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Source: *Copeia*, 2010(3):388-396. 2010.

Published By: The American Society of Ichthyologists and Herpetologists

DOI: 10.1643/CH-09-152

URL: <http://www.bioone.org/doi/full/10.1643/CH-09-152>

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Low Genetic Differentiation among Populations of the Great Plains Toad (*Bufo cognatus*) in Southern New Mexico

Jeremy M. Jungels^{1,2}, Kerry L. Griffis-Kyle³, and Wiebke J. Boeing¹

We examined the genetic population structure for the Great Plains Toad (*Bufo cognatus*) in the Chihuahuan Desert of southern New Mexico in order to discern at what spatial scale genetic differentiation is apparent. In addition, we tested whether habitats in the Chihuahuan Desert of southern New Mexico differed in their resistance to gene flow in *B. cognatus*. We used microsatellites to estimate genetic differentiation in populations that varied in distance from 1 to 60 km. Of 120 pairwise tests of genetic differentiation, 44 were significant. However, differentiation was low between all sites ($F_{ST} = 0.0\text{--}0.087$), almost all of the genetic variation being within populations (96.3%). Compared to published studies of other anuran species, populations of *B. cognatus* in southern New Mexico are among the most genetically homogenous anuran species. Significant isolation by distance did occur over all populations despite the genetic similarity, suggesting that differentiation does occur at a broader scale. In addition, several landscape-based models of gene flow were produced and tested against the allelic data. A community model assigned each plant community a different level of resistance to gene flow. This model was not found to describe the estimated genetic variation between populations better than simple Euclidean distance. However, the river model, which assigned low resistance to the aquatic habitats including the Rio Grande, described the estimated genetic variation better than Euclidean distance, suggesting that the Rio Grande, and potentially other rivers throughout the toad's range, may act as a route of dispersal for *B. cognatus*, reducing genetic differentiation among distant populations.

HERE is a wide range in the spatial scale at which genetic differentiation occurs in species due to a combination of demographic and environmental factors (Jehle et al., 2005; Lowe, 2009; Nobre et al., 2010). By examining the population genetic structure and gene flow of a species, we may gain insight into some of its demographic properties such as its rate of dispersal. The environment through which dispersal occurs, however, can also influence gene flow. Some landscape features may offer greater resistance to gene flow than others, thereby altering the rate of migration (m) between some subpopulations. Genetic differentiation may be affected by environmental variables such as agriculture (Johansson et al., 2005), mountain ridges (Monsen and Blouin, 2004; Funk et al., 2005), headwater sources (Castric et al., 2001; Pritchard et al., 2007), urban areas (Hitchings and Beebe, 1997; Rowe et al., 2000), river crossings (Spear et al., 2005), large bodies of water (Lee-Yaw et al., 2009), plant communities (Keyghobadi et al., 1999; Spear et al., 2005), community structure (Spear and Storfer, 2008), roads (Lesbarreres et al., 2006), and other anthropogenic barriers (Pritchard et al., 2009).

Amphibians have historically been assumed to have low dispersal distances (<1 km) and high fidelity to discreet breeding sites, although current views are now bringing these assumptions into question (Smith and Green, 2005). Consequently, our initial expectations were that amphibians would exhibit genetic differentiation at small scales, especially in an arid system with a seemingly inhospitable terrestrial matrix. Some genetic studies support this hypothesis documenting large differences in allele frequencies between nearby breeding ponds (Lampert et al., 2003; Andersen et al., 2004), while others have found little or no difference between ponds (Barber, 1999; Newman and Squire, 2001; Burns et al., 2004).

For many amphibian species, landscape features in part determine population structure. For instance, Funk et al. (2005) have shown that mountain ridges act as barriers to gene flow in the Columbia Spotted Frog (*Rana luteiventris*). Likewise, plant communities can vary in their resistance to gene flow for some species (Spear et al., 2005; Stevens et al., 2006). Mark-recapture and orientation studies have also found that plant communities differed in their resistance to the movement of amphibians (Rothermel and Semlitsch, 2002; Mazerolle and Desrochers, 2005).

Arid and semi-arid environments may represent a challenge to dispersing amphibians due to the potential for high evaporative water loss through their semi-permeable skin (Duellman and Trueb, 1994; Bartelt and Peterson, 2005). Even so, little is known about the movement of amphibians in arid environments, and there is little work examining whether habitats in an arid and semi-arid environment may differ in their resistance to amphibian movement or dispersal either through the direct measurement of movements or indirectly by estimating gene flow from genetic data (but see Chan and Zamudio, 2009; Wang, 2009).

Our purpose here was to examine the population genetic structure of the Great Plains Toad (*Bufo cognatus*) in the semi-arid environment of the Chihuahuan Desert in southern New Mexico. The Great Plains Toad has a range far beyond the Chihuahuan Desert, as far north as the southern prairies of Canada, making within-species comparisons possible in different environments. In New Mexico the toad breeds explosively at ephemeral pools that develop after monsoon rains, characteristic of the breeding aggregations for anurans in southern New Mexico. In addition, the Great Plains Toad is common and easily captured at breeding sites, making it ideal for genetic study. Specifically, we sought to discern the spatial scale at which population genetic differentiation begins to occur for this species in

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Submitted: 17 August 2009. Accepted: 22 March 2010. Associate Editor: M. J. Lannoo.

© 2010 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CH-09-152

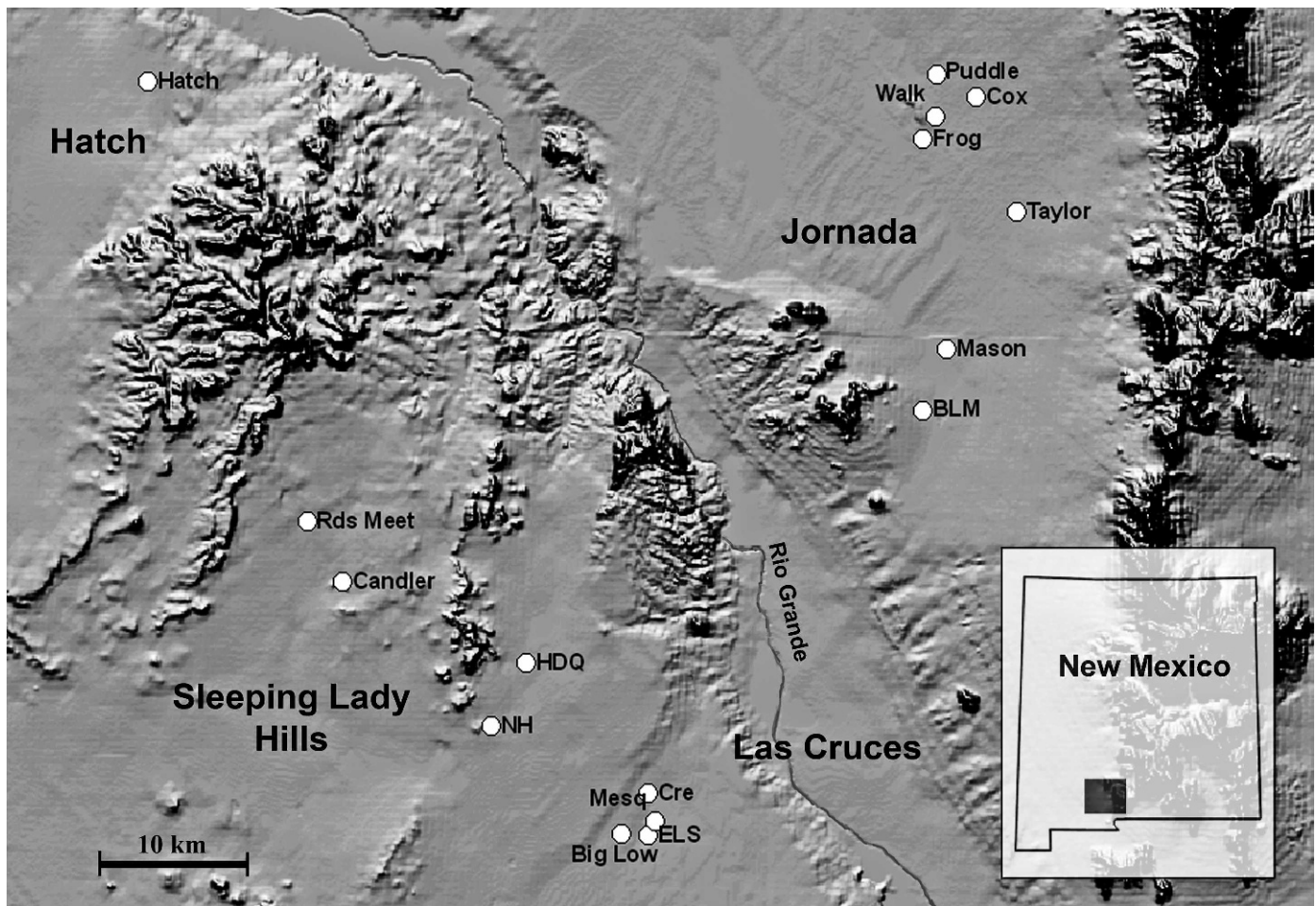


Fig. 1. Map of study area. Sampling sites are marked as circles.

southern New Mexico. In addition, we tested whether the gene flow between these toad populations is influenced by different levels of resistance of the plant communities in the Chihuahuan Desert. We hypothesize that genetic differentiation occurs with distance, that different plant communities exhibit different resistances to *Bufo cognatus* dispersal, and that waterways may act as long distance dispersal conduits.

MATERIALS AND METHODS

Study area.—We selected three surveying areas for this study in Doña Ana County, southern New Mexico. Survey areas included the Jornada Long Term Ecological Research Site and areas of Bureau of Land Management (BLM) land managed for livestock, one near Hatch, NM and another around the Sleeping Lady Hills west of Las Cruces, NM (Fig. 1). We located potential anuran breeding sites, including playas and earthen stock tanks for cattle, using the BLM's GIS database, 7.5 minute topographic maps and by querying the Southwest Regional Gap Analysis Project (SWreGAP) habitat layer in ArcGIS 9.1. We surveyed 37 sites, 19 (51.1%) of which had Great Plains Toads.

Landscape measurements.—We utilized Feature Analyst 4.1 (Visual Learning Systems, Inc., 2006. http://www.featureanalyst.com/feature_analyst/publications/manuals/FA4.1_manual_arcgis.pdf) in order to classify Advanced

Spaceborn Thermal Emission and Reflection Radiometer (ASTER) satellite imagery. We used imagery from the Visible and Near-Infrared subsystem of the ASTER satellite at a resolution of 15 meters. Images available for the sites were taken on 30 September 2003 and 3 November 2004. The images were cut to the dimensions of the three primary survey areas used in the study and split into nine different components. These components were subsequently combined to form five cover classifications based on structure. The combined classifications included Grassland, Mesquite, Creosote, Playa, and Mixed Succulent Desert Scrub.

Molecular analysis.—Whenever present at anuran breeding sites, we captured *B. cognatus* by hand or net, and the outer posterior right toe was clipped and preserved in ethanol. Of the 19 sites, three did not have enough individuals to obtain enough material. Thus, we have 16 different sites for which genetic analyses were conducted. Specimens were digested using proteinase K at 70°C, and DNA was extracted using a QIAGEN DNeasy blood and tissue kit.

Five microsatellite primer pairs developed previously by Gonzalez et al. (2004) for *B. cognatus* were used in polymerase chain reactions (PCR) to amplify fragments. PCR amplification was performed using an Eppendorf Mastercycler following the protocol recommended by the manufacturer for use with the paq5000 DNA polymerase. We determined fragment length using an ABI 3100 Genetic Analyzer and the Rox500 size standard. Peakscanner version

Table 1. Resistance Values Used in Resistance Maps for All Models of Dispersal.

Landscape	Resistance value		
	Habitat	River	Combined
Creosote	40	40	40
Desert scrub	40	40	40
Grassland	50	40	50
Mesquite	10	40	10
Aquatic/riparian	40	1	1

1.0 (Applied Biosystems) was used to visually score the allele size of fragments and separate into bins of two base pairs each. For each locus we sequenced one amplified product to assure that the fragments contained the same base pair repeats as the individuals sampled by Gonzalez et al. (2004) and to ascertain the number of repeats. Amplified product was first purified using a Qiagen QIAquick Gel Extraction kit. Sequencing was done on an ABI 3100 Genetic Analyzer by the Molecular Biology molecular analysis services at New Mexico State University.

Statistical analyses.—We ran all molecular tests in ARLEQUIN 3.1 (Excoffier et al., 2005) unless otherwise noted. Allele frequencies and observed and expected heterozygosities for each locus and population were estimated. We tested for departure from Hardy-Weinberg equilibrium (HWE) within each population for each locus. As this involves a large number of tests (16 for each locus), we used a sequential Bonferroni correction (Rice, 1989) to adjust the overall significance level to 0.05. Linkage disequilibrium tests were run between loci using a likelihood-ratio test (Slatkin and Excoffier, 1996).

We estimated both pairwise F_{ST} and R_{ST} values because each of these estimators of gene flow have been shown to give the most realistic estimates in certain cases with microsatellite data (Balloux and Goudet, 2002). The significance of these values was tested based on a permutation test (Raymond and Rousset, 1995; Goudet et al., 1996). We used an analysis of molecular variance (AMOVA) to partition the observed allelic variation into within population, between population, and between region components (Excoffier et al., 1992). The effective number of migrants per generation ($N_e m$) was estimated using the private alleles method (Slatkin, 1985) using Genepop 3.4.

Straight line (Euclidean) distances between all pairs of sites sampled were calculated in ArcGIS 9.1. We developed several landscape resistance maps in order to model alternative hypotheses about the influence of landscape on dispersal in *B. cognatus*. For these maps we assigned a resistance value to each landscape feature, representing the relative cost of dispersing across that feature. Resistance maps such as this have been used increasingly to adapt behavioral information on organisms into a distance model (Adriaensen et al., 2003). Three such models were developed. One, the community model, assigned a resistance value to each of the vegetation cover types in the vegetation map produced for the study area using Feature Analyst. These resistance values were based on an association of Great Plains Toads with mesquite habitat (unpubl. data). A second model, the river model, assigned a low resistance value to aquatic/riparian cover types relative to a higher

Table 2. Number of Alleles and Observed and Expected Heterozygosity for Populations in This Study and Populations from Northwest Texas.

	This study ($n = 289$)		Gonzalez et al. (2001) ($n = 119$)	
	No. of alleles	H_O/H_E	No. of alleles	H_O/H_E
IYY	17	0.65/0.87	24	0.90/0.90
IDDD	22	0.62/0.86	51	0.56/0.95
IKK	25	0.32/0.93	20	0.89/0.85
IHHH	20	0.69/0.88	33	0.84/0.94
ICCC	1	0.0/0.0	22	0.79/0.90

resistance value for all terrestrial cover types. This model was called the river model because isolated aquatic sites such as stock tanks and playa lakes had little impact in the model, whereas the Rio Grande had a large impact by functioning as a dispersal route connecting distant populations to one another. A third model, the combined model, assigned resistance values based on both of these models (Table 1).

Where gaps existed in our own habitat maps, we used the landcover GIS layer produced by the SWReGAP for Doña Ana County. The landcover types were simplified into the same categories as in our own maps and then assigned a corresponding resistance value. In cases where a cover type did not easily fall into one of these categories, we assigned the median resistance value of 40. The overall occurrence of these habitats however was minimal (<1%).

We used Pathmatrix 1.1 (Ray, 2005), an extension for ArcView 3, in conjunction with the resistance maps to compute least-cost distances between each pair of breeding sites used in our molecular analysis. The least-cost distance is defined as the minimum distance in cost units used to move from source to target point.

We examined the relationship of R_{ST} and F_{ST} to the Euclidean distance and least-cost distance for each of the models with Mantel and partial Mantel tests using PASSAGE 1.1.2.3 (Rosenberg, 2001) with 10,000 permutations. We used Mantel tests to examine the relationship between the linearized pairwise F_{ST} and R_{ST} matrices and the matrices for Euclidean and least-cost distances. In addition, partial Mantel tests were used to examine the correlation between the least-cost distances and the genetic differentiation estimators while holding Euclidean distance constant in order to isolate the effect of cover type in the correlation.

RESULTS

Microsatellites.—Optimized annealing temperatures were different than those used by Gonzalez et al. (2004), possibly due to the use of *Pyrococcus*-derived DNA polymerase (paq5000) and the resulting different PCR protocol. Of the microsatellite loci used, one (ICCC) was found to be monomorphic for the populations in this study, though the same locus was highly polymorphic (20 alleles) in the 119 individuals surveyed by Gonzalez et al. (2004) from playa lakes in northwestern Texas (Table 2). The remaining loci were highly polymorphic (17–25 alleles, average = 21). Allelic richness was lower in southern New Mexico for four out of the five loci. Within-population allelic richness ranged from one to 14 over all loci (average = 8.33; Table 3). Due to poor amplification and much lower heterozygosity

Table 3. Summary of Genetic Variation at the Loci Used in This Study. Significant deviations from Hardy-Weinberg proportions are in bold.

Site (<i>n</i>)	Allelic richness				Observed/expected heterozygosity			
	IYY	IDD	IKK	IHH	IYY	IDD	IKK	IHH
Big Low (16)	13	11	6	8	.81/.94	.53/.89	.18/.80	.80/.86
BLM (19)	9	11	1	9	.83/.88	.74/.88	Monomorphic	.74/.83
Candler (4)	5	2	2	7	.75/.86	.33/.33	0/.67	.75/.96
Creosote (3)	4	5	2	4	.67/.87	1.0/.93	.33/.33	.67/.87
Cox (30)	8	14	12	9	.93/.73	.68/.89	.35/.92	.90/.87
ELS (11)	10	9	8	10	.70/.91	.55/.84	.11/.90	.82/.90
Frog (32)	13	13	15	11	.59/.91	.60/.91	.29/.89	.75/.86
Hatch (28)	14	12	1	10	.64/.85	.61/.90	Monomorphic	.57/.85
HDQ (29)	11	14	15	13	.59/.85	.57/.90	.22/.92	.78/.88
Mason (18)	10	8	6	8	.72/.88	.61/.77	.17/.89	.81/.85
Mesquite (18)	10	10	2	10	.78/.90	.82/.87	.00/.67	.67/.89
NH (7)	6	7	2	9	.29/.77	.43/.90	1.0/1.0	.71/.93
Puddle (35)	8	13	11	8	.71/.82	.69/.89	.26/.91	.66/.83
Roads meet (5)	5	5	6	5	.80/.82	.60/.82	1.0/1.0	.40/.82
Taylor (29)	14	12	14	9	.56/.86	.74/.89	.46/.90	.55/.81
Walk (5)	5	5	1	7	.40/.84	.40/.84	Monomorphic	.60/.91
Mean	9.06	9.44	6.38	8.56	.65/.87	.62/.86	.32/.93	.69/.88
Total (289)	17	22	25	20				

than expected (Table 3), the locus IKK was suspected of having a null, or non-amplifying, allele in our populations and was eliminated from further calculations. In comparison, heterozygosity was higher than expected in the Texas population studied by Gonzalez et al. (2004). Two other loci exhibited deviations from Hardy-Weinberg expectations in several populations, and the presence of a null allele at these loci cannot be ruled out. In these two loci, however, at least one population showed a heterozygote excess and there was clearly no across the board deficiency. No allele at any locus dominated any population, with the highest frequency for any allele in a population among the three remaining loci being 0.229. A non-random association of alleles across loci (linkage disequilibrium) was significant for only three populations (Frog, Hatch, and Walk). Two of these were at the loci IYY and IDD and one at the loci IYY and IHH.

Population differentiation.—Based on pairwise F_{ST} estimates, only one breeding site lacked significant differentiation from all others (Cre), and this site had an extremely small sample size (three, the minimum necessary for such a test). Of 120 pairwise permutation tests, 44 were significant when F_{ST} was used as the estimator of genetic differentiation and 29 were significant when R_{ST} was used as the estimator ($\alpha = 0.05$ in all cases). The magnitude of differentiation, however, was generally low (Table 4) with pairwise F_{ST} values ranging from 0 to 0.087. Seventy-five percent of the significant differences in allele frequencies were between breeding sites from different regions within our study area. However, two sites (Frog and Cox) did show significant though small differentiation ($F_{ST} = 0.032$) despite being separated by less than 5 km. The variation in values was considerably greater for R_{ST} than F_{ST} , and the two estimates did not generally agree. F_{ST} averaged over populations was similar for all sites ranging from 0.030 to 0.039. Global F_{ST} was 0.032. F_{ST} for individual loci averaged over all populations ranged from 0.017 to 0.049. The results of the AMOVA showed that very little genetic variation was between sites (1.35%) or between regions (2.36%), almost

all of it occurring within populations (96.3%). Global F_{IS} was 0.189, with population specific F_{IS} ranging from -0.069 to 0.491. The effective number of migrants per generation (Nm), calculated using the private alleles method and corrected for population size, was 2.94. The mean frequency of private alleles was 0.051.

Landscape analysis.—Linearized F_{ST} was positively related to Euclidean distance ($P = 0.007$; $r = 0.363$; Mantel test; Fig. 2). In addition, linearized F_{ST} was positively related to least-cost distance for the community model ($P = 0.006$; $r = 0.354$; Mantel test), the river model ($P = 0.002$; $r = 0.413$; Mantel test), and the combined model ($P = 0.002$; $r = 0.440$; Mantel test). The partial Mantel tests that examined the relationship between least-cost distance for each of the models while controlling for Euclidean distance was significant for the river model ($P = 0.05$; $r = 0.2144$; 100,000 permutations) and the combined model ($P = 0.027$; $r = 0.280$) but not for the community model ($P = 0.284$).

Because four breeding sites had very few samples (≤ 5) and subsequently high variance in pairwise F_{ST} , we also ran the Mantel tests excluding these sites. Results were similar though the relationships were strengthened; the correlations for F_{ST} were significant with Euclidean distance ($P = 0.002$; $r = 0.420$; Mantel test) and with least-cost distance for the combined model ($P < 0.001$; $r = 0.499$; Mantel test), community model ($P = 0.007$; $r = 0.3621$; Mantel test), and river model ($P < 0.001$; $r = 0.435$; Mantel test). The partial Mantel for least-cost distance from the combined model while controlling for Euclidean distance was still significant ($P = 0.05$; $r = 0.304$); however, it was not significant for the community model only ($P = 0.430$) and the river model only ($P = 0.196$).

When R_{ST} was used as the estimator of genetic differentiation, none of the correlations with Euclidean or least-cost distances were significant. However, modeling has suggested that because of high variance R_{ST} is often not the preferred statistic especially when gene flow is high (Balloux and Goudet, 2002) as suggested by our data.

Table 4. Estimates of Pairwise F_{ST} (below Diagonal) and R_{ST} (above Diagonal) Averaged over Loci IHH, IY, and IDD. Values in bold were statistically significant after sequential Bonferroni correction.

	Big Low	BLM	Candler	Cox	Cre	ELS	Frog	Hatch	HDQ	Mason	Mesq	NH	Puddle	Rds meet	Taylor	Walk
Big Low																
BLM	0.030															
Candler	0.048	0.000														
Cox	0.033	0.039														
Cre	0.012	0.016	0.071													
ELS	0.024	0.022	0.025	0.058												
Frog	0.005	0.018	0.028	0.032	0.020	0.028										
Hatch	0.008	0.053	0.030	0.068	0.034	0.040	0.015									
HDQ	0.023	0.026	0.012	0.046	0.019	0.026	0.020	0.037								
Mason	0.031	0.010	0.056	0.046	0.066	0.046	0.034	0.075	0.047							
Mesq	0.005	0.010	0.001	0.047	0.030	0.013	0.018	0.008	0.007	0.043						
NH	0.038	0.031	0.027	0.033	0.040	0.027	0.037	0.052	0.009	0.050	0.029					
Puddle	0.043	0.032	0.033	0.006	0.026	0.052	0.013	0.053	0.034	0.057	0.048	0.017				
Rds meet	0.049	0.039	0.005	0.087	0.036	0.043	0.018	0.024	0.041	0.086	0.023	0.050	0.047			
Taylor	0.035	0.003	0.048	0.042	0.012	0.045	0.016	0.070	0.020	0.025	0.041	0.043	0.022	0.068		
Walk	0.040	0.036	0.082	0.038	0.026	0.041	0.033	0.046	0.056	0.079	0.014	0.065	0.049	0.046	0.061	

DISCUSSION

We found that genetic differences of different *Bufo cognatus* breeding aggregates were small with a maximum F_{ST} value of 0.087; however, 37% of pairwise comparisons were significant. Thus, we were able to find a significant isolation by distance, supporting our first hypothesis. We did not find a significant difference in dispersal ability of *B. cognatus* among different plant communities, leading us to reject our second hypothesis. However, our results suggest that the Rio Grande may act as an effective dispersal route for the Great Plains Toad, agreeing with our third hypothesis.

Microsatellites.—There were large differences in allelic richness and heterozygosity between the Great Plains Toads in this study and those in the Gonzalez et al. (2004) study in northwestern Texas. This is despite a distance of only around 600 km between the two areas, one order of magnitude greater than the largest distance between sites in this study. While this Euclidean distance is not great, the differences may be explained by the existence of two mountain ranges separating the study sites and the lack of connection by any temporary or permanent waterways.

Genetic structure.—While not entirely indistinguishable from one another, or panmictic, the Great Plains Toad breeding sites covered in this study are characterized by low levels of genetic differentiation. This was also found by Chan and Zamudio (2009) in *B. cognatus* for populations separated by 20–60 km, approximately the same scale as in our study, as well as by Masta et al. (2002) for *B. woodhousii* covering a much larger scale (>1,000 km). We found 30 other studies that documented both genetic distances (average or pairwise F_{ST} or equivalent) and geographic distances between sites. Genetic differentiation occurred at a smaller scale than in our study for 17 out of the 23 species. The remaining six species exhibited differentiation at a scale that is consistent with our results for *B. cognatus*. No paper reported a complete lack of differentiation occurring at the scale of this study (<100 km) for any species. While we cannot rule out a reporting bias (i.e., that researchers who do not find restricted gene flow among the populations they study are less likely to publish), *B. cognatus* appears to be more genetically homogenous than many anuran species.

Explaining the pattern of genetic variability among any group of populations can be difficult, as many demographic and environmental and historical factors affect gene flow and differing population histories may lead to the same current pattern (Felsenstein, 1982; Excoffier, 2001; Masta et al., 2002; Chan and Zamudio, 2009). Great Plains Toads exhibit several demographic properties that may lead to high levels of gene flow across the spatial scale studied. Multiple dispersal events per generation ($N_m > 1$) among disparate Great Plains Toad breeding sites in southern New Mexico could explain the low but extant levels of genetic differentiation seen in this study. Our $N_e m$ estimate (2.94) using the private alleles method is above that threshold and supports the hypothesis of multiple dispersals.

Site fidelity is often assumed to be widespread among amphibians in the literature (Marsh and Trenham, 2001; Smith and Green, 2005). However, only a few studies have tested this assumption of site fidelity (Marsh and Trenham, 2001), and while several species have shown almost complete site fidelity for adults (Berven and Grudzien, 1990; Driscoll, 1999), others have shown significant num-

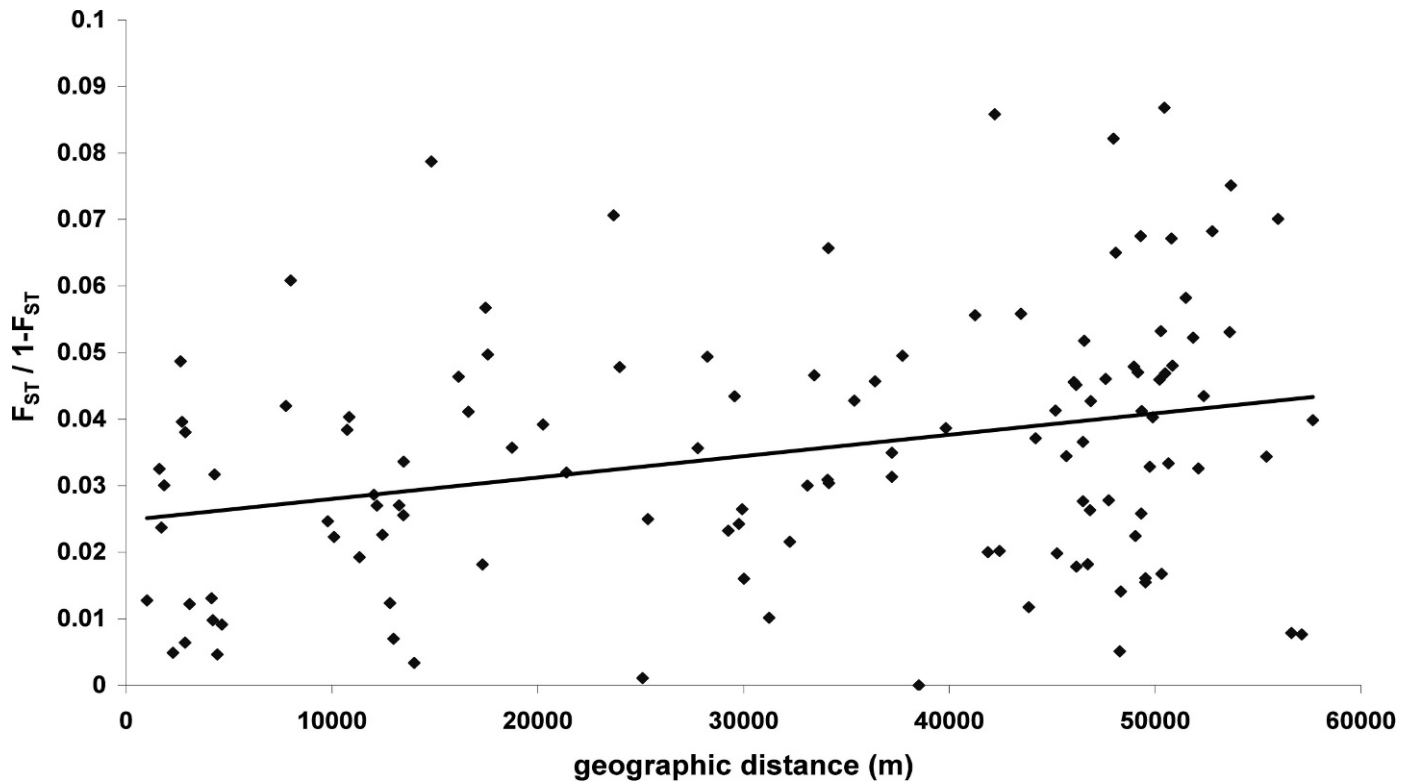


Fig. 2. Linearized F_{ST} plotted against geographic distance between all pairs of sampling sites. The solid line represents the best-fit linear regression ($P = 0.007$, $r = 0.3628$, 10,000 permutations).

bers of dispersers (15–25%) between years (Oldham, 1966; Reading et al., 1991; Sinsch, 1992). The degree to which *B. cognatus* exhibits site fidelity is unknown (but see Krupa, 1994). Furthermore, in a highly stochastic pond environment, such as the playas and stock tanks of the Chihuahuan Desert, it would be adaptive for amphibians to be capable of moving between several potential breeding localities (Fortuna et al., 2006; Chan and Zamudio, 2009). Having low site fidelity in the context of an unpredictable environment may allow amphibian populations to be robust to periods of drought as breeding can occur opportunistically in those areas that receive sufficient precipitation and runoff.

Bufo cognatus may be capable of long distance migration. Migration of distances greater than 10 km have been recorded in several anuran species (Stumpel and Hanekamp, 1986; Vos et al., 2000; Hayes et al., 2001), though such long distance dispersal has been observed in only about 7% of anuran species in the literature (Smith and Green, 2005). *Bufo cognatus* has been documented moving up to 1036 m when migrating seasonally between breeding and wintering grounds (Ewert, 1969). Dispersal, however, can involve faster and more directed movements than these seasonal migrations for some species (see Van Dyck and Baguette, 2005 for a review). Bragg and Brooks (1958) documented highly directional and long-distance movement in mass congregations of juvenile *B. cognatus* and estimated that these toads could disperse several miles within 24 hours.

Great Plains Toads were present in over half (51.1%) of potential breeding sites surveyed in this study and in many of them were abundant. Great Plains toads were found to be one of the three most common anurans in the high plains of Texas (Anderson et al., 1999) and in our study area were also among the most abundant anuran species. Given a large

population size, genetic drift would be expected to work more slowly and the equilibrium state between drift and migration would shift towards a state of genetic homogeneity (Wright, 1969).

Our study does not address the possibility that the lack of genetic differentiation in *B. cognatus* is related to likely recent (11,000–15,000 ybp) range expansions into this area. Vegetation and climate before this time were not suitable for the toad, and fossil evidence of its presence is absent (Martin and Mehringer, 1965; van Devender and Spaulding, 1979; Holman, 1995; Holmgren et al., 2003). However, Chan and Zamudio (2009) suggest that we should still see evidence of local population differentiation as a result of restricted population connectivity, and because we do not, the patterns are a result of current gene flow and not historic range expansions. Gene flow can thereby be active (toad movement) or inactive (human induced).

In order to ascertain the actual causes of the relatively homogenous genetic structure observed in these Great Plains Toad populations, further study is needed. This research could focus on demographic properties relevant to gene flow such as the level of site fidelity or rates of dispersal.

Landscape cover analysis.—Our analysis did not support the hypothesis that plant communities in the Chihuahuan Desert in south-central New Mexico differ in their resistance to gene flow in *B. cognatus*. We found indications of a positive association between the presence of breeding Great Plains Toads at ephemeral pools and mesquite cover in the surrounding landscape (Jungels, unpubl. data); however, a plant community that best supports survival may not be the most conducive to long distance dispersal (Van Dyck and

Baguette, 2005). In addition, the vegetation cover in the Chihuahuan Desert of southern New Mexico has seen radical changes in the last hundred years with the loss of grasslands and subsequent invasion of scrublands (Wilson and MacLeod, 1991). Allelic variation between populations in *B. cognatus* may not reflect these recent changes due to the slowness of genetic drift.

The Rio Grande appears to act as a low resistance route of dispersal; however, we use caution in interpreting these results for several reasons. First, our results correlating least-cost distance for the river model with F_{ST} while holding Euclidean distance constant was of only borderline significance. After eliminating populations at which only a small number of genetic samples were taken from this analysis, it became non-significant. Secondly, estimates of genetic differentiation between populations were made with only a small number of microsatellite loci (three), limiting the strength of the estimates. However, the loci used were highly polymorphic, and modeling has shown that polymorphism in microsatellite loci is as important as the number of loci used in determining the precision of differentiation estimates (Kalinowski, 2002). Finally, allele frequencies in a group of populations may be strongly affected by the history of gene flow and not represent current levels (Crow and Aoki, 1984). This is because genetic drift is often a slow process and that it may not currently be in equilibrium with gene flow. However, unlike vegetation cover in the Chihuahuan Desert, the Rio Grande, while modifications and changes of course have occurred, has been relatively stable for a long period of time. As such, we cautiously interpret our results as the Rio Grande connecting distant populations of *B. cognatus* as a route of dispersal, which suggests that rivers in general could act as dispersal routes throughout the range of *B. cognatus*.

ACKNOWLEDGMENTS

We are indebted to C. Gopalan for the use of her lab as well as her kindness and courtesy. A great deal of technical support was graciously offered by C. Steele in GIS and C. Ross in the isolation and amplification of DNA. We owe many thanks to D. Cowley for his valuable edits and suggestions and W. Gould for his quick response and assistance. We thank D. Alkov for long nights of field assistance and humor. This project was made possible through funding from the National Science Foundation-funded ADVANCE Institutional Transformation Program at New Mexico State University, fund #NSF0123690 and T&E, Inc.

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