

# Population differentiation of temperate amphibians in unpredictable environments

LAUREN M. CHAN and KELLY R. ZAMUDIO

Department of Ecology and Evolutionary Biology, Corson Hall, Cornell University, Ithaca, NY 14853-2701, USA

## Abstract

Amphibians are a globally distributed and diverse lineage, but much of our current understanding of their population genetic structure comes from studies in mesic temperate habitats. We characterize the population genetic structure of two sympatric explosive breeding amphibians in the southwestern deserts of the United States: the Great Plains toad (*Anaxyrus cognatus*) and Couch's spadefoot toad (*Scaphiopus couchii*). For both species, we find limited genetic differentiation even between populations in adjacent valleys separated by dispersal barriers such as mountainous habitats. To understand how population genetic patterns in these two arid-adapted species compare to taxa in more mesic environments, we computed a standardized measure of population differentiation for *A. cognatus*, *S. couchii*, and for pond-breeding amphibians that inhabit mesic temperate environments. Our results indicate that the arid-adapted species have lower population genetic structure at fine and moderate scales than most other amphibian species we surveyed. We hypothesize that stochasticity in the availability of appropriate breeding sites as well as landscape homogeneity may result in increased population connectivity in desert-adapted frogs. Future work examining fine-scale population structure in amphibians from a diversity of habitats will test the generality of our findings. Intraspecific comparisons among localities with varied seasonality and habitats will be particularly useful for investigating the interaction between species-typical population dynamics and environmental characteristics as determinants of population connectivity in pond-breeding amphibians.

*Keywords:* *Anaxyrus*, *Bufo*, connectivity, desert, pond-breeding, *Scaphiopus*

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## Introduction

Patterns of local population genetic structure reflect the interaction between organismal characteristics and landscape features (e.g. Manel *et al.* 2003; Funk *et al.* 2005; Coulon *et al.* 2006; Clark *et al.* 2008). The degree to which populations are connected by gene flow depends in part on the ecological requirements, physiological tolerances, and behavioural ecology of each species, as well as aspects of the landscape. Identifying the spatial scale at which population genetic structure is evident in any species is an important step in linking landscape and taxon-specific characteristics to patterns of differentiation. These data have important implications for

evolution and conservation because they reveal potential mechanisms underlying differentiation and allow us to better predict the consequences of landscape alteration.

For some species, it is reasonable to predict the scale of population genetic structure based on generalized attributes common to a broader taxonomic group (e.g. Zickovich & Bohonak 2007; Sherman *et al.* 2008). Amphibians are generally expected to have limited dispersal due to small body size and their reliance on moist environments to prevent desiccation (Blaustein *et al.* 1994). Moderate levels of juvenile dispersal (~20%) can result in genetic connectivity among local breeding ponds (Breden 1987; Berven & Grudzien 1990), but strong fidelity of adults to breeding sites across years (Gamble *et al.* 2007) often results in small genetic neighborhoods (Berven & Grudzien 1990). Indeed, many amphibian

Correspondence: Lauren M. Chan, Department of Biology, Box 90338, Duke University, Durham, NC 27708, USA.  
Fax: (919) 660 7293; E-mail: lauren.chan@duke.edu

species examined to date exhibit limited genetic connectivity across moderate geographic distances (e.g. Tallmon *et al.* 2000; Brede & Beebee 2004; Funk *et al.* 2005; Zamudio & Wicczorek 2007).

Amphibians are found on every continent except Antarctica; they inhabit highly seasonal northern and southern latitudes and reach particularly high diversity in the tropics (Duellman 1999). This ecological variety is paralleled by diversity in mating system and life-history characteristics (Wells 1977; Petranksa 1998; Bruce *et al.* 2000; Haddad & Prado 2005; Zamudio & Chan 2008). Despite this diversity, studies of gene flow and fine-scale population divergence in amphibians have largely been confined to temperate amphibians that inhabit seasonal environments with predictable spring rains and mild summers with respect to temperature and/or humidity. We know considerably less about the genetic connectivity among populations of explosively breeding amphibians that live in hot, arid environments and depend on stochastic summer rains for reproduction. The stochastic occurrence and persistence of breeding ponds across years in arid habitats may select against site fidelity and favour increased dispersal (Low 1976; Roff 2002; Ronce 2007). Topographic complexity reduces individual movement in some mesic taxa (Funk *et al.* 2005; Giordano *et al.* 2007), but desert habitats inhabited by pond-breeding amphibians are often found in flat desert valleys with few barriers to gene flow. Thus, for amphibians adapted to low-elevation desert habitats, we might expect that the reliance on ephemeral and temporally stochastic ponds will increase genetic connectivity and lower genetic structure at fine spatial scales. Characterizing the population genetic structure in desert amphibians and examining how these taxa compare to species in more mesic habitats will help us better understand how organismal biology and environmental variation interact to shape the distribution of genetic diversity among amphibian populations.

In this study, we examine population genetic structure in two co-occurring anurans common to the deserts of the southwestern United States. The Great Plains toad (*Anaxyrus 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). *Anaxyrus cognatus* occurs from southern Canada to central Mexico (Bragg 1940a; Stebbins 2003; Graves & Krupa 2005), whereas *S. couchii* is a more arid-adapted species (Mayhew 1965; Woodward & Mitchell 1991) restricted to desert habitats from the southwestern United States to central Mexico (Stebbins 2003).

Despite a focus on the population biology and community ecology of desert anurans (Newman 1987; Sullivan 1989; Woodward & Mitchell 1991), few data on

individual movement in these amphibians exist and relatively little is known about their activity outside of the breeding season. Ruibal *et al.* (1969) found aestivating spadefoot toads close to desert breeding ponds suggesting that adults of *S. couchii* might be site faithful. However, anecdotal reports of *A. cognatus* from grassland sites in Oklahoma suggest substantial juvenile dispersal, including mass unidirectional movements (Smith & Bragg 1949; Bragg & Brooks 1958) and the presence of postmetamorphic individuals up to a mile from the nearest known breeding site (Bragg 1940b). In California, Mayhew (1965) found *S. couchii* subadults over a quarter mile from the nearest water. Juvenile mortality can be high in amphibians (Creusere & Whitford 1976) and it is unclear how these movements might translate to gene flow among ponds in arid landscapes. Here, we estimate the population genetic structure in these two desert amphibians at fine and moderate geographic scales and compare the patterns in these species to those in other temperate amphibians using a standardized measure of differentiation. Our goals are to examine the degree of population differentiation in these two desert taxa, determine how it differs from that of species in other environments, and investigate potential mechanisms contributing to these patterns. Future comparative work examining fine-scale population genetic structure in amphibians from a diversity of habitats will test the generality of our findings.

## Methods

### *Tissue sampling and laboratory protocols*

During the summer breeding seasons of 2002–2005, we collected tissue samples of *Anaxyrus cognatus* and *Scaphiopus couchii* breeding populations in southeastern Arizona and southwestern New Mexico from four lowland valleys. 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 (Fig. 1). We sampled 11 and 9 breeding aggregations of *A. cognatus* and *S. couchii* respectively (Fig. 1; Appendix I); toe clips were sampled from adults and preserved in 100% EtOH for subsequent genetic analysis. Five additional collection localities were not sampled at breeding aggregations: we collected tissues from individuals found along short stretches of road for both species at one site at the northern tip of the San Simon Valley (BA) and for *A. cognatus* at two sites in Sulphur Springs Valley (KU and DC). We also included two population samples of *S. couchii* collected as tadpoles from ephemeral ponds in the Animas Valley (DY and MR). Samples within the

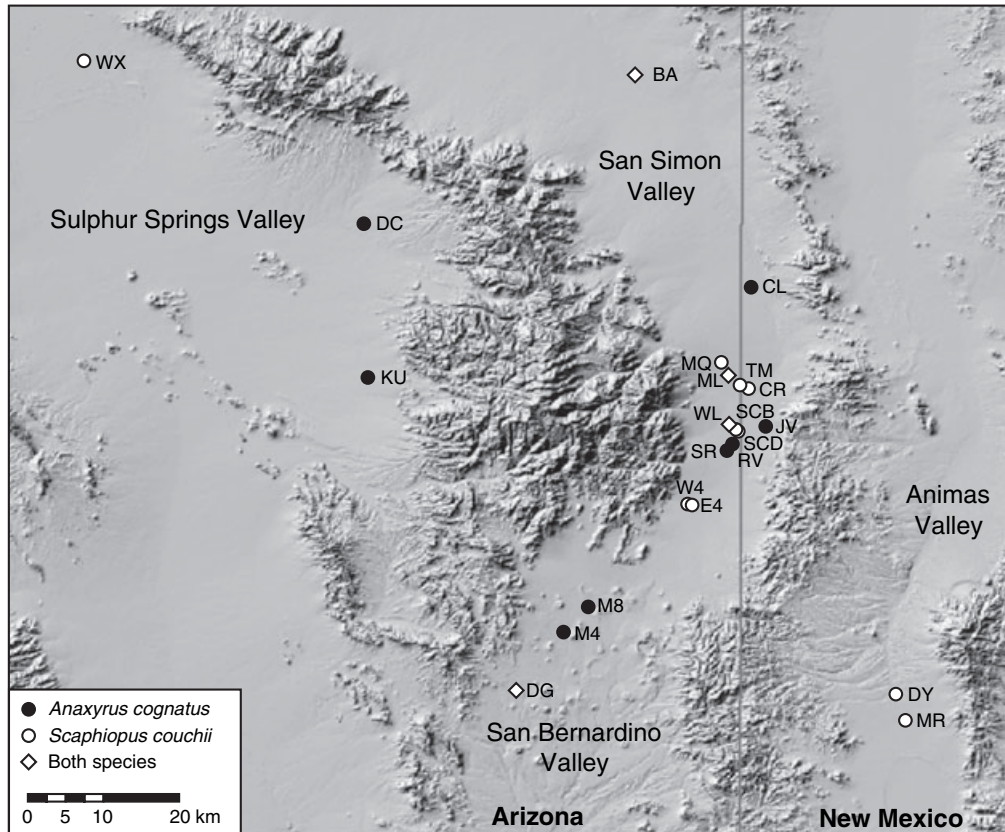


Fig. 1 Collection localities for *Anaxyrus cognatus* and *Scaphiopus couchii* across four desert valleys in southern Arizona and New Mexico. Bottom of the map is the border with Mexico.

San Simon/San Bernardino Valleys spanned a distance of 0.5–82.5 km for both species; between valley distances ranged from 20.4 to 63.8 for *A. cognatus* and 34.0 to 127.0 km for *S. couchii*.

Whole genomic DNA was isolated from tissue samples with a standard Chelex extraction consisting of ~1 mm<sup>3</sup> of tissue in 150 µL of a 5% Chelex solution (Chelex-100; Bio-Rad) incubated with 19 µg Proteinase K at 55 °C for 180 min. and 99 °C for 10 min. The supernatant from extractions was used directly as template for amplification of microsatellite loci via polymerase chain reactions (PCR). We genotyped all individuals of *A. cognatus* and *S. couchii* at 12 and 11 species-specific microsatellite loci, respectively, following protocols previously published for both species (Gonzales *et al.* 2004; Chan 2007a, b).

#### Microsatellite data

With three exceptions, each locality was sampled once during a single breeding period. For *A. cognatus* at the localities RV and SCD and *S. couchii* at ML, we had samples from multiple years and grouped samples across years to increase sample sizes. In those cases,

we first tested for temporal structure across years using a log-likelihood *G*-test for genotypic variance in GENEPOP v3.4 (Raymond & Rousset 1995; Goudet *et al.* 1996). We tested for departures from Hardy–Weinberg equilibrium (HWE) and for evidence of linkage disequilibrium within populations in GENEPOP. A Markov chain method (Guo & Thompson 1992) with 5000 dememorization steps and 100 batches of 5000 iterations per batch was used to determine the significance of all tests. We calculated average expected heterozygosity ( $H_E$ ) across all loci for each species using GENALEX v6 (Peakall & Smouse 2006). In addition, we 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 and therefore will not reflect population level genetic diversity (Chan 2007c). To determine whether the two *S. couchii* population samples collected as tadpoles (DY and MR) consisted of closely related individuals, we compared relatedness within each larval sample to relatedness among adults from all other collection localities. We computed

Queller & Goodnight's (1989) estimate of relatedness for all individuals sampled in GENALEX (Peakall & Smouse 2006). We 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

We used FSTAT 2.9.3 (Goudet 1995) to estimate  $F$ -statistics ( $F_{IS}$ ,  $F_{ST}$  and  $F_{IT}$ ; Weir & Cockerham 1984) over all loci and samples and among population pairs. Because small sample sizes can bias overall estimates of  $F_{ST}$ , we also estimated overall  $F$ -statistics for a reduced data set omitting localities with fewer than 15 sampled individuals. Mean  $F_{IS}$ ,  $F_{ST}$ , and  $F_{IT}$  and associated standard errors were determined by jackknifing over loci (Goudet 1995). Exact  $P$ -values for each pairwise  $F_{ST}$ -estimate were determined by randomizing multi-locus genotypes between the two populations. For each species, we determined table wide significance at  $\alpha = 0.05$  after sequential Bonferroni correction for multiple comparisons.

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 neighbouring populations. For each species, we examined the relationship among pairwise values of the natural logarithm of geographic distance and genetic distance, as  $F_{ST}/(1 - F_{ST})$  (Rousset 1997) for the full data sets as well as the reduced data set omitting localities with <15 sampled individuals. We conducted Mantel (1967) tests with 10 000 permutations on the geographic and genetic distance matrices using the Isolation-by-Distance Web Service (IBDWS; Jensen *et al.* 2005) to determine whether the slope of the reduced major axis (RMA) regression was significantly greater than zero ( $P < 0.05$ ).

#### Individual-based analyses

Analyses based on predefined delineations of populations can obscure patterns of differentiation; individual-based approaches are alternative methods for identifying spatial genetic structure and barriers to gene flow (Manel *et al.* 2003; Latch *et al.* 2006; Waples & Gaggiotti 2006; Rowe & Beebee 2007). We examined population genetic structure without relying on *a priori* delineations of populations using two individual-based approaches: (i) Bayesian assignment methods and (ii) genetic autocorrelation analyses in a spatially explicit framework.

We also used Bayesian clustering methods to infer the patterns of genetic differentiation for both *A. cognatus* and *S. couchii* in the program STRUCTURE 2.1 (Pritchard *et al.* 2000; Falush *et al.* 2003). STRUCTURE uses Markov chain Monte Carlo algorithms to determine the posterior probability that an individual belongs to each of  $K$  clusters under a population model assuming HWE and linkage equilibrium. For each species, we conducted ten replicate runs at each possible value of  $K$  (*A. 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, we used the admixture model where individuals can have a genetic signature from other demes with a uniform prior on the admixture parameter,  $\alpha$ . We used the  $F$ -model (Falush *et al.* 2003) assuming correlated allele frequencies across clusters and did not include collection locality as prior information. We used Bayes' rule to calculate the posterior probability of each  $K$  from the average natural logarithm of the probability of the data at each  $K$  across replicates. We examined the average  $\ln \Pr(X|K)$  across values of  $K$  as well as the individual assignment probabilities at  $K$  with the highest posterior probability to determine the value of  $K$  that best fit these data.

Finally, we 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 predefined classes of geographic distance. To determine whether evidence of autocorrelation existed for either species at fine scales, we examined spatial autocorrelation ( $r_c$ ) at 2-km distance classes from 2 to 40 km. We 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), we 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  $r_c$  for the data did not include  $r_c = 0$ .

#### Standardized genetic differentiation

One goal of comparative studies of genetic differentiation is to understand how geographic genetic structure varies across taxa and across environments. Comparisons across taxa are complicated by differences in the geographic scale and levels of polymorphism at genetic markers that are available for each taxon. We compared genetic differentiation in *A. cognatus* and *S. couchii* to

data for two temperate amphibians from the northeastern United States using a standardized measure of differentiation (Meirmans 2006). The maximum value of genetic differentiation metrics such as  $F_{ST}$  and  $G_{ST}$  is constrained by overall observed heterozygosity of the genetic markers used, such that the maximum possible measure of differentiation decreases with increasing heterozygosity (Hedrick 1999, 2005). We obtained genotypic data for 29 populations of the spotted salamander (*Ambystoma maculatum*) in Tompkins County, New York, USA spanning distances of 1.6–47.1 km (Zamudio & Wicczorek 2007) and for 11 populations of the bullfrog (*Lithobates catesbeianus*) in Ontario, Canada spanning 0.7–50.9 km (Austin *et al.* 2004) and computed a standardized measure of genetic differentiation,  $F_{ST}'$ , (Hedrick 2005; Meirmans 2006) to compare the estimates of differentiation across species. Following the methods described in Meirmans (2006), we used the program RECODE to alter each of the four data sets so that every population had a unique set of alleles and used FSTAT 2.9.3 (Goudet 1995) to calculate the maximum genetic differentiation ( $F_{STmax}$ ) 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 estimated pairwise values of  $F_{ST}$  from the original data sets by the corresponding  $F_{STmax}$ . Negative values of standardized differentiation in pairwise comparisons were set to zero (Meirmans 2006). For each species, we used RMA regression to examine the relationship between pairwise natural logarithm of geographic distance ( $d$ ) and the standardized  $F_{ST}'$ . We used 10 000 permutations of the Mantel test implemented in IBDWS to determine whether the slope of the regression for each species was significantly greater than zero.

We used two methods of bootstrap replication to estimate the 95% CI around the slope and intercept of the RMA regression for each species (Bohonak 2002). The first method constructed each of 10 000 bootstrap replicates by drawing randomly from all population pairs with replacement. The second, more conservative approach, constructed bootstrap replicates by only considering unique and nonoverlapping population pairs such that each data set contained  $p/2$  or  $(p - 1)/2$  populations pairs for even and odd numbers of populations,  $p$ , respectively. Bootstrap replicates were performed in R (R Development Core Team 2009) using the package LMODEL2 (Legendre 2008) to calculate the slope and intercept of the RMA regression. We considered the slopes of the RMA regressions to be significantly different from one another if the 95% CI did not overlap.

We also compared the geographic scales of genetic differentiation inferred for species in this study to those reported in other temperate amphibians. We surveyed

the literature for studies of temperate, pond-breeding amphibians that 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, we recorded overall  $F_{ST}$ ,  $H_E$ , and the range of geographic distances between sampled localities; for two studies, we broke down the data set to compare localities within low elevation sites (Funk *et al.* 2005; Giordano *et al.* 2007). When overall genetic differentiation estimates were not reported, we computed mean pairwise  $F_{ST}$  and the mean  $H_E$  (weighted by population sample sizes) across all localities; for studies where locality-specific values were not included, we used the range of values reported rather than a single value. The range of geographic distances among sampled sites was taken directly from published studies, estimated using coordinates published therein, or obtained from the authors (Table 3).

To estimate standardized  $F_{ST}'$ , original  $F_{ST}$ -values must be divided by  $F_{STmax}$  calculated from the raw data set using the method described above. Because we did not have the genotypic data for the studies surveyed from the literature, we verified that  $H_E$  was a good predictor of  $F_{STmax}$  by using linear regression to examine the relationship between direct estimates of  $H_E$  and  $F_{STmax}$  for the four species for which we had genotypic data sets (*A. cognatus*, *S. couchii*, *A. maculatum*, and *L. catesbeianus*). We then used this linear model to extrapolate  $F_{STmax}$  from published values of  $H_E$  for each study from our comparative survey. We divided the overall or the mean pairwise  $F_{ST}$  by the estimated  $F_{STmax}$  to obtain an overall  $F_{ST}'$  for each species; in instances where a range of  $H_E$  and/or  $F_{ST}$  were available rather than a single estimate, we calculated the range of possible  $F_{ST}'$ -values.

## Results

### Microsatellite data

We did not find any significant variation in population genotypic frequencies across years for localities RV, SCD and ML and therefore combined samples across years for those sites ( $P$ -value for pairwise comparison ranged from 0.165 to 0.416). Genotypic frequencies within *Anaxyrus cognatus* populations differed significantly from expectations under HWE in only three of 183 estimates after sequential Bonferroni correction for multiple comparisons ( $P < 0.00029$  at  $\alpha = 0.05$ ). For *Scaphiopus 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 loci for *A. cognatus* and

*S. couchii* were at equilibrium and not biased by null alleles. Only a single pair of loci (BC52.12 and BC60.20) showed evidence of LD after Bonferroni corrections for *A. cognatus*. However, these loci showed evidence of disequilibrium at one site (WL) suggesting that factors other than linkage might explain this pattern. We did not find evidence of LD in pairwise comparisons of loci within populations for *S. couchii* indicating that these markers were segregating independently.

The microsatellite loci had an average of 20.8 alleles/locus in *A. cognatus* (range 7–43) and 14.6 alleles/locus in *S. couchii* (range 4–46). 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 12 genes in HP-RARE (Kalinowski 2005). Mean allelic richness was similar across all ponds for each species. Allelic richness ranged from 3.55 to 9.19 alleles/locus (mean = 6.54) for *A. 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 *A. cognatus* than *S. couchii* and ranged from 0.069 to 0.546 alleles/locus (mean = 0.247) and from 0.141 to 0.302 alleles/locus (mean = 0.209) respectively. Similarly, average  $H_E$  was higher for populations of *A. cognatus* (weighted average = 0.819, range = 0.774–0.843) compared to *S. couchii* (weighted average = 0.709, range = 0.667–0.729).

Mean pairwise relatedness for the tadpole samples of *S. couchii* was not significantly greater than expected for MR ( $P_{MR} = 0.454$ ) and only weakly significant for DY ( $P_{DY} = 0.052$ ) under a null hypothesis of equal relatedness among all individuals from all sampled localities. Given these results, we assumed that these samples were reflective of adult allele frequencies and included them in subsequent analyses using the full data sets.

#### Genetic diversity and differentiation

Estimates of genetic differentiation among ponds were generally low for both *A. cognatus* and *S. couchii* (*A. cognatus*:  $F_{IS} = 0.057 \pm 0.024$ ,  $F_{ST} = 0.006 \pm 0.001$ ,  $F_{IT} = 0.062 \pm 0.024$ ; *S. couchii*:  $F_{IS} = 0.033 \pm 0.019$ ,  $F_{ST} = 0.003 \pm 0.001$ ,  $F_{IT} = 0.036 \pm 0.018$ ). Omitting localities with small sample sizes (including DY with weakly significant larval relatedness) did not alter the results for *A. cognatus* and only did so slightly for *S. couchii* ( $F_{IS} = 0.035 \pm 0.022$ ,  $F_{ST} = 0.003 \pm 0.002$ ,  $F_{IT} = 0.038 \pm 0.022$ ). Tests of pairwise differentiation among sampling localities were significant for 22 of 91 comparisons for *A. cognatus* and two of 66 comparisons for *S. couchii* (Table 1). For *A. cognatus*, significant estimates of pairwise  $F_{ST}$  between sampling localities within the San Simon/San Bernardino Valleys ranged from 0.006 to

0.017 (mean = 0.0097) and pairwise estimates for sampling localities from different valleys were 0.007–0.033 (mean = 0.0155). For *S. couchii*, we found significant differentiation between only two pairs of localities from different valleys (WX & ML,  $F_{ST} = 0.012$ ; WX & DY,  $F_{ST} = 0.023$ ; Table 1).

Despite the low levels of differentiation overall, we found statistical support for a pattern of isolation by distance in both *A. cognatus* and *S. couchii*. The natural logarithm of pairwise geographic distance was correlated with genetic differentiation [ $F_{ST}/(1 - F_{ST})$ ] for both species and although the slopes were relatively shallow, both were significantly greater than zero in Mantel tests (Fig. 2).

#### Individual-based estimates of genetic differentiation

Bayesian assignment methods in STRUCTURE (Pritchard *et al.* 2000; Falush *et al.* 2003) did not reveal any further population structure than did the pairwise population  $F_{ST}$ -estimates. For *S. couchii*, the highest posterior probability was for  $K = 1$  and examination of the  $\ln L$  scores and individual assignment plots confirm this finding. For *A. cognatus*, we inferred  $K = 2$ , however, the average  $\ln L$  score at  $K = 2$  (–20936.39) was only marginally greater than the  $\ln L$  score for  $K = 1$  (–20950.0). The individual assignment probability plot at  $K = 2$  for *A. cognatus* shows that nearly all individuals are admixed (Fig. 3) indicating no true geographic structure.

Tests of spatial autocorrelation at 2-km distance class intervals did not support genetic structure at fine spatial scales for either species corroborating the STRUCTURE results. Mean  $r_c$  were not significantly different from zero or permuted values under the null hypothesis of no spatial autocorrelation (Fig. 4) and following the criteria outlined by Peakall *et al.* (2003).

#### Comparisons among temperate amphibians

Overall standardized population differentiation ( $F_{ST}'$ ) was higher in *A. cognatus* than *S. couchii*, corroborating the trend observed in direct estimates of  $F_{ST}$ . Both *Ambystoma maculatum* and *Lithobates catesbeianus* showed the values of standardized  $F_{ST}'$  (Table 2), that were approximately an order of magnitude greater than those for *A. cognatus* and *S. couchii*. Both desert species still showed a pattern of isolation by distance when using the standardized index  $F_{ST}'$  (*A. cognatus*:  $F_{ST}' = -0.260 + 0.030d$ ,  $P = 0.028$ ; *S. couchii*  $F_{ST}' = -0.171 + 0.019d$ ,  $P = 0.028$ ). Standardized genetic differentiation was positively correlated with the natural logarithm of distance for *A. maculatum* ( $F_{ST}' = -1.566 + 0.186d$ ,  $P < 0.001$ ) and for *L. catesbeianus* ( $F_{ST}' = -0.604 + 0.076d$ ,

**Table 1** Pairwise estimates of  $F_{ST}$  for *Anaxyrus cognatus* and *Scaphiopus couchii* across sampling localities above the diagonal and pairwise geographic distances (km) among populations below the diagonal.  $F_{ST}$  significantly different from zero after Bonferroni correction at  $\alpha = 0.05$  are indicated in bold

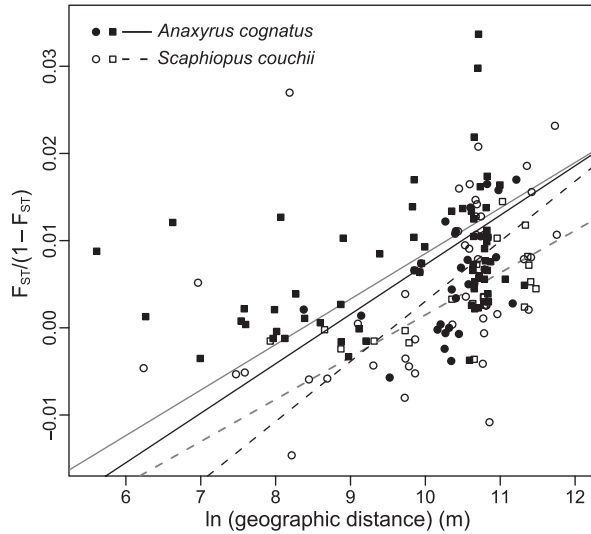
	KU	DC	BA	CL	ML	JV	WL	SCD	SCB	RV	SR	M4	M8	DG
<i>Anaxyrus cognatus</i>														
KU	—	0.006	<b>0.011</b>	<b>0.033</b>	<b>0.013</b>	0.006	<b>0.007</b>	<b>0.010</b>	<b>0.021</b>	<b>0.012</b>	0.007	0.014	0.005	0.002
DC	20.4	—	<b>0.014</b>	<b>0.029</b>	<b>0.016</b>	0.008	<b>0.014</b>	<b>0.010</b>	0.017	<b>0.010</b>	0.004	0.016	0.008	0.006
BA	50.0	36.1	—	0.013	0.007	0.008	<b>0.006</b>	<b>0.009</b>	0.007	<b>0.007</b>	<b>0.003</b>	<b>0.017</b>	0.003	0.005
CL	44.7	44.3	31.2	—	0.008	0.014	<b>0.017</b>	<b>0.010</b>	0.007	<b>0.007</b>	<b>0.009</b>	<b>0.016</b>	0.010	<b>0.016</b>
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	—
	WX	BA	MQ	ML	TM	CR	WL	E4	W4	DG	DY	MR		
<i>Scaphiopus couchii</i>														
WX	—	0.014	0.008	<b>0.012</b>	0.018	0.008	0.007	0.008	0.005	0.005	<b>0.023</b>	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	—		

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 I.

$P = 0.049$ ). For the bootstrapping test including all populations, the two-tailed 95% CI for the slopes of the regressions were nonoverlapping for all species pairs except *A. cognatus* and *S. couchii* (Fig. 5). In the more conservative, individual pairs bootstrap approach, no two species were significantly different from one another using the 95% CI (Fig. 5), but the trends for slopes of the four species were in the same direction. The 95% CI for *A. cognatus* and *S. couchii* were broadly overlapping between the two desert-adapted species independent of the bootstrap approach. These results suggest that at any given geographic distances *A. maculatum* is more differentiated than *L. catesbeianus* and those two mesic species show higher differentiation than *A. cognatus* and *S. couchii*.

We found a tight correlation between  $H_E$  and  $F_{STmax}$  for the four species data sets in this study ( $R^2 = 0.99$ ;  $P = 0.0024$ ;  $F_{STmax} = 1.016-1.042 H_E$ ) justifying the use

of this relationship to approximate  $F_{STmax}$  for other amphibian species. Approximations of  $F_{ST}'$  for published studies of population genetic differentiation in amphibians ranged from 0.018 to 0.667 (Table 3). The lowest and highest values in this range were measured for populations of *Rana 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 3). Among the remaining studies,  $F_{ST}'$  ranged from 0.023 in *Rana luteiventris* at fine scales (0.1–1.6 km) to 0.537 in *Bufo bufo*, also at fine scales (5.5–14.4 km). Overall, the levels of genetic differentiation in many amphibian taxa surveyed were high in comparison to *A. cognatus* and *S. couchii* (Table 3). Only five 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 & Blouin 2004), *R. luteiventris* (Funk *et al.* 2005), *R. temporaria*

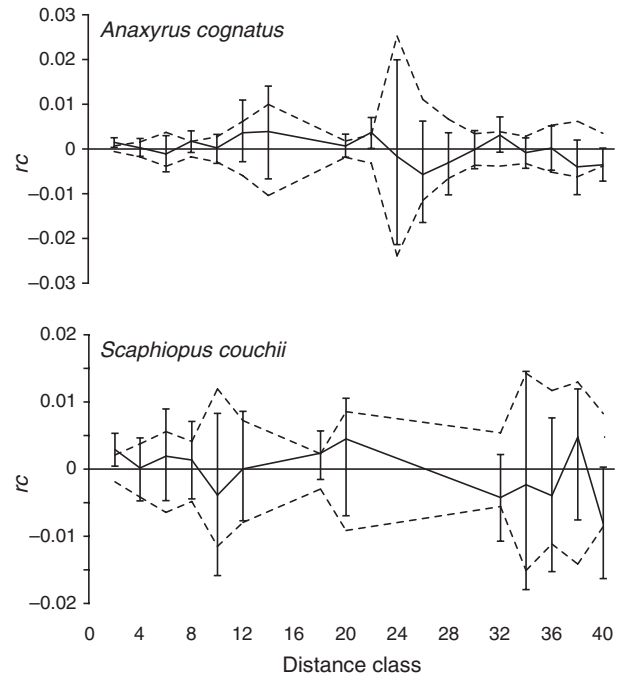


**Fig. 2** Reduced major axis regression of pairwise genetic divergence and natural logarithm of geographic distance for complete data sets (all points) and data sets including only populations with more than 15 samples (squares). *Anaxyrus cognatus* (filled symbols, solid regression lines): complete data set  $F_{ST}/(1 - F_{ST}) = -0.0495 + 0.0057 [\ln(\text{distance})]$ ,  $P = 0.025$ ; reduced data set  $F_{ST}/(1 - F_{ST}) = -0.0436 + 0.0052 [\ln(\text{distance})]$ ,  $P = 0.031$ . *Scaphiopus couchii* (open symbols, dashed regression lines): complete data set  $F_{ST}/(1 - F_{ST}) = -0.0658 + 0.0069 [\ln(\text{distance})]$ ;  $P = 0.011$ , reduced data set  $F_{ST}/(1 - F_{ST}) = -0.0469 + 0.0048 [\ln(\text{distance})]$ ;  $P = 0.008$ .

(Johansson *et al.* 2006), and *L. sylvaticus* (Newman & Squire 2001). These taxa showed  $F_{ST}'$ -values  $\leq 0.05$ ; however, these studies focused primarily at spatial scales of 20 km or less.

**Discussion**

*Anaxyrus cognatus* and *Scaphiopus couchii* show lower genetic differentiation, at equal or larger spatial scales, than many of the species we surveyed, suggesting that arid environments may be more conducive to amphibian dispersal than previously believed. Our results do not exclude the possibility that extrinsic factors such as post-Pleistocene range expansion into the desert southwest or response to more recent habitat alteration may



**Fig. 4** Spatial autocorrelation at 2-km distance classes for *Anaxyrus cognatus* (top) and *Scaphiopus couchii* (bottom). Solid lines are the mean and 95% CI generated with 999 bootstrap replicates and dashed lines are the upper and lower 95% CI for the null distribution of no spatial genetic structure generated by 999 permutations of the data across distance classes.

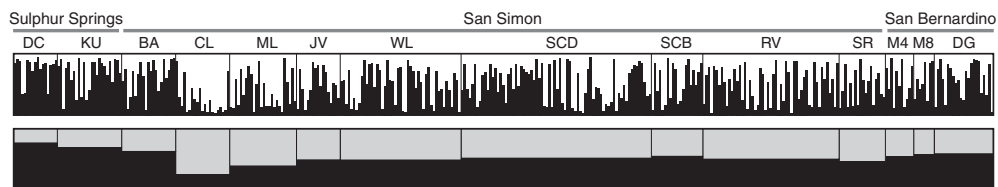
**Table 2** Expected heterozygosity ( $H_E$ ) and mean  $F_{ST}$  and  $F_{STmax} \pm SE$  after jackknifing over samples and loci within each species

Species	$H_E$	$F_{ST}$	$F_{STmax}$	$F_{ST}'$
<i>Anaxyrus cognatus</i>	0.819	0.006 $\pm$ 0.001	0.165 $\pm$ 0.032	0.036
<i>Scaphiopus couchii</i>	0.709	0.003 $\pm$ 0.001	0.274 $\pm$ 0.061	0.011
<i>Ambystoma maculatum</i> *	0.657	0.073 $\pm$ 0.010	0.339 $\pm$ 0.020	0.215
<i>Lithobates catesbeianus</i> †	0.670	0.041 $\pm$ 0.009	0.314 $\pm$ 0.070	0.131

Standardized genetic differentiation ( $F_{ST}'$ ) is calculated as  $F_{ST}/F_{STmax}$ .

\*Zamudio & Wieczorek (2007).

†Austin *et al.* (2004).



**Fig. 3** Genetic membership from Bayesian assignment tests in STRUCTURE for *Anaxyrus cognatus* individuals (top) and populations (bottom). Collection localities are delineated by thin vertical black lines; locality names correspond to those listed in Appendix I and Fig. 1; horizontal grey bars unite localities within the same desert valley.



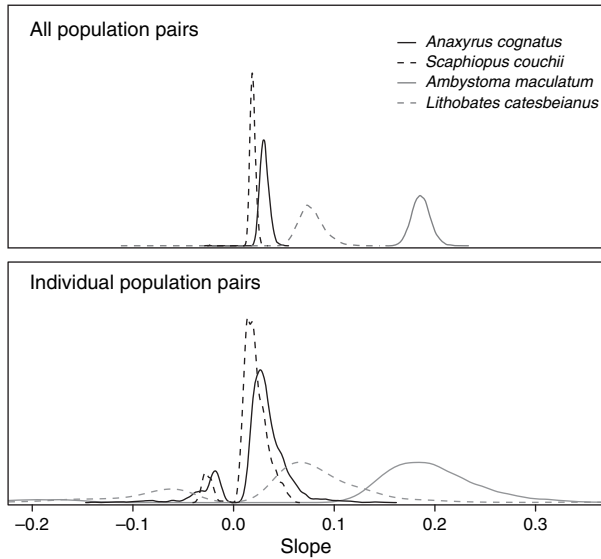


Fig. 5 Sampling distributions for slopes from RMA regressions of bootstrap replicates. Bootstrap analyses including all population pairs (top) and only independent population pairs (bottom).

play a role in the population genetic structure of these two species. Phylogeographic studies of *Anaxyrus woodhousii* (Masta *et al.* 2003) and *Spea bombifrons* (Rice & Pfennig 2008) based on mitochondrial DNA sequence data have inferred range expansion into the southwestern deserts from the Great Plains. Based on vegetation and climate reconstructions (Martin & Mehringer 1965; van Devender & Spaulding 1979; Holmgren *et al.* 2003) and fossil remains (Holman 1995), *A. cognatus* and *S. couchii* were likely absent from the sampled region as recently as 11 000–15 000 ybp and thus, may also be relatively recent colonizers. However, we find high allelic diversity at microsatellite loci in addition to widespread genetic connectivity indicating that historical processes are insufficient to explain completely the pattern we observed in these arid-adapted species. In other species of temperate amphibians that have recently expanded to occupy their current ranges, there is still evidence of local population differentiation due to restricted population connectivity (e.g. Palo *et al.* 2004; Pabijan & Babik 2006). This indicates that the low genetic structure we see in *A. cognatus* and *S. couchii* reflects current patterns of gene flow and not simply recent historical range expansions.

Contemporary anthropogenic changes to the landscape associated with agriculture may increase the density of breeding ponds and contribute to the overall pattern we find here. However, increased breeding sites should only translate to such pronounced genetic connectivity if individuals have low site fidelity and a high propensity for dispersal. The low population genetic differentiation we see in *A. cognatus* and *S. couchii*

indicates pronounced inter-pond connectivity. Our results contradict our general expectations based on the ecological features of temperate amphibian species, but they do agree with the expectations for population connectivity and life-history evolution in cases where resource availability is unpredictable (see Roff 2002; Ronce 2007). Gene flow in these species may be maintained as the combined result of physiological and behavioural adaptations to an arid environment, landscape homogeneity and reliance on a spatiotemporally stochastic resource for reproduction.

#### Desert amphibians

Although patterns of genetic structure in the two desert anurans we surveyed are not identical, both species maintain the high levels of genetic diversity with limited population divergence at this spatial scale. Widespread genetic connectivity among populations of each of these two desert species is in stark contrast to predictions based on our understanding of anuran biology. We do not expect deserts to be conducive to movement in organisms that rely on moist environments for survival and aquatic habitats for reproduction. However, physiological adaptations of both *A. cognatus* and *S. couchii* to arid environments, presumably evolved due to the selective advantage of summer breeding and foraging activities and a long aestivation period, may contribute to extensive population connectivity. Both species have high dehydration tolerances (McClanahan 1964, 1972; Hillman 1980) and high aerobic capacities (Hillman 1976) in comparison to other temperate anurans. A consequence of these adaptations is an increased capacity for prolonged activity through long stretches of arid habitats.

Substantial gene flow in desert anurans may also be facilitated by the habitat homogeneity of the desert valley landscape. Habitat complexity and heterogeneity contribute to isolation due to increased barriers to gene flow (Wiens 2001) and not surprisingly, landscape features correlate with patterns of fine-scale genetic structure in many taxa (e.g. Hedgecock 1978; Hitchings & Beebee 1997; Funk *et al.* 2005; Spear *et al.* 2005; Giordano *et al.* 2007; Storfer *et al.* 2007). The creosote and mesquite dominated valley floors where *A. cognatus* and *S. couchii* occur lack the topographic relief and heterogeneity that typically restrict gene flow. Thus, population connectivity in *A. cognatus* and *S. couchii* may be maintained via dispersal among ponds according to a stepping stone model of migration, as suggested by the pattern of isolation by distance we find here. Populations may be connected at large geographic distances and across valleys through high and continuous levels of gene flow among neighbouring populations. Indeed,

**Table 3** Summary of studies of population differentiation in temperate amphibians

Species	Scale (km)	$F_{ST}$	$H_E$	$F_{ST}'$	Ref.
<b>Anura</b>					
Family Bufonidae					
<i>Anaxyrus cognatus</i>	0.3–82.5	0.006	0.819	0.036	1
<i>Bufo bufo</i>	5.5–14.4	0.016	0.683	0.053	2
	5.5–14.4	0.032	0.527	0.068	2
	2.5–12	0.222*	0.579†	0.537	3
<i>Epidalea calamita</i>	2.0–3.6	0.06	0.242–0.376	0.079–0.096	4
	2.0–16.0	0.224	0.242–0.376	0.293–0.359	4
	0.5–9.0	0.111	0.242–0.376	0.145–0.178	4
Family Hylidae					
<i>Litoria aurea</i>	5–80	0.034	0.779†	0.166	5
Family Ranidae					
<i>Lithobates catesbeianus</i>	0.7–50.9	0.041	0.67	0.131	1, 6
<i>Lithobates sylvaticus</i>	< 0.5–20	0.014	0.44–0.50	0.025–0.028	7
<i>Rana arvalis</i>	0.3–7.6	0.052	0.378	0.084	8
	0.3–150	0.065	0.384	0.105	8
<i>Rana cascadae</i>	1–23	0.010–0.260	0.450–0.730	0.018–0.356	9
	26–670	0.040–0.520	0.450–0.780	0.073–0.667	9
<i>Rana luteiventris</i>	0.4–4.6‡	0.059*	0.571†	0.139	10
	0.4–6.2‡	0.015*	0.653†	0.046	10
	0.1–1.6‡	0.012*	0.453†	0.023	10
	1.2–3.1‡	0.092*	0.314†	0.134	10
<i>Rana temporaria</i>	2.5–12	0.050*	0.669†	0.156	3
	5–20	0.018–0.051	0.48–0.67	0.047–0.146	11**
Family Scaphiopodidae					
<i>Scaphiopus couchii</i>	0.5–127	0.003	0.709	0.011	1
<b>Caudata</b>					
Family Ambystomatidae					
<i>Ambystoma macrodactylum</i>	0.4–31.2‡	0.035*	0.664†	0.108	12
<i>Ambystoma maculatum</i>	1.6–47.1	0.073	0.657	0.215	1, 13
	<0.02–55.6	0.041	0.692	0.139	14
<i>Ambystoma tigrinum melanostictum</i>	0.5–50	0.240	0.320†	0.255	15, 16
Family Salamandridae					
<i>Triturus cristatus</i>	0.4–5.9	0.07	0.611†	0.184	17
<i>Triturus marmoratus</i>	0.2–6.4	0.11	0.448†	0.200	17

1. This study; 2. Scribner *et al.* 1994; 3. Brede & Beebe 2004; 4. Rowe *et al.* 2000; 5. Burns *et al.* 2004; 6. Austin *et al.* 2004; 7. Newman & Squire 2001; 8. Vos *et al.* 2001; 9. Monsen & Blouin 2004; 10. Funk *et al.* 2005; 11. Johansson *et al.* 2006; 12. Giordano *et al.* 2007; 13. Zamudio & Wicczorek 2007; 14. Purrenhage *et al.* 2009; 15. Spear *et al.* 2005; 16. S. Spear, personal communication; 17. Jehle *et al.* 2005.

\* $F_{ST}$  calculated as the average of all pairwise values.

† $H_E$  calculated as the weighted average across populations.

‡Distances calculated from published values.

\*\*Range of  $F_{ST}$ ,  $H_E$ , and  $F_{ST}'$  across nine subregions reported for this study.

studies examining amphibians at low and high elevations have found greater connectivity among sites in valleys compared to sites in mountainous regions (e.g. Funk *et al.* 2005; Giordano *et al.* 2007; Zamudio & Wicczorek 2007).

Finally, the habitat requirements for reproduction in these desert environments may result in selection for dispersal across breeding years. *Anaxyrus cognatus* and *S. couchii* are dependent on ephemeral ponds that fill with summer rains and avoid breeding in permanent pond with higher densities of predators (Bragg 1940b;

Woodward 1983). Breeding at ephemeral sites decreases mortality due to predation thereby increasing the chance of larval survivorship; however, the distribution of these habitats is unpredictable from year to year. Adults of both species breed for multiple years (Rogers & Harvey 1994; Tinsley & Tocque 1995; Sullivan & Fernandez 1999), but ponds do not reliably fill year after year (Anderson *et al.* 1999; Chan 2007c). The lack of genetic structure in *A. cognatus* and *S. couchii* indicates that the selective environment for these desert amphibians differs from that experienced by mesic species;

desert-adapted species may experience negative selection for philopatry or site fidelity, and greater selection for continued individual dispersal and/or higher survival/reproductive success in postdispersal life stages.

#### Differences between *A. cognatus* and *S. couchii*

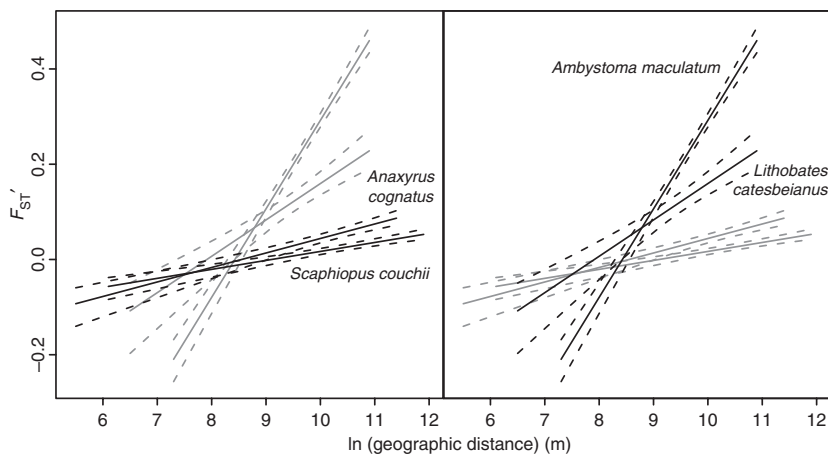
Observed differences in the patterns of genetic differentiation between our two focal desert species suggest that species characteristics might contribute to the levels of gene flow and the maintenance of genetic diversity. We did not detect significant genetic structure at fine scales for either species, however, *A. cognatus* populations are structured within the San Simon/San Bernardino Valleys as well as between these valleys and the Sulphur Springs Valley at distances >18 km. In contrast, *S. couchii* showed significant differentiation among valleys, but not within valleys. While we are unable to say with certainty that gene flow is unequal between these species, differences in ecology and behaviours that influence movement are congruent with this observed pattern of genetic structure. Increased dispersal ability and increased propensity of individuals to move large distances are possible explanations for the slightly lower estimates of standardized population differentiation among *S. couchii* populations in comparison to *A. cognatus*. Although both species are adapted to xeric conditions relative to other temperate amphibians, *S. couchii* is the most desert-adapted species of the two. The dehydration tolerance of *S. couchii* is higher than that of *A. cognatus* (McClanahan 1972; Hillman 1980) and these physiological differences may contribute to the slight differences in genetic structure.

Our two focal species also differ in the probability of successful reproduction following dispersal (i.e. gene flow) because of differences in mating system and life-history traits. Both species are explosive aggregate

breeders (Wells 1977) with mating activity at a particular pond occurring in one to three consecutive nights. The ephemeral ponds these desert species utilize for breeding can evaporate quickly and this may influence the opportunities for breeding for each species. Because *S. couchii* has an extremely short larval development time (8–13 days; Mayhew 1965), they can breed in a wide variety of water-bodies from shallow puddles to larger ponds. In contrast, *A. cognatus* is constrained to breeding only in larger ponds because of a longer larval period (25–45 days; Bragg 1940a). Limited breeding opportunities due to site availability may be compounded by low probabilities for individual reproductive success. *Anaxyrus cognatus* typically has a more highly male-biased sex ratio at mating ponds, such that many males may not mate successfully in any given year; likewise, their longer larval period may translate to higher probabilities of offspring mortality.

#### Comparisons of *A. cognatus* and *S. couchii* with other temperate amphibians

After standardizing for overall levels of genetic diversity, the genetic differentiation ( $F_{ST}$ ) in *A. cognatus* and *S. couchii* remains approximately an order of magnitude lower than those for bullfrogs (*Lithobates catesbeianus*) and spotted salamanders (*Ambystoma maculatum*) at comparable spatial scales (Table 2; Fig. 6). *Lithobates catesbeianus* and *A. maculatum* are also pond-breeding amphibians, but they inhabit temperate deciduous forests. Although it seems counterintuitive, our data suggest that movement by amphibians in some mesic environments is more restricted than in arid deserts. The difference between the desert and mesic species is most pronounced at moderate and large spatial scales (>5 km; Fig. 6) and it is at these scales that the low differentiation in *A. cognatus* and *S. couchii* is most remarkable.



**Fig. 6** Reduced major axis regression between natural logarithm of geographic distance and standardized genetic differentiation for four species of amphibians. Confidence intervals (95%) were generated by 10 000 bootstrap replicates across all population pairs with replacement. Both panels contain the same for regressions with desert species emphasized in black in the left panel and the mesic species emphasized in the right panel.

Our survey of the literature for temperate, pond-breeding amphibians corroborates our empirical results; many temperate mesic-adapted species have higher levels of  $F_{ST}'$  at smaller spatial scales than either *A. cognatus* or *S. couchii* (Table 3). Several taxa do show very low measures of  $F_{ST}'$  at small spatial distances of several kilometres such as *Rana luteiventris* for comparisons of populations up to 6.2 km apart and *Bufo bufo* across 5.5–14.4 km of arable pasture (Table 3). *Lithobates sylvaticus* populations inhabiting prairie wetlands were the only taxon surveyed to exhibit low levels of population genetic differentiation at 20 km ( $F_{ST}' \sim 0.025$ ). Although these species studies do not demonstrate low differentiation at greater spatial scales, they suggest that at local scales, montane valleys, pastures, and prairie habitats may be conducive to among pond movement. Thus, landscape homogeneity may be a general feature that contributes to the high levels of gene flow and connectivity in a number of amphibians, not just desert species (Newman & Squire 2001). Future research focusing within species and comparing patterns of fine-scale genetic differentiation among localities with different habitat characteristics will be especially informative about the importance of environmental variation to local adaptation and population genetic structure.

We find similar levels of genetic structure across distantly related taxa suggesting that these ecological factors, rather than shared evolutionary history, are more likely to contribute to these patterns of differentiation. Although we explicitly compare levels of divergence among the four species for which we have genetic data, it is important to recognize that our broader comparison is descriptive and not a statistical test of among species variation in genetic diversity or population genetic structure. As more fine-scale population genetic data become available, studies that formally test for differences among species and incorporate corrections for phylogenetic effects will help us identify specific organismal traits and aspects of the environment that shape population genetic structure.

The genetic diversities of these two desert anurans challenge our view of amphibian population structure and suggest that desert environments may permit and perhaps even favour higher degrees of dispersal and gene flow despite temporally restricted activity periods and harsh xeric conditions. We expected that the loss of genetic diversity within ponds due to reproductive skew and environmental stochasticity could result in more pronounced genetic structure, but these effects are mitigated by gene flow and possibly also by overlapping generations (Nunney 1993). Simulation-based models have been useful in determining the influence of reproductive failure on the population persistence of marbled salamanders (*Ambystoma 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 dynamics and environmental stochasticity. Characterizing the distribution of genetic diversity among populations of desert amphibians contributes to a more complete perspective on how organismal ecology and local adaptation interact to shape population genetic structure, and ultimately, patterns of speciation in a diversity of habitats.

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This study was part of L.M.C.'s PhD thesis that focused on the consequences of organismal ecology to spatial and temporal patterns of genetic diversity in amphibians and reptiles. L.M.C. is currently a postdoctoral researcher at Duke University studying the phylogeography and population genetics of Malagasy vertebrates. Current research in the Zamudio Laboratory focuses on systematics, population and conservation genetics, and the evolution of mating systems in amphibians and reptiles.

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## Appendix I

Collection locality names, abbreviation, latitude and longitude. The sample sizes of *Anaxyrus cognatus* ( $N_{Ac}$ ) and *Scaphiopus couchii* ( $N_{Sc}$ ) are given for each locality

Locality name	Abbreviation	Latitude	Longitude	$N_{Ac}$	$N_{Sc}$
Sulphur Springs Valley					
Dos Cabezas	DC	32.05821499	-109.4964261	17	0
Kuykendall Road	KU	31.87526800	-109.4913286	25	0
Willcox Ditch	WX	32.25192419	-109.8289155	0	21
San Simon Valley					
Barnes Road	BA	32.23626758	-109.1761260	21	20
Corner Pond II	CR	31.86209681	-109.0390503	0	24
Culvert 12	CL	31.98250280	-109.0357943	21	0
E409 Pond	E4	31.72378894	-109.1063836	0	12
Javelina Pond	JV	31.81714348	-109.0187584	17	0
Mesquite Pond	MQ	31.89328926	-109.0715750	0	12
Miller Pond	ML	31.87766309	-109.0618377	26	71
River Pond	RV	31.79638000	-109.0580300	53	0
Sky Ranch 80 Pond	SR	31.78843808	-109.0646844	18	0
Sulphur Canyon Bufo Pond	SCB	31.81238896	-109.0519215	20	0
Sulphur Canyon Ditch	SCD	31.81394728	-109.0541386	74	0
Tomberlin Pond	TM	31.86631838	-109.0490225	0	9
Willow Ponds	WL	31.81386963	-109.0596753	47	19
W409 Pond	W4	31.72481399	-109.1116287	0	20
San Bernardino Valley					
Dangerous Ditch	DG	31.50529942	-109.3174678	23	32
Mile 394 Pond	M4	31.57302125	-109.2592193	11	0
Mile 398 Pond	M8	31.60272379	-109.2295881	8	0

**Appendix I** *Continued*

Locality name	Abbreviation	Latitude	Longitude	$N_{Ac}$	$N_{Sc}$
Animas Valley					
Day Pond	DY	31.49881517	-108.8638295	0	13
Marsh Pond	MR	31.46811251	-108.8525518	0	6