F_{ST}. However, *B. cognatus* shows some genetic structure within the San Simon/San Bernardino Valleys as well as between these valleys and Sulphur Springs Valley for populations separated by distances greater than 18 km. In contrast, *S. couchii* showed significant differentiation only among valleys (> 80 km through low elevations), but not within valleys. Higher levels of gene flow observed in *S. couchii* compared to *B. cognatus* may be explained by greater vagility and dispersal ability in *S. couchii*, greater survival and reproductive success post-dispersal in *S. couchii*, and/or larger effective population sizes and thus, less genetic drift in *S. couchii*.

Scaphiopus couchii occurs primarily in arid regions of the United States and Mexico and is highly adapted to xeric environments (Blair, 1976; Mayhew, 1965; Woodward and Mitchell, 1991). This species has a suite of adaptations to desert environments including rapid and plastic development in quickly drying ponds (Newman, 1988) and adult water conservation and resistance to desiccation (McClanahan, 1964; Shoemaker *et al.*, 1969). *Bufo cognatus* can tolerate arid environments and can conserve water efficiently (McClanahan, 1964; Walker and Whitford, 1970), but in contrast to *S. couchii* occurs in a variety of habitats from Canada to Mexico (Graves and Krupa, 2005; Sullivan, 2005) and is not strictly a desert species. Greater physiological tolerances of desert conditions in *S. couchii* may translate to higher rates of gene flow and population connectivity.

More mating opportunities and greater larval survivorship to metamorphosis may lead to higher reproductive success post-dispersal in *S. couchii* and contribute to differences between these two species in genetic structure. *Scaphiopus couchii* is able to reproduce in waterbodies of many sizes including small ponds and puddles because of a short larval development time (8 – 13 days; Mayhew 1965), but *B. cognatus* is

constrained to breeding only in larger ponds because of a comparatively long larval period (25 – 45 days; Bragg, 1940b). At these breeding sites, both species can be abundant, but because *B. cognatus* has a more strongly male-biased sex skew, few adults are reproductively successful in comparison to *S. couchii* (Chan, Chapter 2). Thus, in *B. cognatus* limited breeding opportunities due to larval habitat requirements are compounded by high levels of reproductive skew. Larval mortality can also exacerbate the effects of reproductive skew (Hedgecock, 1994) such that offspring survival may also influence the degree to which dispersing individuals contribute to gene flow among regions. Competition, predation, and pond drying can each lead to high levels of larval mortality (Bragg, 1940a, 1945; Woodward, 1983) and reduce adult reproductive success. Because of the difference in larval development time, survival in *S. couchii* is likely to be greater than that of *B. cognatus* increasing the chance that dispersal will contribute to gene flow. Even if movement among ponds for these two species are similar, breeding habitat requirements and larval survival are likely to contribute to greater reproductive success in *S. couchii* following dispersal to new areas and consequently, higher levels of gene flow.

Finally, life history traits and demographic factors may also contribute to larger effective population sizes in *S. couchii* relative to *B. cognatus* reducing the impact of genetic drift and degree of population differentiation. *Scaphiopus couchii* has a less male-biased sex ratio and lower rates of larval mortality than *B. cognatus* which should increase effective population size and lower the rate of divergence due to genetic drift. Genetic estimates of effective breeding size and effective population size do not differ systematically between these species (Chan, Chapter 4) suggesting that effective size may not explain the patterns of population genetic structure. Variation among sites in these genetic estimates of effective size for each species indicates that ponds may differ considerably in sex-ratios and breeding group sizes

and that the relative effective sizes for each species fluctuate temporally and spatially. Demographic estimates of effective population size that are able to consider temporal and spatial variation in breeding aggregation characteristics may better reflect average breeding opportunities for these species across site and across years and help to determine the role that effective population size plays in these patterns of genetic differentiation.

Desert amphibians

The lack of substantial genetic structure in these two desert species indicates high levels of movement and gene flow among pops and is in stark contrast to predictions based on our understanding of the biology of these species. Desert environments are not expected to be conducive to movement in organisms that rely on moist environments for survival and aquatic habitats for reproduction. Despite high reproductive skew and low effective breeding sizes at some sites (Chan, Chapter 2, 4), both *S. couchii* and *B. cognatus* are able to maintain high levels of genetic diversity with very little among population divergence. Desert habitats may lack barriers to dispersal, but characteristics of these two anurans are also likely to contribute to the lack of genetic differentiation; investigating other desert anurans would allow us to assess the relative importance of habitat and organismal traits to patterns of population genetic structure.

Low genetic differentiation in desert anurans may be partially explained by landscape homogeneity in desert valleys. Habitat complexity and heterogeneity contribute to isolation by presenting barriers to gene flow (Wiens, 2001) and not surprisingly, landscape features correlate with patterns of fine-scale genetic structure in many taxa (e.g. Funk *et al.*, 2005; Hedgecock, 1978; Hitchings and Beebee, 1997; Spear *et al.*, 2005; Storfer *et al.*, 2006). The creosote and mesquite dominated valley

floors where *B. cognatus* and *S. couchii* occur lack the topographic relief and heterogeneity of habitat types usually correlated to restricted gene flow. Connectivity and gene flow in *B. cognatus* and *S. couchii* may occur through dispersal among ponds according to a stepping stone model of migration as suggested by the pattern of isolation by distance. Populations may be connected at large geographic distances and across valleys through high and continuous levels of gene flow among neighboring populations.

Even for amphibian taxa in which long distance dispersal is documented, genetic differentiation exists at fine-scales (e.g. Funk *et al.*, 2005; Newman and Squire, 2001) emphasizing individual dispersal is not equal to gene flow (Peacock and Ray, 2001). Movements by adults from ~0.5 km to 2 km (e.g. Bragg, 1940c; Funk *et al.*, 2004; Smith and Green, 2006; Trenham *et al.*, 2001) and dispersal distances of over 5 km by recently metamorphosed individuals and subadults (Dole, 1971; Funk *et al.*, 2004; Newman and Squire, 2001; Sinsch, 1997) suggest that long distances dispersal is not uncommon among amphibians (Smith and Green, 2005). Natal philopatry and site fidelity across breeding years (Greenberg and Tanner, 2005a; Greenberg and Tanner, 2005b; see Table 2 in Smith and Green, 2005) may help to promote genetic differentiation in these species.

High vagility and/or high rates of dispersal required to explain the pattern of limited genetic structure in *B. cognatus* and *S. couchii* are not unreasonable. The physiological capacity for prolonged activity is higher in *B. cognatus* and *S. couchii* compared to that of ranid frogs potentially related to burrowing activity (Seymour, 1973). Thus, higher metabolic scope in these desert species may facilitate long distance movements. Despite a focus on the population biology and community ecology of desert anurans (e.g. Newman, 1987; Sullivan, 1989; Woodward and Mitchell, 1991) few data on individual movement in desert amphibians exist. Bragg

(1940c) noted the presence of post-metamorphic *B. cognatus* half a mile to a mile from the nearest known breeding site in Oklahoma. In California, Mayhew (1965) found *S. couchii* subadults over a quarter mile from the nearest water. Additionally, Smith and Bragg (1949) and Bragg and Brooks (1958) noted mass unidirectional movements of *B. cognatus* individuals in Oklahoma populations. Movement, and hence gene flow, may be a consequence of nightly foraging activities following adult reproduction or metamorphosis in juveniles; Smith and Bragg (1949) found that toads collected from mass movements typically had full stomachs and they observed subadults stopping at ants nests to forage. Thus, not only are individuals capable of long distance dispersal, but such movements may be common.

Habitat requirements for reproduction in these desert environments may also contribute to high levels of gene flow in these species. *Bufo cognatus* and *S. couchii* are completely dependent on ephemeral ponds that fill with summer rains and often avoid the few permanent ponds that exist in this region and tend to have higher densities of predators (Woodward, 1983). While ephemeral breeding sites are likely to increase the chance of larval survivorship to metamorphosis, dependence on this habitat feature means that the distribution of suitable breeding sites is not predictable from year to year. Adults of both *B. cognatus* and *S. couchii* breed for multiple years (Rogers and Harvey, 1994; Sullivan and Fernandez, 1999; Tinsley and Tocque, 1995). but ponds which fill in some years do not necessarily fill in other years (Anderson *et al.*, 1999). Therefore, in this environment, philopatry and site fidelity should be selected against because individuals able to disperse and find breeding sites each year will have greater opportunities of successful reproduction. The unpredictability of breeding sites across years may select for behaviors that also promote gene flow in habitats characterized by environmental stochasticity.

Comparisons with other temperate amphibians

The degree of population genetic structure in *B. cognatus* and *S. couchii* is much lower than that described for other temperate amphibians. Even after standardizing for overall levels of genetic diversity, the genetic differentiation (F_{ST}') in these species remains approximately an order of magnitude lower than those for Bullfrogs (*R. catesbeiana*) and Spotted Salamanders (*A. maculatum*) at comparable spatial scales (Table 5.5; Figure 5.6). Like *B. cognatus* and *S. couchii*, *R. catesbeiana* and *A. maculatum* are pond-breeding amphibians, but they inhabit deciduous forests indicating that movement in the north-temperate regions is more restricted than in the arid deserts of southwestern North America. These results are corroborated by my survey of the literature for temperate, pond breeding amphibians; most species have considerably higher levels of F_{ST}' at smaller spatial scales than either *B. cognatus* or *S. couchii* (Table 5.6). Several taxa do show very low measures of F_{ST}' (*R. cascadae,* Monsen and Blouin, 2005; *B. bufo*, Scribner *et al.*, 1994), but only at fine spatial scales and the levels of standardized differentiation in these species at short geographic distances are comparable to what I find in *B. cognatus* and *S. couchii* only at distance of 50 – 130 km. *Rana sylvatica* inhabiting prairie wetlands was the only surveyed study with a low degree of population genetic differentiation at 20 km ($F_{ST}' \sim 0.025$; Newman and Squire, 2001). In comparison to the montane and forested habitats of some taxa surveyed here, prairie wetlands are likely to be more spatially homogeneous much like desert landscapes. Desert amphibians stand apart from the other taxa included here with very weak differentiation at wide geographic distances. However, such low differentiation in a prairie population of *R. sylvatica* suggests that landscape homogeneity may be a general habitat feature that contributes to high levels of gene flow and connectivity in a number of amphibians, not just desert species.

Patterns of genetic diversity in these two desert anurans challenge our view of amphibian population structure and suggest that desert environments may allow for high levels of movement and gene flow despite temporally restricted activity periods and harsh xeric conditions. The loss of diversity within ponds due to reproductive skew and environmental stochasticity was expected to exacerbate genetic structure, but is compensated for by gene flow and overlapping generations. Simulation based models have been useful in determining the influence of reproductive failure on the population persistence of Marbled Salamanders (*A. opacum*) (Taylor *et al.*, 2005). Applying a similar approach to these desert breeding anurans may elucidate the role of dispersal and overlapping generations in maintaining genetic diversity and overall genetic homogeneity in these taxa given pronounced within population processes and environmental unpredictability.

Over the last century, southwestern deserts have been modified for agriculture and urban development (Gray *et al.*, 2004). While these populations do not appear to be declining, both species are likely to be sensitive to human altered landscapes. Habitat connectivity is especially important for species with high dispersal (Funk *et al.*, 2004) and these data suggest that both *B. cognatus* and *S. couchii* may be negatively affected by habitat fragmentation despite currently low genetic structure. Bragg (1940c) noted that *B. cognatus* is closely associated with grasslands, avoids wooded and urban areas, and suffers high mortality along paved roads. Woodward (1983) found that permanent ponds, such as cattle tanks, were less likely support breeding aggregations of *S. couchii*. These patterns of population structure suggest that juvenile dispersal away from natal ponds and adult movement among ponds are important components of the life history of these toads. *Bufo cognatus* and *S. couchii* maintain high genetic diversity and avoid population divergence despite potentially low levels of reproductive success within ponds (Chan, Chapter 2, 3). Alterations to

the landscape that affect dispersal rates, movement patterns, and reproductive success may have significant consequences for genetic diversity and population differentiation in desert anurans.

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Appendix 1. Tissue samples and collection localities for mitochondrial DNA (mtDNA) and microsatellite datasets (μsat). Individuals used in population-based microsatellite analyses are listed by population assignment. Collection series abbreviations: sgr – L. Chan; DL – D. Laurencio; sar – L. Chan; TJH – T. J. Hibbitts; MSB – Museum of Southwestern Biology, MTH – M. T. Hill, TCWC – Texas Cooperative Wildlife Collection.

Sample ID	Latitude	Longitude	Dataset
Sceloporus graciosus			
sgr03	N 36° 03' 29.3"	W 107° 00' 30.4"	mtDNA
sgr04	N 36° 04' 06.7"	W 106° 59' 50.3"	mtDNA
Sceloporus arenicolus			
DL902	N 33° 01' 16.3"	W 103° 55' 08.9"	mtDNA
DL912	N 33° 01' 14.8"	W 103° 55' 06.6"	mtDNA
DL914	N 33° 01' 15.3"	W 103° 55' 09.8"	mtDNA
DL915	N 32° 31' 38.4"	W 103° 05' 41.2"	mtDNA
DL916	N 33° 41' 41.3"	W 103° 38' 34.7"	mtDNA
DL924	N 33° 41' 08.5"	W 103° 42' 20.6"	mtDNA
DL925	N 32° 08' 50.4"	W 102° 46' 29.7"	mtDNA
DL944	N 31° 44' 39.7"	W 102° 54' 11.5"	mtDNA
DL957	N 31° 44' 38.9"	W 102° 54' 11.2"	mtDNA
DL958	N 32° 31' 35.0"	W 103° 03' 49.4"	mtDNA
sar0381	N 32° 33' 26.7"	W 103° 07' 41.4"	mtDNA
sar0383	N 32° 33' 26.6"	W 103° 19' 11.4"	mtDNA
TJH789	N 32° 31' 33.7"	W 103° 03' 52.4"	mtDNA
MSB57654	N 32° 36' 07.9"	W 103° 48' 03.4"	mtDNA & μsat
MSB57659	N 32° 34' 15.4"	W 103° 36' 05.6"	μsat
MSB57684	N 33° 12' 36.6"	W 103° 59' 27.0"	μsat
Kenna			
MSB57609	N 33° 53’ 06.2”	W 103° 57’ 28.2”	mtDNA & μsat
MSB57610	N 33° 53’ 06.2”	W 103° 57’ 28.2”	mtDNA & μsat
MSB57613	N 33° 52’ 06.9”	W 103° 57’ 31.1”	mtDNA & μsat
MSB57694	N 33° 43’ 15.1”	W 103° 49’ 16.5”	mtDNA & μsat
MSB57704	N 33° 45’ 22.4”	W 103° 54’ 04.3”	mtDNA & μsat
MSB57705	N 33° 45’ 22.4”	W 103° 54’ 04.3”	mtDNA & μsat
sar0023	N 33° 50’ 19.1”	W 103° 50’ 58.7”	mtDNA & μsat
sar0024	N 33° 50’ 19.1”	W 103° 50’ 58.7”	mtDNA & μsat
sar0027	N 33° 50’ 19.1”	W 103° 50’ 58.7”	mtDNA & μsat
sar0028	N 33° 50’ 19.1”	W 103° 50’ 58.7”	mtDNA & μsat
sar0030	N 33° 50’ 19.1”	W 103° 50’ 58.7”	mtDNA & μsat
MTH266	N 33° 51’ 52.6”	W 103° 56’ 06.3”	μsat
sar0021	N 33° 50’ 19.1”	W 103° 50’ 58.7”	μsat
sar0022	N 33° 50’ 19.1”	W 103° 50’ 58.7”	μsat
sar0025	N 33° 50’ 19.1”	W 103° 50’ 58.7”	μsat
sar0026	N 33° 50’ 19.1”	W 103° 50’ 58.7”	μsat
sar0029	N 33° 50’ 19.1”	W 103° 50’ 58.7”	μsat
sar0031	N 33° 50’ 19.1”	W 103° 50’ 58.7”	μsat
sar0032	N 33° 50’ 19.1”	W 103° 50’ 58.7”	μsat
sar0033	N 33° 50’ 19.1”	W 103° 50’ 58.7”	μsat
sar0361	N 33° 53’ 00.9”	W 103° 57’ 33.9”	μsat

Appendix 1 (continued).

Sample ID	Latitude	Longitude	Dataset
Site 20			
sar0224	N 33° 42' 24.2"	W 103° 19' 58.4"	mtDNA & µsat
sar0226	N 33° 42' 24.2"	W 103° 19' 58.4"	mtDNA & µsat
sar0227	N 33° 42' 24.2"	W 103° 19' 58.4"	mtDNA & µsat
sar0229	N 33° 42' 24.2"	W 103° 19' 58.4"	mtDNA & µsat
sar0225	N 33° 42' 24.2"	W 103° 19' 58.4"	µsat
sar0228	N 33° 42' 24.2"	W 103° 19' 58.4"	µsat
sar0230	N 33° 42' 24.2"	W 103° 19' 58.4"	µsat
sar0231	N 33° 42' 24.2"	W 103° 19' 58.4"	µsat
sar0232	N 33° 42' 24.2"	W 103° 19' 58.4"	µsat
sar0233	N 33° 42' 24.2"	W 103° 19' 58.4"	µsat
sar0234	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0235	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0236	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0237	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0238	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0239	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0240	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0241	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0242	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0243	N 33° 41' 41.7"	W 103° 19' 35.9"	µsat
sar0244	N 33° 41' 54.9"	W 103° 19' 47.3"	µsat
Site 106			
sar0019	N 33° 27' 48.5"	W 103° 39' 38.9"	mtDNA & µsat
sar0050	N 33° 27' 48.5"	W 103° 39' 38.9"	mtDNA & µsat
sar0054	N 33° 27' 48.5"	W 103° 39' 38.9"	mtDNA & µsat
sar0016	N 33° 27' 48.5"	W 103° 39' 38.9"	µsat
sar0017	N 33° 27' 48.5"	W 103° 39' 38.9"	µsat
sar0035	N 33° 27' 48.5"	W 103° 39' 38.9"	µsat
sar0051	N 33° 27' 48.5"	W 103° 39' 38.9"	µsat
sar0052	N 33° 27' 48.5"	W 103° 39' 38.9"	µsat
sar0053	N 33° 27' 48.5"	W 103° 39' 38.9"	µsat
Camp			
sar0002	N 33° 27’ 16.7”	W 103° 47’ 17.1”	mtDNA & µsat
sar0003	N 33° 27’ 16.7”	W 103° 47’ 17.1”	mtDNA & µsat
sar0004	N 33° 27’ 16.7”	W 103° 47’ 17.1”	mtDNA & µsat
sar0200	N 33° 26’ 52.2”	W 103° 48’ 00.8”	mtDNA & µsat
sar0218	N 33° 27’ 15.7”	W 103° 47’ 06.2”	mtDNA & µsat
sar0307	N 33° 26’ 10.5”	W 103° 49’ 10.4”	mtDNA & µsat
MTH270	N 33° 23’ 31.9”	W 103° 53’ 57.5”	µsat
MTH275	N 33° 23’ 18.2”	W 103° 54’ 11.5”	µsat
MTH276	N 33° 23’ 51.6”	W 103° 54’ 05.6”	µsat
sar0001	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0005	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0006	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0007	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0008	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0009	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0010	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat

Appendix 1 (continued).

Sample ID	Latitude	Longitude	Dataset
Camp (continued)			
sar0011	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0012	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0013	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0014	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0015	N 33° 27’ 16.7”	W 103° 47’ 17.1”	µsat
sar0036	N 33° 27’ 15.7”	W 103° 47’ 06.2”	µsat
sar0037	N 33° 27’ 15.7”	W 103° 47’ 06.2”	µsat
sar0038	N 33° 27’ 13.7”	W 103° 47’ 29.0”	µsat
sar0039	N 33° 27’ 11.7”	W 103° 47’ 43.9”	µsat
sar0040	N 33° 27’ 04.7”	W 103° 47’ 35.1”	µsat
sar0041	N 33° 27’ 04.7”	W 103° 47’ 35.1”	µsat
sar0042	N 33° 27’ 04.7”	W 103° 47’ 35.1”	µsat
sar0043	N 33° 27’ 11.7”	W 103° 47’ 43.9”	µsat
sar0045	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0046	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0047	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0048	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0049	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0055	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0201	N 33° 26’ 52.2”	W 103° 48’ 00.8”	µsat
sar0215	N 33° 27’ 04.7”	W 103° 47’ 35.1”	µsat
sar0216	N 33° 27’ 04.7”	W 103° 47’ 35.1”	µsat
sar0217	N 33° 26’ 55.8”	W 103° 46’ 41.8”	µsat
sar0219	N 33° 26’ 52.2”	W 103° 48’ 00.8”	µsat
sar0220	N 33° 27’ 11.7”	W 103° 47’ 43.9”	µsat
sar0221	N 33° 27’ 11.7”	W 103° 47’ 43.9”	µsat
sar0223	N 33° 27’ 13.6”	W 103° 47’ 51.6”	µsat
sar0245	N 33° 27’ 06.4”	W 103° 47’ 17.7”	µsat
sar0246	N 33° 27’ 13.7”	W 103° 47’ 29.0”	µsat
sar0247	N 33° 27’ 04.7”	W 103° 47’ 35.1”	µsat
sar0248	N 33° 26’ 52.2”	W 103° 48’ 00.8”	µsat
sar0249	N 33° 27’ 13.6”	W 103° 47’ 51.6”	µsat
sar0250	N 33° 27’ 11.7”	W 103° 47’ 43.9”	µsat
sar0251	N 33° 26’ 24.3”	W 103° 48’ 46.8”	µsat
sar0267	N 33° 27’ 04.7”	W 103° 47’ 35.1”	µsat
sar0268	N 33° 27’ 15.7”	W 103° 47’ 06.2”	µsat
sar0269	N 33° 27’ 13.7”	W 103° 47’ 29.0”	µsat
sar0270	N 33° 27’ 02.2”	W 103° 48’ 01.2”	µsat
sar0271	N 33° 26’ 32.0”	W 103° 48’ 14.7”	µsat
sar0272	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0274	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0275	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0276	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0277	N 33° 26’ 32.0”	W 103° 48’ 14.7”	µsat
sar0278	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0279	N 33° 27’ 15.7”	W 103° 47’ 06.2”	µsat
sar0280	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0281	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat

Appendix 1 (continued).

Sample ID	Latitude	Longitude	Dataset
Camp (continued)			
sar0282	N 33° 27’ 13.7”	W 103° 47’ 35.8”	µsat
sar0283	N 33° 27' 16.7"	W 103° 47' 17.1"	µsat
sar0284	N 33° 27' 04.7"	W 103° 47' 35.1"	µsat
sar0285	N 33° 27' 04.7"	W 103° 47' 35.1"	µsat
sar0286	N 33° 26' 32.0"	W 103° 48' 14.7"	µsat
sar0287	N 33° 27' 13.7"	W 103° 47' 35.8"	µsat
sar0290	N 33° 27' 13.7"	W 103° 47' 35.8"	µsat
sar0291	N 33° 27' 11.7"	W 103° 47' 43.9"	µsat
sar0292	N 33° 27' 04.7"	W 103° 47' 35.1"	µsat
sar0293	N 33° 27' 11.7"	W 103° 47' 43.9"	µsat
sar0294	N 33° 27' 04.7"	W 103° 47' 35.1"	µsat
sar0295	N 33° 27' 13.6"	W 103° 47' 51.6"	µsat
sar0296	N 33° 27' 13.7"	W 103° 47' 35.8"	µsat
sar0297	N 33° 27' 13.7"	W 103° 47' 35.8"	µsat
sar0298	N 33° 27' 04.7"	W 103° 47' 35.1"	µsat
sar0299	N 33° 27' 15.7"	W 103° 47' 06.2"	µsat
sar0300	N 33° 27' 13.7"	W 103° 47' 35.8"	µsat
sar0301	N 33° 27' 16.7"	W 103° 47' 17.1"	µsat
sar0302	N 33° 27' 13.7"	W 103° 47' 35.8"	µsat
sar0303	N 33° 26' 26.3"	W 103° 48' 59.8"	µsat
sar0306	N 33° 26' 26.3"	W 103° 48' 59.8"	µsat
sar0309	N 33° 26' 10.5"	W 103° 49' 10.4"	µsat
sar0310	N 33° 26' 03.0"	W 103° 49' 03.7"	µsat
sar0311	N 33° 26' 03.0"	W 103° 49' 03.7"	µsat
sar0313	N 33° 27' 02.5"	W 103° 51' 43.6"	µsat
sar0314	N 33° 27' 54.0"	W 103° 46' 30.0"	µsat
sar0315	N 33° 27' 20.2"	W 103° 47' 14.2"	µsat
sar0316	N 33° 27' 17.8"	W 103° 47' 28.9"	µsat
sar0317	N 33° 26' 09.8"	W 103° 49' 07.0"	µsat
sar0318	N 33° 27' 14.4"	W 103° 47' 17.8"	µsat
sar0319	N 33° 27' 04.9"	W 103° 47' 30.8"	µsat
sar0320	N 33° 27' 15.2"	W 103° 47' 16.2"	µsat
sar0321	N 33° 27' 05.9"	W 103° 51' 53.5"	µsat
sar0322	N 33° 27' 17.4"	W 103° 47' 22.4"	µsat
sar0323	N 33° 27' 48.1"	W 103° 46' 29.1"	µsat
sar0324	N 33° 28' 06.2"	W 103° 46' 19.4"	µsat
sar0325	N 33° 28' 06.5"	W 103° 47' 35.2"	µsat
sar0326	N 33° 27' 18.5"	W 103° 47' 25.1"	µsat
sar0327	N 33° 27' 18.1"	W 103° 47' 29.4"	µsat
sar0328	N 33° 28' 06.6"	W 103° 46' 21.8"	µsat
sar0329	N 33° 27' 30.7"	W 103° 47' 21.8"	µsat
sar0330	N 33° 26' 02.9"	W 103° 49' 05.2"	µsat
sar0331	N 33° 26' 25.5"	W 103° 49' 05.8"	µsat
sar0332	N 33° 27' 16.2"	W 103° 47' 22.1"	µsat
sar0333	N 33° 27' 15.2"	W 103° 47' 17.1"	µsat
sar0334	N 33° 27' 16.3"	W 103° 47' 29.4"	µsat
sar0335	N 33° 27' 15.2"	W 103° 47' 16.9"	µsat
sar0336	N 33° 26' 23.5"	W 103° 49' 01.3"	µsat

Appendix 1 (continued).

Sample ID	Latitude	Longitude	Dataset
Camp (continued)			
sar0338	N 33° 26' 28.4"	W 103° 49' 04.1"	µsat
sar0339	N 33° 26' 24.1"	W 103° 49' 02.8"	µsat
sar0340	N 33° 26' 27.6"	W 103° 49' 13.0"	µsat
sar0341	N 33° 26' 21.6"	W 103° 49' 11.6"	µsat
sar0342	N 33° 26' 21.6"	W 103° 49' 11.7"	µsat
sar0343	N 33° 26' 23.6"	W 103° 49' 11.6"	µsat
sar0344	N 33° 26' 31.3"	W 103° 49' 11.8"	µsat
sar0345	N 33° 27' 17.5"	W 103° 47' 13.5"	µsat
sar0346	N 33° 27' 17.0"	W 103° 47' 36.6"	µsat
sar0347	N 33° 27' 15.0"	W 103° 47' 16.6"	µsat
sar0349	N 33° 27' 18.3"	W 103° 47' 29.7"	µsat
sar0352	N 33° 23' 37.1"	W 103° 51' 43.4"	µsat
sar0353	N 33° 23' 46.9"	W 103° 51' 43.7"	µsat
sar0354	N 33° 23' 37.1"	W 103° 51' 43.4"	µsat
sar0357	N 33° 26' 54.4"	W 103° 48' 05.7"	µsat
sar0358	N 33° 27' 05.5"	W 103° 48' 02.3"	µsat
sar0359	N 33° 26' 54.5"	W 103° 47' 57.6"	µsat
sar0360	N 33° 26' 54.4"	W 103° 48' 05.7"	µsat
sar0362	N 33° 25' 49.0"	W 103° 48' 18.8"	µsat
sar0363	N 33° 26' 59.2"	W 103° 47' 30.4"	µsat
sar0364	N 33° 26' 59.2"	W 103° 47' 30.4"	µsat
sar0365	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0366	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0367	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0368	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0369	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0370	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0371	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0372	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0373	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
sar0375	N 33° 26' 59.2"	W 103° 47' 30.4"	µsat
sar0376	N 33° 26' 59.2"	W 103° 47' 30.4"	µsat
sar0377	N 33° 26' 59.2"	W 103° 47' 30.4"	µsat
sar0378	N 33° 26' 43.1"	W 103° 47' 53.9"	µsat
TCWC10411	N 33° 25' 05.0"	W 103° 50' 48.1"	µsat
Site 67			
sar0252	N 32° 54' 16.4"	W 103° 55' 32.1"	mtDNA & µsat
sar0253	N 32° 54' 16.4"	W 103° 55' 32.1"	mtDNA & µsat
sar0254	N 32° 54' 16.4"	W 103° 55' 32.1"	mtDNA & µsat
sar0255	N 32° 54' 16.4"	W 103° 55' 32.1"	mtDNA & µsat
sar0256	N 32° 54' 16.4"	W 103° 55' 32.1"	mtDNA & µsat
Loco Hills			
MSB57646	N 32° 42’ 32.9”	W 103° 48’ 22.7”	mtDNA & µsat
sar0204	N 32° 47’ 01.0”	W 103° 48’ 53.9”	mtDNA & µsat
sar0206	N 32° 47’ 01.0”	W 103° 48’ 53.9”	mtDNA & µsat
sar0208	N 32° 47’ 01.0”	W 103° 48’ 53.9”	mtDNA & µsat
MSB57640	N 32° 46’ 54.5”	W 103° 48’ 50.7”	µsat
sar0203	N 32° 47’ 01.0”	W 103° 48’ 53.9”	µsat

Appendix 1 (continued).

Sample ID	Latitude	Longitude	Dataset
Loco Hills (continued)			
sar0207	N 32° 47' 01.0"	W 103° 48' 53.9"	µsat
sar0209	N 32° 47' 01.0"	W 103° 48' 53.9"	µsat
sar0210	N 32° 47' 01.0"	W 103° 48' 53.9"	µsat
sar0211	N 32° 47' 01.0"	W 103° 48' 53.9"	µsat
sar0212	N 32° 47' 01.0"	W 103° 48' 53.9"	µsat
sar0213	N 32° 47' 01.0"	W 103° 48' 53.9"	µsat
sar0214	N 32° 47' 01.0"	W 103° 48' 53.9"	µsat
sar0348	N 32° 47' 01.0"	W 103° 48' 53.9"	µsat
sar0355	N 32° 47' 34.2"	W 103° 48' 55.5"	µsat
sar0356	N 32° 47' 34.0"	W 103° 48' 55.8"	µsat
sar0379	N 32° 47' 01.5"	W 103° 49' 50.5"	µsat
Site 28			
sar0259	N 32° 31' 26.2"	W 103° 5' 51.2"	mtDNA & µsat
sar0261	N 32° 31' 26.2"	W 103° 5' 51.2"	mtDNA & µsat
sar0262	N 32° 31' 26.2"	W 103° 5' 51.2"	mtDNA & µsat
sar0264	N 32° 31' 26.2"	W 103° 5' 51.2"	mtDNA & µsat
sar0265	N 32° 31' 26.2"	W 103° 5' 51.2"	mtDNA & µsat
sar0266	N 32° 31' 26.2"	W 103° 5' 51.2"	mtDNA & µsat
MSB57661	N 32° 31' 26.2"	W 103° 5' 51.2"	µsat
sar0258	N 32° 31' 26.2"	W 103° 5' 51.2"	µsat
sar0260	N 32° 31' 26.2"	W 103° 5' 51.2"	µsat
sar0263	N 32° 31' 26.2"	W 103° 5' 51.2"	µsat

Appendix 2. Locality names and abbreviations for *Bufo cognatus* and *Scaphiopus couchii* sites.

Locality Name	Abbreviation
Barnes Road	BA
Big Rig	BR
Corner Pond II	CR
Crown	CW
Culvert 12	CL
Dangerous Ditch	DG
Dangerous Pool	DP
Deering Pond	DE
E409 Pond	E4
East Willow	EW
End Road	ER
Javelina Pond	JV
Lava Pond	LV
Mesquite Pond	MQ
Mile 394 Pond	M4
Mile 398 Pond	M8
Miller Pond	ML
North Willow	NW
River's Pond	RV
River's Road	RR
River's Tank	RT
Sky Ranch 80 Pond	SR
South Willow	SW
Stuck Pond	ST
Sulphur Canyon Bufo Pond	SCB
Sulphur Canyon Ditch	SCD
Sulphur Canyon Tank	SCT
Tomberlin Pond	TM
W409 Pond	W4
Willow Ponds (adults)	WL

Appendix 3. Final run parameters for sibship clustering analyses in PARENTAGE. Larval stages analyzed are indicated by T, for tadpoles, M for metamorphs, and C for tadpoles and metamorphs combined.

Locality	Stage	# Chains	Heating Temperatures	Step Size	# Discarded Samples	# Recorded Samples
B. cognatus						
DG	T	6	4, 3, 2.3, 1.5, 1.2, 1	250	2500	5000
EW	T	1	1	500	1000	5000
NW	T	2	1.2, 1	100	1000	5000
JV	T	2	1.5, 1	250	2000	5000
SCD	T	1	1	100	2500	5000
SR	T	4	2, 1.5, 1.2, 1	50	5000	5000
ST	T	2	1.5, 1	500	1000	5000
DG	M	2	1.5, 1	250	2500	5000
EW	M	2	1.5, 1	50	5000	5000
NW	M	4	4, 2.5, 1.5, 1	50	5000	5000
SCD	M	6	4, 3, 2.5, 1.8, 1.2, 1	25	10000	5000
SR	M	2	1.5, 1	500	1000	5000
ST	M	2	1.2, 1	500	1000	5000
DG	C	8	4, 3.5, 3, 2.5, 2, 1.5, 1.2, 1	10	5000	5000
ST	C	4	3, 2, 1.2, 1	25	5000	5000
SR	C	4	2, 1.5, 1.2, 1	25	5000	5000
S. couchii						
BR	T	4	4, 1.5, 1, 0.8	500	1000	5000
CR	T	1	1	500	1000	5000
CW	T	4	4, 1.5, 1, 0.8	500	1000	5000
DG	T	4	4, 2.5, 1.5, 1	50	5000	5000
DP	T	6	4, 3, 2, 1.5, 1.2, 1	50	5000	5000
DE	T	5	4, 2.5, 1.5, 1	50	5000	5000
EW	T	5	2, 1.5, 1.2, 1	50	5000	5000
SW	T	1	1	250	2000	5000
LV	T	2	1.5, 1	500	1000	5000
SCB	T	1	1	500	1000	5000
SCD	T	6	4, 3, 2, 1.5, 1.2, 1	50	5000	5000
SCT	T	4	2, 1.5, 1.1, 1	50	10000	5000
SR	T	7	6, 4, 3, 2, 1.5, 1.2, 1	100	5000	5000
ST	T	2	1.5, 1	50	5000	5000
DG	M	2	1.5, 1	50	5000	5000
EW	M	4	2, 1.5, 1.2, 1	50	5000	5000
SW	M	4	4, 2.5, 1.5, 1	50	5000	5000
SCD	M	7	4, 3, 2.5, 2, 1.5, 1.2, 1	50	5000	5000
SCT	M	6	4, 3, 2, 1.5, 1.2, 1	100	5000	5000
SR	M	1	1	500	1000	5000
ST	M	4	4, 2.5, 1.5, 1	50	5000	5000
SCT	C	4	4, 2.5, 1.5, 1	25	5000	5000
SR	C	6	6, 4.5, 2.5, 1.5, 1.2, 1	25	10000	5000