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Citation	Mims, M. C., Phillipsen, I. C., Lytle, D. A., Kirk, E. E. H., & Olden, J. D. (2015). Ecological strategies predict associations between aquatic and genetic connectivity for dryland amphibians. <i>Ecology</i> , 96(5), 1371-1382. doi:10.1890/14-0490.1.sm
DOI	10.1890/14-0490.1.sm
Publisher	Ecological Society of America
Version	Version of Record
Terms of Use	http://cdss.library.oregonstate.edu/sa-termsfuse

Ecological strategies predict associations between aquatic and genetic connectivity for dryland amphibians

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Abstract. The study of how population genetic structure is shaped by attributes of the environment is a central scientific pursuit in ecology and conservation. But limited resources may prohibit landscape genetics studies for many threatened species, particularly given the pace of current environmental change. Understanding the extent to which species' ecological strategies—their life histories, biology, and behavior—predict patterns and drivers of population connectivity is a critical step in evaluating the potential of multi-taxa inference in landscape genetics. We present results of a landscape genetic study of three dryland amphibians: the canyon treefrog (*Hyla arenicolor*), red-spotted toad (*Anaxyrus punctatus*), and Mexican spadefoot (*Spea multiplicata*). These species characterize a range of ecological strategies, driven primarily by different water dependencies, enabling amphibian survival in arid and semiarid environments. We examined a suite of hypothesized relationships between genetic connectivity and landscape connectivity across species. We found a positive relationship between population differentiation and water dependency, e.g., longer larval development periods and site fidelity for reliable water sources. We also found that aquatic connectivity is important for all species, particularly when considered with topography (slope). The effect of spatial scale varied by species, with canyon treefrogs and Mexican spadefoots characterized by relatively consistent results at different scales in contrast to the stark differences in results for red-spotted toads at different scales. Using ecological information to predict relationships between genetic and landscape connectivity is a promising approach for multi-taxa inference and may help inform conservation efforts where single-species genetic studies are not possible.

Key words: *anurans; canyon treefrog (Hyla arenicolor); dryland ecology; landscape genetics; life histories; Mexican spadefoot (Spea multiplicata); red-spotted toad (Anaxyrus punctatus); traits.*

INTRODUCTION

Mounting concern for the survival of species in human-modified environments has strengthened interest in how species biology and landscape heterogeneity interact to structure populations. Population genetic structure is a fundamental consideration in applied conservation (Allendorf and Luikart 2007), which has led to greater research emphasis on integrating population genetics with emerging spatial methodologies to elucidate the landscape genetics of species (Manel et al. 2003). Substantial progress has been made in the last decade toward quantifying associations between landscape features and genetic connectivity of single species, and interest in explicitly testing for generalizations across multiple species is increasing (Manel and Holderegger 2013). Multispecies inference may be a promising way to identify emerging patterns of landscape influence on population connectivity across taxa and to reveal transferable relationships according to biological attri-

butes such as dispersal ability (Richardson 2012) and life history (Bradbury et al. 2008, Hughes et al. 2013). Ecological strategies—the life history, biology, and behavior of a species—may provide a means to generalize associations between genetic connectivity and structural connectivity (i.e., the physical linkages between habitat patches via a landscape feature such as riparian networks or ridgelines [Taylor et al. 1993]), or what is commonly considered “landscape connectivity.” The potential for generalized, multi-taxa relationships between genetic and landscape connectivity is important in light of limited management resources or logistical complications that render single species studies unfeasible for many species of conservation concern.

Aquatic habitat supports a range of obligate aquatic species; some species require perennial water for the duration of their life cycle, whereas others require water for only a short period to fulfill a portion of their life cycle. The water requirements, dispersal abilities, and other traits of aquatic species coupled with the distribution (spatial and temporal) of aquatic habitat may be major factors that influence the population structure of a diverse array of species including plants (Imbert and Lefèvre 2003, Nilsson et al. 2010), insects

Manuscript received 11 March 2014; revised 30 July 2014; accepted 7 October 2014; final version received 4 November 2014. Corresponding Editor: T. J. Valone.

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(Hughes et al. 2013, Robertson et al. 2014), fishes (Hughes et al. 2013), amphibians (Richardson 2012), and birds (Kingsford et al. 2004, Cadena et al. 2011). In arid and semiarid (hereafter “dryland”) environments characterized by sparse, sometimes unpredictable, and often temporary, aquatic habitat, the link between aquatic species ecology and aquatic habitat on the landscape in driving population structure may be more pronounced due to the arid terrestrial matrix between aquatic habitats (Finn et al. 2007, Phillipsen and Lytle 2013). Conversely, the need to reach temporally and spatially variable aquatic habitat may decouple the link between the aquatic habitat and population genetic structure for aquatic species with high dispersal ability (Chan and Zamudio 2009, Cadena et al. 2011).

Quantifying genetic connectivity among populations of aquatic animals, and determining how this is influenced by landscape factors such as hydrology, is critical for conservation planning now and in the future. Worldwide threats to water security for society and freshwater diversity require efficient and effective conservation planning (Vörösmarty et al. 2010). In recent decades, amphibians have declined globally as a result of major threats including habitat loss, disease, and nonnative species (Stuart et al. 2004, Sodhi et al. 2008). These ubiquitous challenges for amphibians worldwide are also true of anurans (frogs and toads) in dryland environments. In the American Southwest, habitat loss is an ongoing challenge as limited perennial water is often appropriated for human use, and groundwater pumping results in continued reduction in surface water availability (Marshall et al. 2010, Jaeger et al. 2014). Current demand for and conflict over these resources foreshadows increasing demand and decreasing availability as climate change increases aridity in the region (Seager et al. 2007).

Anurans native to this region utilize a variety of life history and behavioral strategies to survive in a harsh, arid landscape. Some species are specialists that live in either perennial or ephemeral freshwater habitats, and others occupy niches intermediate to these two extremes. If these anurans have dispersal limitations that correspond with their habitat requirements, the patterns of population structure among these species may be as diverse as their ecological strategies related to water use.

In this study, we examine the relationship between genetic and structural connectivity for three dryland anurans common to the American Southwest: the canyon treefrog (*Hyla arenicolor*), the red-spotted toad (*Anaxyrus punctatus*), and the Mexican spadefoot (*Spea multiplicata*). These species represent a range of water dependencies typical of dryland anurans and thus provide a unique opportunity to investigate whether ecological strategies, in this case defined by water requirements, can be used to generalize the associations between genetic and structural connectivity. Although canyon treefrogs have unique behavioral adaptations thought to minimize evaporative water loss, adults

require frequent (possibly daily) access to water (Snyder and Hammerson 1993). The larval period is estimated to range between 6 and 11 weeks (Zweifel 1961, Stebbins 2003). Red-spotted toads are common near intermittent and ephemeral water sources in the Mojave and Sonoran deserts. They show evidence of some site fidelity, returning to similar stream sections and even burrows when displaced (Weintraub 1974). The larval period of red-spotted toads is estimated to last between 4 and 8 weeks (Tevis 1966, Brennan and Holycross 2006). Mexican spadefoots are ephemeral specialists that breed in temporary pools and ponds filled by summer monsoon thunderstorms. Vibrations from rainfall and/or thunder are thought to signal emergence of spadefoot toads that aestivate in burrows deep underground and emerge to breed in ephemeral pools (Dimmitt and Ruibal 1980). The larval period of Mexican spadefoots ranges between 2 and 4 weeks (Buchholz and Hayes 2000).

Our primary objective was to test whether water dependency, a defining element of aquatic species' ecological strategies, provides a means to generalize the likely mechanisms, and hence patterns, of anuran population structure. First, we hypothesized that genetic population connectivity was inversely related to water requirements, the primary driver of species ecology in an environment in which desiccation risk is a considerable threat for aquatic species (Table 1). We also expected that water availability explained a greater degree of population genetic structure (Fig. 1C) as species-specific water requirements increased. Land cover, topography, and geographic distance were expected to have heterogeneous effects across species. We predicted that terrestrial resistance (canopy cover and urban land use) would be most important for canyon treefrogs (Fig. 1B), the species with the highest desiccation risk for which permeability of the matrix between breeding sites may drive genetic connectivity. We also expected that topographic resistance (slope) would limit gene flow for red-spotted toads and Mexican spadefoots, whereas canyon treefrogs are adept climbers and may disperse more easily across complex topography (Fig. 1D). Alternatively, canyon treefrog populations may be so isolated and gene flow so low that genetic drift results in genetically isolated populations (Fig. 1A). Finally, we predicted an isolation-by-distance pattern (Fig. 1E) or panmixia pattern (Fig. 1F) for Mexican spadefoots where gene flow is likely to be diffuse and relatively unimpeded by landscape factors.

METHODS

The study region is the Madrean Sky Islands of southeastern Arizona (USA), characterized by many high mountain ranges separated by arid valley scrubland. The region's landscape is remarkably heterogeneous with large gradients in elevation, water permanence, precipitation, vegetation, and temperature. Our study focused on the Huachuca Mountains and

TABLE 1. Genetic and landscape connectivity hypotheses.

Hypotheses	Species			Description	Species predictions	
	CT	RT	MS		Population genetics patterns	Landscape drivers and genetic connectivity correlation (+/-)
Isolated populations	X			High differentiation; no relationship with distance.	All populations distinct from one another; high population differentiation.	None: local processes (e.g., genetic drift) dominate.
Terrestrial	X			Canopy cover promotes genetic connectivity; development reduces connectivity.	Populations cluster within areas of high canopy cover; distinct populations in developed areas.	Canopy cover (+) and urban land use (-).
Aquatic	X	X		Connectivity correlated with hydrologic network (streams, ponds), precipitation.	Populations cluster along riparian networks and in areas with high precipitation.	Hydrologic network: streams and ponds (+); precipitation (+).
Topography		X	X	Slope (rise over run) negatively correlated with genetic connectivity.	Isolated populations in areas with steep terrain; high connectivity along flat ground.	Topography: slope (-).
Isolation by distance			X	Differentiation correlated with distance.	Population differentiation increases proportionally with distance.	Distance (+).
Panmixia			X	Low differentiation; no relationship with distance.	Little/no population differentiation.	None: migration/connectivity dominate.

Notes: Hypothesized genetic connectivity models for each species (CT, canyon treefrog; RT, red-spotted toad; and MS, Mexican spadefoot) are indicated by X. Hypotheses are visualized in Fig. 1.

surrounding mountain ranges and valleys, including the Santa Rita, Whetstone, Dragoon, and Mule Mountains (see Plate 1). Summer monsoon rains and flash floods in our study region are seasonally predictable but spatially variable. Some areas may receive rain early in the season and stay wet during the entire monsoon while others may receive no rain all season. For these reasons, we used an opportunistic but spatially stratified sampling approach in order to maximize the chance of finding amphibians while attempting to balance the extent of our study, the density of sample locations, and the evenness of sampling locations on the landscape. Adult and larval amphibians were sampled during the spring and summer monsoon seasons of 2010, 2011, and 2012. A single toe clip (adult) or tail clip (larvae) was taken from each individual for DNA extraction and genotyping. Where possible, sampling sites were visited across multiple years in order to maximize the chance of sampling multiple families. Other sites consisted of multiple isolated pools within 1 km of each other to maximize the chance of sampling multiple families. Additional sampling details and effects of sampling strategy are reported in Appendix A.

Genetic methods

Microsatellite marker information, genotyping details, marker screening procedures, and sibling removal procedures for larval samples are provided in Appendix A. Briefly, population genetic diversity estimates of expected heterozygosity (H_E), observed heterozygosity (H_O), and allelic richness (AR) were estimated with the

program MSA 4.05 (Dieringer and Schlötterer 2003). We estimated effective population size (N_e) using the linkage disequilibrium method of Waples and Do (2008), as implemented in NeEstimator V2 (Do et al. 2014). Global genetic differentiation for each species was estimated using G'_{ST} , a standardized measure of genetic differentiation appropriate for multiple species comparisons (Hedrick 2005). Pairwise genetic distance (between each pair of sample sites) was calculated using D_{ps} , a method of measuring genetic differentiation based on proportion of shared alleles (Bowcock et al. 1994). Both G'_{ST} and D_{ps} were calculated with MSA 4.05. Individual-based hierarchical population structure was analyzed using the Bayesian clustering program STRUCTURE 2.3.4 (Pritchard et al. 2000). The most likely number of genetic clusters (K) for each species was determined using the delta- K method (Evanno et al. 2005). Genetic clustering methods are further described in Appendix A, and genotype data are available through figshare (see *Data availability*).

Landscape genetics

Hypothesized landscape connectivity surfaces were built using CIRCUITSCAPE (McRae 2006). CIRCUITSCAPE uses circuit theory to simulate gene flow (i.e., “current”) through a resistance surface in which landscape features hypothesized to promote gene flow are assigned low resistances, and landscape features hypothesized to inhibit gene flow are assigned high resistances. CIRCUITSCAPE allows gene flow across multiple pathways and reports pairwise summations of

resistance between sampling locations. Modeling multiple pathways is appropriate for dryland anurans with high dispersal ability (Chan and Zamudio 2009). A geographic information system (ArcGIS 10.1; Environmental Systems Research Institute, Redlands, California, USA) was used to catalog and manipulate landscape data, and landscape resistance models, data, and sources are described in detail in Appendix B. Nine landscape resistance surfaces in four broad structural connectivity categories were examined (Tables 2 and B1). The first category was terrestrial and included three resistance surfaces: Canopy (resistance decreased with canopy cover), Urban (resistance increased with development), and LandCov (combination of Canopy and Urban for which resistance was lowest with high canopy cover and highest for high development). The second category was aquatic and included three resistance surfaces: Stream (resistance was lowest for perennial/intermittent streams and ponds, moderate for ephemeral streams, and highest for areas with no aquatic habitat), PrecipET (resistance decreased as summer precipitation–evapotranspiration increased), and AvgWat (combination Stream and PrecipET for which resistance was lowest where both precipitation was high and aquatic habitat was available, and for which resistance was highest for dry areas with no aquatic habitat). The third category was topography and included Slope (resistance increased with slope). The fourth category was isolation by distance and included pairwise Euclidean distance between sampling locations (Euclidean) and one uniform, non-zero resistance surface (Null).

We evaluated relationships between pairwise genetic distance and pairwise landscape resistances using a mixed-effects modeling approach (van Strien et al. 2012). Through mixed-effects modeling, explanatory variables (pairwise landscape resistances) are treated as fixed effects, and sampling locations are treated as random effects to account for nonindependent values in distance matrices (Yang 2004). Model fit was evaluated with the R^2_{β} statistic (Edwards et al. 2008) that compares a model with fixed and random effects (pairwise landscape distance or resistance and sampling location) to a null model with only the random effect (sampling location) and an intercept. Comparison of model performance metrics, such as P values, allows for evaluating significant differences between models; however, no formal method of comparison has been developed or agreed upon for evaluating R^2_{β} values of different models. Therefore, we compared R^2_{β} values to one another directly as is common to-date in studies using this approach (van Strien et al. 2012). We also used multiple regression with distance matrices (MRDM; Holzhauser et al. 2006, Balkenhol et al. 2009) as a complementary method to evaluate relationships between genetic and structural connectivity. MRDM methods are described in Appendix B. All analyses were performed in R version 2.14.0 (R Development Core Team 2012), using a modified version of lme4 (Bates et

al. 2014) for mixed-effects modeling, PBKRTEST (Halekoh and Højsgaard 2012) for R^2_{β} calculation as described in van Strien et al. (2012), and ecodist (Goslee and Urban 2007) for MRDM.

RESULTS

We found support for the hypothesis that population structure (genetic differentiation) was correlated with species' water requirements. Global genetic differentiation was highest for canyon treefrogs ($G'_{ST} = 0.57$), intermediate for red-spotted toads ($G'_{ST} = 0.31$), and lowest for spadefoots ($G'_{ST} = 0.09$). We found strong hierarchical clustering for canyon treefrogs (Fig. 2A) with spatial clustering by mountain range (Fig. 2D). Red-spotted toads had moderate hierarchical structure (Fig. 2B) with complex spatial patterns of genetic connectivity (Fig. 2E). Mexican spadefoots had little hierarchical structure (Fig. 2C) with diffuse spatial clustering (Fig. 2F). Delta- K results for all species and genetic clusters are included in Appendix A. Sampling information and population genetic metrics are summarized in Table 2 and described in full in Appendix A.

Landscape resistances and distances in three of four categories (terrestrial, aquatic, and isolation by distance) were moderately to highly correlated for each species (Appendix B: Table B3). We evaluated correlated landscape resistances independently in a mixed-effects modeling framework to insure that collinear variables were not included in the same model. Mixed-effects models revealed a large difference in the strength of correlation between genetic and structural connectivity across species. R^2_{β} values for canyon treefrogs were more than double those for red-spotted toads and Mexican spadefoots (Table 3). Within species, R^2_{β} values for canyon treefrogs were highest for models in the terrestrial and aquatic categories, with AvgWater having the highest correlation across models ($R^2_{\beta} = 0.70$). For red-spotted toads, Urban had the strongest correlation with genetic connectivity ($R^2_{\beta} = 0.33$). However, R^2_{β} for other terrestrial resistances (Canopy and LandCov) were lower than overall aquatic resistances, and Urban was highly correlated ($r > 0.9$) with Null (isolation by distance category). Thus, the R^2_{β} for urban resistance may reflect the effects of null resistance rather than a true terrestrial effect. Finally, Mexican spadefoot genetic connectivity was most closely related to Euclidean distance in the isolation by distance category ($R^2_{\beta} = 0.31$).

We also examined relationships between genetic and structural connectivity within major genetic clusters for each species. For canyon treefrogs, we examined two nested clusters: the western cluster (CT-W) and the Huachuca Mountains cluster (CT-H; Fig. 2D). Results for the two nested clusters did not differ greatly from results for all canyon treefrog sites, though the support for isolation by distance increased marginally at finer scales (Appendix B: Table B4). The same was true for the only major genetic cluster of Mexican spadefoots (MS-E, Fig. 2F) in which results differed only slightly by

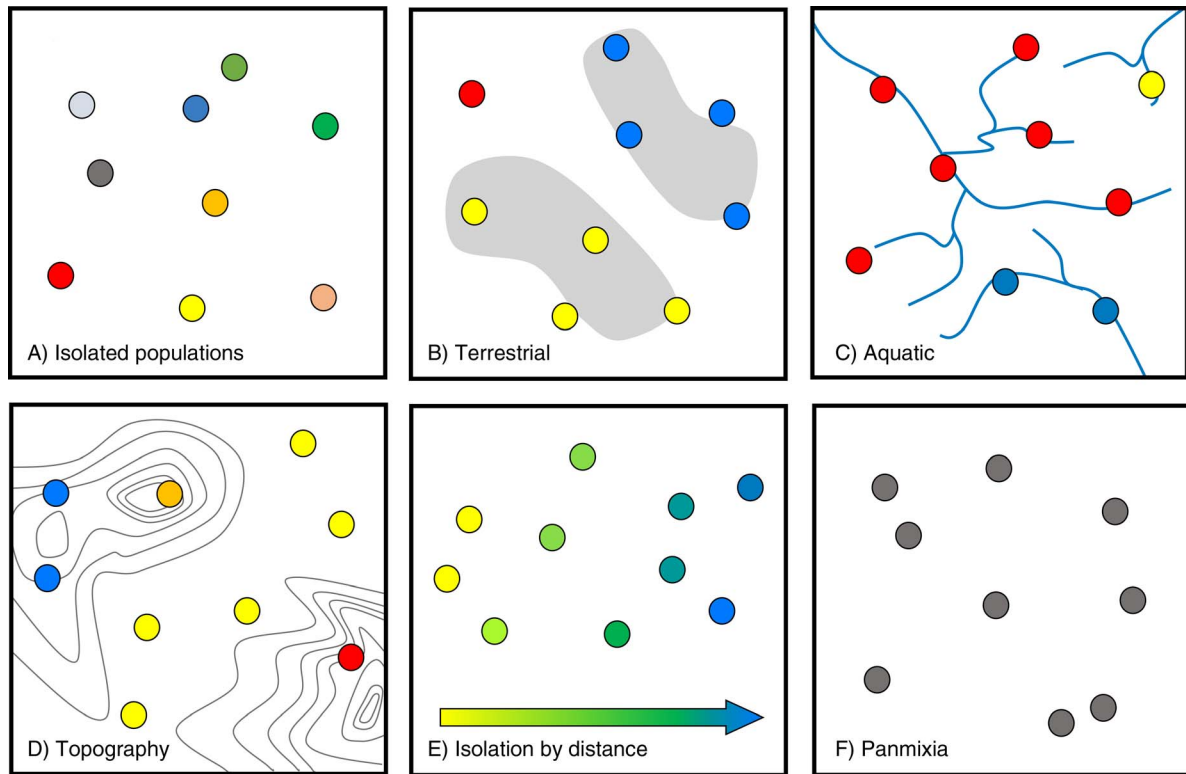


FIG. 1. Genetic connectivity hypotheses (see Table 1). Populations (circles) of the same color are genetically similar. (A) Isolated populations with no landscape drivers of population differentiation; rather, local processes such as genetic drift drive genetic differentiation of populations. (B) Terrestrial describes genetic connectivity associated with low-resistance land cover, such as areas with high canopy cover (shown in green). (C) Aquatic describes connectivity within riparian networks (shown as blue lines) or in areas with high precipitation. (D) Topography is characterized by high connectivity along flat ground (low slope) and high differentiation of populations separated by high slope, as shown with brown contour lines. (E) Isolation by distance describes an increase in genetic differentiation between populations as distance increases or along a cline (arrow). (F) Panmixia is characterized by genetically similar populations across the sampling range with no relationship to landscape.

scale (Appendix B: Table B4). However, the two major genetic clusters for red-spotted toads were spatially complex, with one spatially clustered group (the Huachuca Mountains group, RT-H) and a larger, spatially diffuse group (the northern group, RT-N; Fig. 2E). Hierarchical structure was not found in RT-H and was moderate for RT-N. We found strong support for a relationship between genetic structure and

topographic resistance for RT-N. Conversely, genetic structure of populations in the RT-H group had no relationship with landscape structure, indicating panmictic population structure (Table 3).

Combining topographic resistance with other landscape resistances provides a context for interpreting how the physical template (topography) and landscape features (e.g., streams, canopy cover, and urbanization)

TABLE 2. Sample size, microsatellite information, and population genetics metrics for each species.

Species	Sample size				Average n			Loci		Population genetics			
	N	n	n , no sibs	n , with parents	\bar{n}	\bar{n} , no sibs	\bar{n} , with parents	Count	AR	H_E	H_O	\bar{N}_e	G'_{ST}
Canyon treefrog	15	575	175	202	42.2	11.7	13.5	12	5.09	0.73	0.74	30.7	0.57
Red-spotted toad	15	660	233	252	41.1	15.9	16.8	14	5.29	0.76	0.74	83.0	0.31
Mexican spadefoot	26	1163	781	784	38.3	30.0	30.2	8	5.26	0.67	0.67	5951.7	0.09

Notes: Sample sizes are given as number of sampling locations (N), total individuals (n), n with all but one full sibling (sib) from each family removed, n with reconstructed parents included (described in Appendix A), and average n (\bar{n}) per sampling location with all siblings, with only one full sibling, and with reconstructed parents. Microsatellite information includes loci count and average allelic richness (AR, averaged across populations and adjusted for smallest sample size). Population genetic metrics include expected heterozygosity (H_E) and observed heterozygosity (H_O) calculated as averages of all populations, with reconstructed parents; median effective population size across populations (\bar{N}_e); and overall population differentiation (G'_{ST}). More information, including population genetic metrics by population and locus, is included in Appendix A.

interact and affect genetic connectivity. Topographic resistance (slope) was not correlated with other landscape resistance surfaces, and for that reason we evaluated couplet models combining topography with landscape resistances in other categories (terrestrial, aquatic, and isolation by distance). R^2_{β} values for couplet models were higher than single resistance models across all species, and R^2_{β} values increased by the widest margin for red-spotted toads and Mexican spadefoots (Table 3). Perhaps most notably, the best couplet model for each species and within the northern red-spotted toad (RT-N) genetic cluster included both topographic and aquatic resistances. Canyon treefrogs had equivalent and high R^2_{β} values (0.85) for the couplet models combining topography + aquatic resistance and topography + isolation by distance. However, rather than the expected negative correlation with genetic connectivity, topographic resistance was positively correlated with genetic connectivity for canyon treefrogs. This was also true for the two finer-scale genetic clusters examined for canyon treefrogs (Appendix B: Table B4). This may indicate gene flow across high slope areas, such as mountain ranges and ridgelines, rather than across flat landscapes. Urbanization resistances were highly correlated with uniform resistance values (the isolation by distance hypothesis) for both genetic clusters of canyon treefrogs due to very low urban development within the spatial extent of those clusters, and R^2_{β} values for models incorporating urbanization resistances should be interpreted with this in mind.

Finally, MRDM results for each species and their genetic clusters largely corroborated mixed-effects modeling results, although there were some differences. Canyon treefrog genetic distances were most highly correlated with uniform landscape resistance, with the highest support for uniform landscape resistance (isolation by distance hypothesis) model. When accounting for high collinearity between the uniform and aquatic resistances within genetic clusters of canyon treefrogs (variance inflation factor [vif] greater than or near to 10), conservative interpretation of the MRDM results also supports the uniform resistance-only model for both genetic clusters. For red-spotted toads (both overall and for the northern group), the best model included low resistance along river networks and ponds, topographic resistance, and uniform resistance. However, the correlation with uniform resistance was negative, indicating no support for the isolation-by-distance hypothesis. Finally, we found support for the aquatic hypothesis for Mexican spadefoots at multiple spatial scales. Results are summarized in Appendix B.

DISCUSSION

Characterizing the influences of species ecology and the landscape on genetic connectivity among populations contributes to fundamental ecological and evolutionary knowledge and is an important part of successful conservation of species (Allendorf and Luikart 2007,

Manel and Holderegger 2013). Our findings highlight the utility and potential of species' ecological traits, in our case water dependency, in characterizing relationships between genetic and structural (landscape) connectivity across taxa. We found a positive relationship between population differentiation and increasing water requirements across three aquatic species. When considered independently, landscape drivers of genetic connectivity were largely predicted by hypothesized models built upon knowledge of water requirements, a defining characteristic of species ecology for desert amphibians. Aquatic connectivity had the strongest relationship with genetic connectivity across species when landscape drivers were combined with topography. This supports the notion that in arid environments, water and aquatic habitat are major factors in gene flow and landscape permeability for all aquatic species, from perennial to ephemeral specialists.

Desert anurans utilize a range of ecological strategies to survive in areas characterized by a spatial mosaic of perennial, intermittent, and ephemeral waters embedded in an arid landscape. Larval development periods restrict species' breeding habitats, and desiccation risk is a known driver of amphibian movements in arid environments (Tingley and Shine 2011). High mobility is one possible strategy for capitalizing on unpredictable availability of water and may drive high genetic connectivity observed in some desert anurans in the American Southwest (Mexican spadefoots in this study; Chan and Zamudio 2009). The high mobility of Mexican spadefoots and other ephemeral specialists in the region (e.g., Couch's spadefoot, *Scaphiopus couchii*, and the Great Plains toad, *Bufo cognatus*) may provide greater resiliency to temporal or spatial changes in habitat availability, and high genetic connectivity between populations may buffer the genetic consequences of some habitat loss.

High mobility, however, is only one end of a spectrum of potential strategies for aquatic desert life; other species may instead exhibit site fidelity to isolated pools with perennial or longer-term intermittent water. Both canyon treefrogs and red-spotted toads exhibit some degree of site fidelity and had greater population structure than Mexican spadefoots. Proximity to water is the hypothesized mechanism by which canyon treefrogs meet necessary water requirements for thermoregulation (Snyder and Hammerson 1993). Red-spotted toads also exhibit nonrandom occupancy of suitable breeding habitat (Dayton and Fitzgerald 2001) and fidelity for particular sites (Turner 1959, Weintraub 1974). These findings support our hypothesis of greater population structure with increasing water requirements. Aquatic habitat in the Sky Islands region is threatened by human water use (Marshall et al. 2010), a warming and drying climate (Seager et al. 2007), and an increased risk of catastrophic disturbances such as extreme fires (Brown et al. 2004). A drier landscape and loss of an already limited number of breeding sites

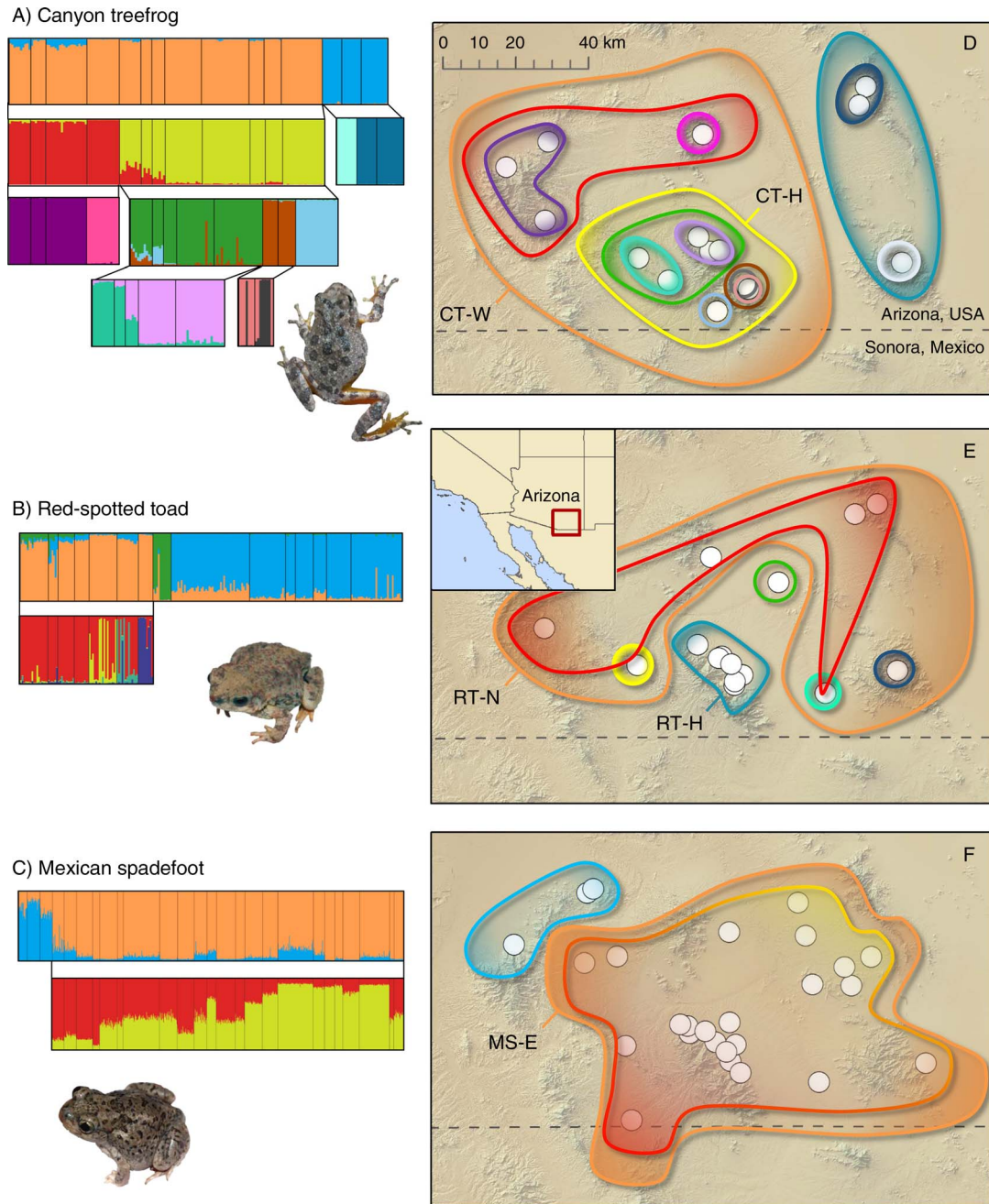


FIG. 2. Spatial and individual hierarchical population structure for each species. Individual-based STRUCTURE results (Pritchard et al. 2000) are shown in panels (A), (B), and (C). Each vertical bar represents one individual. Colors indicate the most likely genetic cluster assignments. Black vertical bars denote individuals from the same sampling locations. Each cluster was hierarchically analyzed for nested structure; nested structure results are shown directly below the original cluster. Hierarchical analyses were repeated until terminal clusters ($K = 1$, where K is the number of genetic clusters) were reached. Panels (D), (E), and (F) show nested outlines that group sampling locations into the genetic clusters shown in panels (A), (B), and (C), with study extent shown in the inset map (panel E). Outline color corresponds to population clusters. Red to yellow shading in panel (F) represents the transition between two clusters shown in panel (C) (Mexican spadefoots). Major genetic clusters include (D) canyon treefrog, west (CT-W) and canyon treefrog, Huachuca Mountains (CT-H); (E) red-spotted toad, north (RT-N) and red-spotted toad, Huachuca Mountains (RT-H); and (F) Mexican spadefoot, east (MS-E).

TABLE 3. Mixed-effects modeling results for each species and major genetic clusters of red-spotted toads (RT-N, north; RT-H, Huachuca Mountains; Fig. 2).

Hypotheses and resistance layers/ distance	R^2_{β} , mixed-effects models				
	CT	RT	MS	RT-N	RT-H
Isolated populations	NS	NS	NS	NS	NS
Terrestrial (TE)					
Canopy	0.68	0.26	0.28	0.06	0.02
Urban	0.68	0.33	0.27	0.06	<0.01
LandCov	0.69	0.27	0.28	0.07	<0.01
Aquatic (A)					
Stream	0.67	0.32	0.28	0.10	<0.01
PrecipET	0.68	0.30	0.28	0.06	<0.01
AvgWater	0.70	0.31	0.28	0.09	<0.01
Topography (T)					
Slope	0.35	0.27	0.29	0.48	0.01
Isolation by distance (IBD)					
Euclidean	0.57	0.14	0.31	0.02	<0.01
Null	0.67	0.30	0.28	0.06	<0.01
Panmixia	NS	NS	NS	NS	Yes
TE + T					
Best of TE + Slope	0.78	0.58	0.49	0.62	0.04
A + T					
Best of A + Slope	0.85	0.61	0.50	0.66	0.03
IBD + T					
Euclidean + Slope	0.76	0.32	0.39	0.37	0.02
Null + Slope	0.85	0.56	0.48	0.36	0.03

Notes: Spatial data are described in full in Appendix B. Isolated populations, TE, A, T, IBD, and Panmixia are single explanatory variable models; the rest are couplet models. Model fit was evaluated with the R^2_{β} statistic (Edwards et al. 2008) that compares a model with fixed and random effects (pairwise landscape distance or resistance and sampling location) to a null model with only the random effect (sampling location) and an intercept. Top R^2_{β} values are highlighted in boldface type. Isolated populations and panmixia hypothesize no landscape effect, indicated by poor model performance across all other models. NS indicates no support for isolated populations and panmixia, "Yes" indicates support. All R^2_{β} correlation coefficients are positive except those in italic text. Slope is negatively correlated with D_{ps} (a method of measuring genetic differentiation based on proportion of shared alleles) for CT in all couplet models, and Euclidean is negatively correlated with D_{ps} for RT-N in the IBT + T couplet model.

may result in a greater risk of loss of genetic diversity for amphibians such as red-spotted toads, canyon treefrogs and others with small population sizes and/or high water requirements (e.g., Chiricahua leopard frogs, *Lithobates chiricahuensis*; Arizona treefrog, *Hyla wrightorum*; and Sonoran tiger salamander, *Ambystoma mavortium stebbinsi*).

Considered independently, the importance of different connectivity models varied across species. As predicted, terrestrial and aquatic connectivity were highly correlated with genetic connectivity for canyon treefrogs in mixed-effects models. But, the null resistance model (isolation by distance) performed similarly, and MRDM models provided strong support for an isolation-by-distance-only model. This indicates that distance alone may be an important driver of population genetic structure in this species. This may be particularly true at the spatial scale of a single mountain range where high elevation, lower temperature, and greater precipitation provide a high density and availability of permeable landscape. This is supported by the marginal increase in support for isolation by distance at a finer scale for canyon treefrogs. Two things may explain this

result. First, distance may in fact be the primary driver of genetic connectivity between populations, particularly within a mountain range. Second, if landscape attributes such as aquatic connectivity are important for dispersal, the high permeability of the landscape may make it difficult to detect the importance of such features using a landscape genetics approach (Cushman et al. 2013).

For red-spotted toads, all mixed-effects models performed similarly with the exception of poorly supported Euclidean distance-only model. However, when major genetic clusters were considered independently, topographic resistance had the highest R^2_{β} for the northern red-spotted toad group (RT-N) as predicted, and the Huachuca group (RT-H) was panmictic, highlighting the variability of important landscape drivers of genetic connectivity introduced by spatial scale and extent of a study. Finally, mixed-effects models supported distance alone (Euclidean) as the best explanatory factor in Mexican spadefoot genetic connectivity.

Topography is the physical template upon which organisms interact with other landscape factors, and high slope is known to reduce genetic connectivity in



PLATE 1. Intermittent stream pools along a tributary to French Joe Canyon in the Whetstone Mountains, Arizona, USA. Seasonal stream pools at this location often contain canyon treefrog tadpoles (*Hyla arenicolor*). This is Site 10 for canyon treefrogs in this study. Photo credit: M. C. Mims.

some amphibians (Lowe et al. 2006). When topographic resistance was considered alongside other landscape factors, aquatic connectivity emerged as a dominant driver of genetic connectivity for all species. However, for canyon treefrogs, the correlation between topographic resistance and genetic connectivity was opposite the expected relationship, with high slope correlated with high genetic connectivity. Though canyon treefrogs have the highest water requirements of the three species we examined, they are relatively well adapted to dry conditions (Snyder and Hammerson 1993). Complex topography and high elevation ridges may not completely inhibit canyon treefrog gene flow, and our results suggest some genetic resilience to temporarily dewatered or disturbed habitat (e.g., from fires or a dry year) at finer spatial scales where individuals may be capable of recolonizing breeding habitat. Furthermore, mountain ranges may provide more refugia and less stressful environmental conditions for dispersing individuals due to wetter monsoon conditions, higher canopy cover, and lower temperatures than valley regions. Similar patterns of isolation by mountain range are described for sympatric insects described as headwater specialists (Finn et al. 2007, Phillipsen and Lytle 2013).

The relationship between genetic and aquatic connectivity for red-spotted toads may be driven by the species' affinity for bedrock pools. In ephemeral areas, adults might disperse along riparian networks in which refugia and the likelihood of finding suitable breeding habitat

are greatest, and they may be deterred by the high slope of canyons, incised channels, or valley walls. Longitudinal connectivity may also be the result of rare but important downstream dispersal when larvae in these shallow bedrock reaches are displaced and washed downstream by flash floods.

Finally, the relationship between genetic and aquatic connectivity in a topographic context for Mexican spadefoots supports the idea that aquatic connectivity may be important for aquatic species with even the most ephemeral water requirements. Mexican spadefoots have the lowest hydrologic requirements of the three species in this study, but aquatic habitat remains a critical part of their life cycle. Furthermore, high slope topography may represent a barrier, may not provide adequate breeding habitat or underground refugia, or may be too energetically costly to navigate.

These findings highlight the potential predictive power of species biology and ecology in understanding population connectivity. Still, there are inherent limitations with this methodology. Gene flow estimates derived from microsatellite data reflect gene flow over the last few decades to centuries and do not always reflect present-day demographic or genetic processes (Waples and Gaggiotti 2006). Secondary contact after removal of a historical barrier may manifest as a cryptic genetic signal unrelated to current landscape processes (Landguth et al. 2010). Our study extent may have encompassed two major lineages of red-spotted toads

that diverged during the late Pleistocene (Bryson et al. 2012). However, studies suggest similar or even increased landscape permeability for at least the last 1000 generations for this species (Holmgren et al. 2003, Pigati et al. 2009). Moreover, global genetic differentiation of red-spotted toads in this study was moderate to low, and with many populations in this study estimated to have modest N_e it is unlikely that the signal from secondary contact is detectable in this study (Whitlock and McCauley 1999).

Challenges stemming from collinearity, resistance surface parameterization, and spatial scale can also complicate detection and interpretation of relationships between genetic and structural connectivity. This is not unique to our study as collinearity among landscape resistance values is a persistent challenge in landscape genetics (Balkenhol et al. 2009). Our ability to account for collinearity in resistance values is limited because formal comparisons of R^2_{β} have yet to be developed. Thus, comparisons of close R^2_{β} values can make interpretation, and determining the “true” landscape driver, difficult. Therefore, though interpreting results in an ecological context does provide valuable insight (Cushman and Landguth 2010), R^2_{β} values must be interpreted with these correlations in mind. Defining landscape resistance values is a recognized challenge in landscape genetics (Spear et al. 2010, Cushman et al. 2013). It is likely that true landscape resistances are more complex than the simple resistance values used in our study, and non-linear relationships may exist between genetic and landscape connectivity (e.g., Peterman et al. 2014). However, our goal was to compare hypothesized relationships between genetic structure of populations and a suite of landscape factors, and we created simple landscape resistance layers that reflect our hypotheses and that were easily compared across species.

Our study’s spatial extent captured meaningful genetic structure across all three species, but landscape effects may change at different spatial scales (Anderson et al. 2010, Murphy et al. 2010). Spatial patterns of genetic diversity indicated clean hierarchical structure for canyon treefrogs and clear isolation-by-distance patterns for spadefoots; however, red-spotted toads displayed spatially complex structure, and genetic connectivity within genetic clusters varied in its relationship to the landscape. Though we do not know the underlying cause of that spatial variability in red-spotted toads, complex effects of spatial scale highlight the need to carefully interpret patterns observed in one portion of a species’ range when drawing management-relevant conclusions outside the study area. The landscape is often heterogeneous across a species’ range, and edge versus central populations may have different population dynamics and undergo different selective pressures (Hardie and Hutchings 2010, Trumbo et al. 2013). The ranges of the three study species extend beyond the area of this study and encompass areas with drier climate, greater urban-

ization, and lower canopy cover than the geographic extent of this study. The variability observed for red-spotted toads emphasizes that relationships between landscape variables and genetic connectivity are not necessarily consistent across a range or at different spatial scales, and relationships may be fundamentally different in areas where the matrix between populations is drier, harsher, or offers fewer refugia.

In conclusion, we found that patterns of population structure, connectivity, and their landscape drivers are predicted by the water dependencies of anurans in dryland ecosystems. Our work supports recent studies highlighting the utility of multispecies inference and ecologically derived hypotheses (e.g., Hughes et al. 2013). Genetic diversity is often a missing component in conservation planning and resource allocation despite its recognized role in species persistence (Laikre et al. 2010). With increasing human demand for aquatic resources in arid environments, environmental change and habitat alteration will likely outpace the resources and time necessary for single-species population genetics studies for many species of conservation concern. When single-species studies are not feasible, the use of species’ ecological information to predict relationships between genetic and structural connectivity may provide a promising alternative.

ACKNOWLEDGMENTS

We extend our sincerest gratitude to the following individuals, agencies, and organizations that helped make this work possible. This study was funded by the Department of Defense Strategic Environmental Research and Development Program (RC-1724). Sheridan Stone assisted with study design, logistics, and sampling efforts throughout the duration of the project. Jessica Hale, Kristen Jaeger, Britta Padgham, Tom Miscione, and Robert Troup provided extensive help with sampling efforts. David Armstrong, Jan Armstrong, Michael Bogan, Ken Charters, Merce Dostale, Caren Goldberg, Matt Kaplan, Lanie Levick, Jesi Miscione, Eric Moody, Mike Sredl, Katherine Strickler, Michael Tarachow, and Eric Wallace assisted with *H. arenicolor* sampling. Brooke Gebow and The Nature Conservancy Ramsey Canyon helped with field work logistics. Martin Schlaepfer and Mike Sredl assisted with study design, Dale Turner provided spatial riparian data for Mexico, and Martin van Strien provided assistance with mixed-effects modeling. Lorenz Hauser, Adam Leaché, Joshua Lawler, and three anonymous reviewers provided helpful comments on the manuscript. Additional funding for M. C. Mims was provided by a National Science Foundation Graduate Research Fellowship (Grant No. DGE-0718124).

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SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A and B are available online: <http://dx.doi.org/10.1890/14-0490.1.sm>

Data Availability

Data associated with this paper have been deposited with figshare: <http://dx.doi.org/10.6084/m9.figshare.1205533>