

Molecular evaluation of the metapopulation structure of
Lowland Leopard Frogs (*Rana yavapaiensis*) in the
Bill Williams River drainage, of western Arizona.

Nicolas Benedict – Ph.D.

University of Denver
Department of Biological Sciences
Denver, CO 80208

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Introduction

Rana yavapaiensis

The genus *Rana*, to which over 300 species have been ascribed (Tanaka *et al.*, 1994), is believed to have diverged from other genera about 40-50 million years ago (Hillis, 1988). Included among these are over two dozen “morphologically conservative” species of Leopard Frog, broadly referred to as the Leopard Frog Complex (*Rana pipiens* Complex) (Hillis *et.al.*, 1983). This classification was largely based on the characterization of allozyme products from 50 gene loci. The species fall into two broadly sympatric groups, delineated as the Alpha and Beta divisions. However, a geographic dichotomy exists in each of these divisions, suggesting further subdivision of these into North and Middle American species (Hillis, 1998). Though revisions to this original taxonomy have been suggested (Benedict, unpublished data; Hillis, personal communication), this phylogeny remains the most current.

The greatest diversity of Leopard Frogs is found in the southwestern United States. Its members are believed to have been highly adaptable to climatic changes and to have been early invaders into newly available habitat for at least the past 11-12,000 years (Holman, 1995). Given that the Sonoran desert is relatively young (<10,000 years)(MacMahon, 1992), their modern range and persistence in geographically isolated habitats is likely a consequence of such an early expansion into these habitats.

Lowland leopard frogs (*Rana yavapaiensis*) were originally described by Platz (1984). Historically they have been found throughout the southwestern United States and into northern Mexico; from eastern California to western New Mexico and from southern Nevada to northern Sonora, Mexico (Sredl, In Press). As is the case with other species of leopard frogs, they have been subject to multiple and severe anthropogenic impacts in the form of habitat alteration and

fragmentation, changing water use practices, and the introduction of exotic species. Additional factors such as the spread of the *chytridiomycosis* fungus also have resulted in large-scale population mortality (Sredl, In Press). Consequently many historical populations have likely become extinct, further isolating extant populations.

Metapopulations

Of relevance to wildlife managers is the extent to which relationships exist between populations in terms of gene flow and do they in effect constitute a larger metapopulation, or “Population of Populations” (Debinski, 1994). Functional metapopulations contain persistent source populations providing individuals for immigration into other populations.

Metapopulations can also include more ephemeral sink populations that do not contribute individuals to other populations in the metapopulation structure. The strength of the metapopulation lies in its resilience to stochastic events which can effectively eliminate individual populations. If the corridors that enable the metapopulation to function remain, these stochastic population extinctions can often be mitigated by re-colonization of individuals from surviving source populations. Thus, the identification of persistent source and ephemeral sink populations, along with the mechanisms and dispersal corridors maintaining them, allows for better management of imperiled species (Sredl, 1997; Soule, 1987).

Amphibian spatial dynamics closely resemble classic metapopulation models (Marsh and Trenham, 2001). Their highly philopatric tendencies coupled with typically poor dispersal ability result in what is often referred to as a “Ponds as Patches” mosaic (Marsh and Trenham, 2001). Short-lived amphibian populations, which undergo population fluctuations in excess of 200-300 fold (Sartorius and Rosen, 2000; Scribner *et al.*, 1997; Beebee, 1996; Weitzell and

Panik, 1993; Pechmann *et al.*, 1991), are particularly susceptible to both stochastic processes and density dependent effects (Sjoren, 1991) resulting in local extinctions in relatively few years (Marsh and Trenham, 2001). Such local extinctions are normal (Sjoren, 1991) and can be mitigated through recolonization from other nearby populations (Marsh and Trenham, 2001; Hecnar and M'Closkey, 1996; Sjoren, 1991).

The long-term persistence of amphibian complexes is thus dependent on assemblages of interconnected, rather than individual populations. This requisite connectivity is often a function of the distribution and composition of surrounding terrestrial habitats, whose usage as suitable distribution corridors is principally a function of land use, topological distances (Reh and Seitz, 1990), climate and hydrology. Though each of these factors plays a roll in our study area, perhaps the most dramatic is that of hydrology.

Study Area

The Santa Maria River is an intermittent stream located principally in the Basin and Range Province of southern Arizona with its headwaters in the central highlands of western Arizona (Chronic, 1983) and flowing southwest into Alamo Reservoir (Figure 1). Portions of this river contain surface water and lush riparian vegetation (cienega) throughout the year. These cienega assemblages typically include alder (*Alnus oblongifolia*), various willow species (*Salix sp.*), velvet ash (*Fraxinus velutina*), cottonwood (*Populus fremontii*), box elder (*Acer negundo*) and walnut (*Juglans major*). Other sections consist of large expanses of alluvial stream-bed that remain dry throughout most of the year. These sections rarely contain surface water, but periodically become flooded during the summer rainy season.

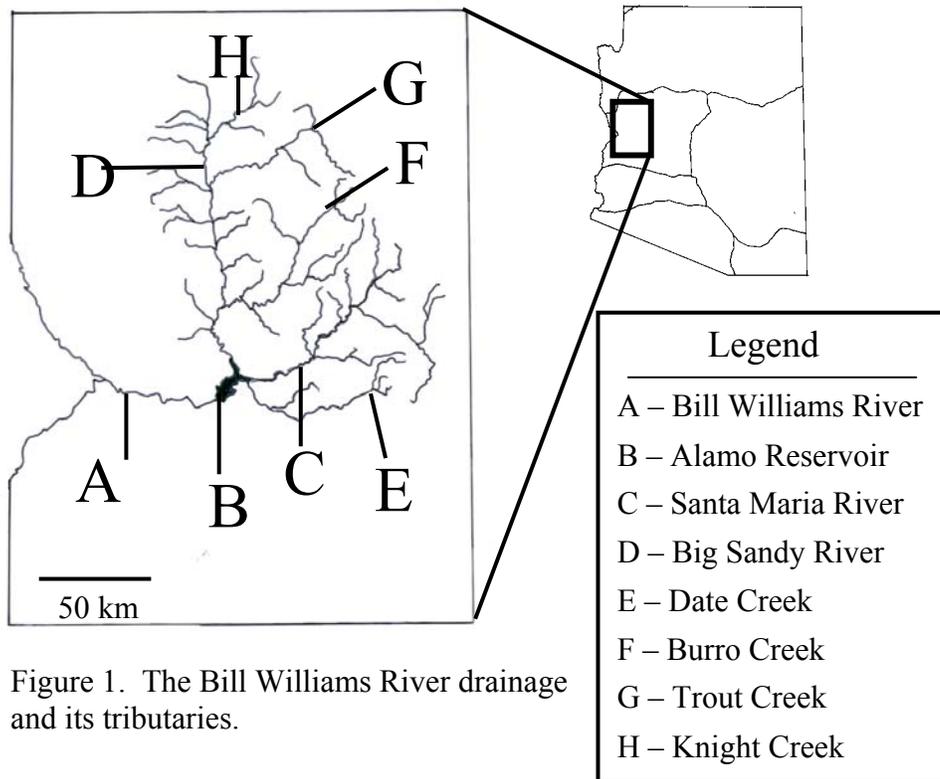


Figure 1. The Bill Williams River drainage and its tributaries.

Tributaries of the Santa Maria River consist chiefly of low-lying streams (~450-600 m) of gentle gradient and higher elevation (~1000 m) springs (Sycamore Spring, Peoples Canyon and Cottonwood Canyon) (Figure 2). In each case persistent stream flow is intermittent and, with the exception of extreme flood events, is not continuous throughout its reaches. These higher elevation sources are typically tinajas (rock bound pools), separated by large expanses of essentially barren rock and interspersed with typical Sonoran Desert-scrub (Brown, 1982).

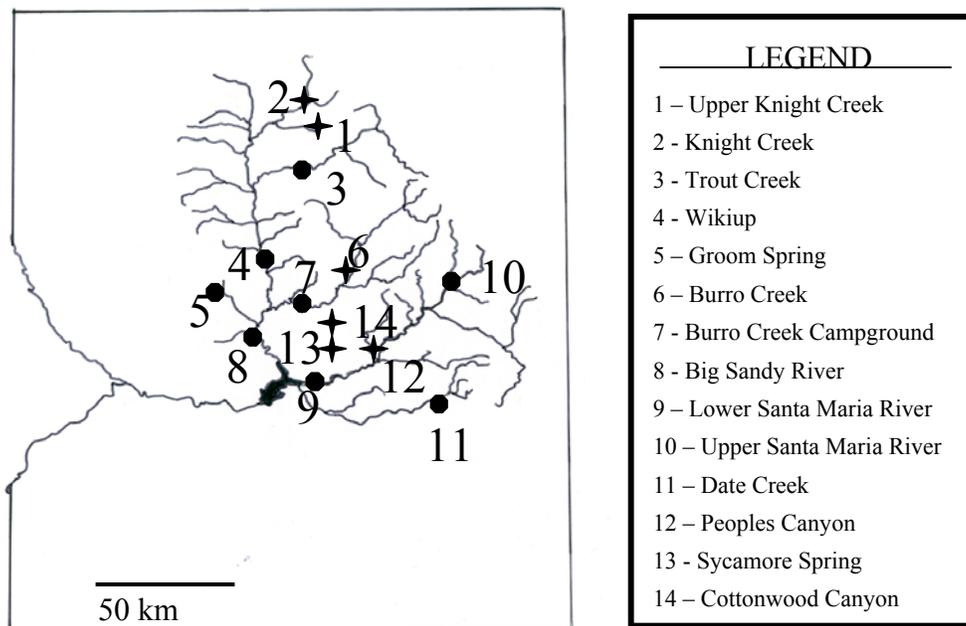


Figure 2. Location of populations sampled.
 ✦ Refers to sites composed of tinajas

The Santa Maria River is also an intermittent stream located in the Basin and Range Province of southern Arizona with its headwaters in the central highlands of western Arizona (Chronic, 1983) and flowing south into Alamo Reservoir (Figure 1). It is similar to the Santa Maria in its associated topography, vegetation, hydrology and scale of both low-lying reaches (Trout Creek, Groom Spring and Wikiup) and higher elevation tributaries (Knight Creek, Upper Knight Creek, and Burro Creek) (Figure 2).

Alamo Reservoir is the result of an artificial impoundment of the Bill Williams River just below the confluence of the Big Sandy and Santa Maria rivers (Figure 1). Its creation eliminated a significant stretch (~ 20 km) of native Sonoran cienega which was contiguous through its confluence with the Big Sandy and Santa Maria rivers. The large open water expanse which replaced it is heavily stocked with non-native predatory fish and is largely contained by a

barren rock landscape interspersed with patchy exotic vegetation types, dominated by saltcedar (*Tamarix chinensis*). The Bill Williams River flows from Alamo Reservoir to its confluence with the Colorado River at Lake Havasu.

Methods

Field Collections

The study area is situated in the Bill Williams River drainage of west-central Arizona. This roughly 16,800 square kilometer region encompasses the Bill Williams River, Alamo Reservoir and large portions of both the Santa Maria River and the Big Sandy River, along with their tributaries (Figure 1).

DNA samples were collected from a total of 16 populations (Figure 2) throughout the study area. This number was subsequently reduced to 14 by pooling three sites from the lower arm of the Santa Maria River. These three sites are located along approximately a 5 km contiguous stretch of persistently open and slow moving water. Given that other *Rana* species have been shown to move up to 3 km/season (Beebee, 1996; Berven and Grudzein, 1990) and that *Rana yavapaiensis* have been documented to move over 2 km (Sredl personal communication), these frogs likely constitute one large breeding population. The number of individuals captured per site ranged from a high of 23 (Groom Spring), to a low of 1 (Burro Creek Campground and Date Creek), with a mean of 9.6 (Table 1). These sites consist of open water river channels (Upper and Lower Santa Maria River, Burro Creek, Burro Creek Campground, Big Sandy River, Wikiup, Trout Creek and Date Creek), higher elevation bedrock

seeps (Cottonwood Canyon, Sycamore Spring, Peoples Canyon, Knight Creek, Upper Knight Creek) and a relatively open cattle pond/spring (Groom Spring).

Table 1. Populations Sampled

Name	n=	Number of Visits
Upper Knight Creek	11	2
Knight Creek	5	2
Trout Creek	11	4
Wikiup	11	3
Groom Spring	23	4
Burro Creek	4	3
Burro Creek Campground	1	4
Big Sandy River	6	6
Lower Santa Maria River	19	6
Upper Santa Maria River	10	3
Date Creek	1	1
Peoples Canyon	10	1
Sycamore Spring	11	1
Cottonwood Canyon	12	2

Samples were collected along open watercourses and from shallow water pools using a fine mesh net, while probing grasses and bushes with a long handled reptile hook. Small pools were systematically searched both using the reptile hook and by hand. Most readily accessible sites were visited multiple times in different seasons (Table 1). Non-destructive sampling was performed by removing small toe-clippings (6-8mm) from frogs and froglets (Heyer *et al.*, 1994; Carey, 1992; Campbell, 1970). These samples were subsequently stored in a preservation buffer (Seutin *et al.*, 1991) at room temperature. In cases where tadpoles were the only available source of tissue, small tail-clippings were used, though DNA extractions from these sources proved to be more problematic.

Location data were either; recorded with a Magellan GPS Color Track (using triangulation from a minimum of five and a maximum of twelve geostationary satellites), provided to us by the Arizona Game and Fish department, or the U.S. Fish and Wildlife service.

Population sizes were estimated based on the number of individuals observed and the habitat size. This estimation does not take into account the relative difficulty of locating frogs at different life stages. Only frogs and froglets were included in the count, as tadpoles do not contribute to the effective population size. Though these counts are extremely crude, they were performed in a consistent manner among the populations surveyed. As such they provide an estimate only of relative population sizes at the time the surveys were made.

DNA preparation and analysis

DNA was extracted using either a phenol-chloroform based protocol, as described by Kahn *et al.* (1999) or the Wizard Genomic DNA Purification System (Promega), following the manufacturers instructions.

Genomic DNA of 16 individuals from 8 geographically distant sites was pooled to maximize the number and sizes of targeted microsatellite fragments for enrichment purposes. This pooled DNA was used in an enrichment procedure in which oligonucleotide linkers were attached to the genomic DNA as described by Hamilton *et al.* (1999), with the following modifications. A 2X overdigestion of genomic DNA was performed using *HaeIII* and *Sau3a*. Hybridization of the linker ligated DNA to a CA(12) biotinylated probe was performed at 65 c, as were the washing steps. Detection of positive clones was performed using the CDP-Star chemiluminescent detection kit (Tropix). Amplification was performed using a MJ Research PTC-2000 peltier thermocycler. Sequencing was performed using the dye terminator cycle

sequencing reaction and visualized with the Beckman-Coulter CEQ2000 automated sequencer, following the manufacturers' instructions.

Primers flanking the microsatellite inserts were designed visually, upon inspection of sequence from positive clones. Subsequent PCR optimization was initially performed on a gradient thermocycler (Eppendorf). Additional optimization to allow for successful amplification on different manufacturers Thermocycler (MJ Research) involved adjusting annealing temperatures. One primer from each pair, that consistently produced clean PCR products, was modified by adding a fluorescent dye (D1, D2, D3, or D4) to its 5' end (Research Genetics). Amplification of samples was then performed incorporating fluorescent primers and visualized using the microsatellite protocol on a Beckman-Coulter CEQ2000 automated sequencer, following the manufacturers' protocol. Alleles were identified using the CEQ-Fragment Analysis Program (version 2.0.0). The software was set to allow for the detection of +A peaks and stutter bands and spurious peaks that were less than 50% of the height of the maximum peak.

Sequencing of a portion of the mitochondrial cytochrome *b* gene was performed using the primers L14841 and H15149, as described in Kocher *et al.* (1989). Initial amplification and visualization followed the protocol of Kahn *et al.* (1999), using the following profile; 32 cycles at 92 c for 40 seconds, 54 c for 1 minute and 72 c for 2 minutes, followed by a final cycle of 72 c for 5 minutes. Double strand products were cleaned using Amicon Microcon-PCR Centrifugal Filter Devices (Millipore), following the manufacturers instructions. The cycle sequencing and subsequent purification of the products was performed using the Beckman-Coulter cycle sequencing kit, following the manufacturers' instructions. Visualization was performed on a CEQ2000 automated sequencer (Beckman-Coulter). Alignment of resultant sequences was done using the DNAsis software package (Hitachi).

Five individuals from each of the Upper Knight Creek, Groom Spring, Cottonwood Canyon, the Upper Santa Maria River and Wikiup populations were sequenced at the mitochondrial cytochrome b locus. These sites were chosen based on their geographic distances and that they encompass the entire study area.

Data Analysis

Data analyses were performed using the Tools for Population Genetic Analysis software package (TFPGA) version 1.3 (Miller, 1997). All of the genetic distance models available in this package were evaluated using the default settings (the only options being the format to output results). Descriptive statistics were obtained from the entire data set and included: allele and heterozygosity frequencies, locus heterozygosity, average heterozygosity per locus and percent polymorphic loci. Hardy-Weinberg equilibrium was evaluated using the exact test option from a set of pooled genotypes. The Mantel test was performed using the genetic distance and each geographic distance measure, incorporating the LOG transform elements of Matrix #1 and #2 options.

Frog locations were added to a GIS database developed with data layers obtained from the Arizona Land Resource Information System (ALRIS). All data layers were obtained in Arcexport format (.e00 file format) for use in Environmental Systems Research Institute (ESRI) ArcView ver. 3.2, ArcInfo ver. 8.02, and ArcInfo GRID ver. 8.02 GIS software. While many data layers were obtained, only the hydrology and topographical layers were used in this study. Because of the extremely ephemeral nature of the streams in the study area, all fourth and fifth order streams were eliminated from the hydrology dataset, except for those directly downstream from a population location. Elevation data was obtained in the form of 12 United States

Geological Survey (USGS) quadrangle Digital Elevation Models (DEM) with 30 meter resolution. All 12 quadrangle datasets were mosaiced in ArcInfo GRID to give a unified elevation dataset for the entire study area.

The GIS was used to assess geographic distances using both overland and stream channel distances. The overland distances between populations were obtained by simply measuring a straight line distance in ArcView. Stream channel distances were obtained by selecting all stream channel segments between two populations and then summing the length of all selected segments in the ArcView database.

Results

Only a single haplotype was identified at the mitochondrial cytochrome *b* locus. This haplotype is identical to what has been identified in other Arizona populations of *R. yavapaiensis* (Coon Creek and Cave Creek, AZ).

Approximately 15,000 colonies were obtained using the microsatellite enrichment technique. Roughly 10% of those were subsequently shown to hybridize to the corresponding dinucleotide probes. Three hundred and eighty potential positives were sequenced, of which 23 contained a matching microsatellite repeat. These repeats often were too short to be informative, and/or contained insufficient flanking sequence from which to design appropriate primers. Additional positives existed from which adequate and unambiguous sequence through the repetitive regions was unobtainable.

Primer pairs were designed for 10 loci. Three of these proved to successfully and consistently amplify polymorphic products in *Rana yavapaiensis*. These were subsequently

named *Rana yavapaiensis*-1 (RY-1), *Rana yavapaiensis*-2 (RY-2) and *Rana yavapaiensis*-3 (RY-3) (Table 2).

Table 2. Microsatellite primers developed for *Rana yavapaiensis*
Repeat sequences are for the smallest repeat units observed

Loci	Primer sequences (5'-3')	Repeat Sequences	Repeat Number	Size Range (b.p.)
RY-1	A: TTAGCTGATTTGCTGCAGAC B: AAGCCGAGTACGCACATCTG	(AC) ₇	5	132-144
RY-2	C: GTGTGCGGCAGGCCATGTGC D: GGCATATCCATTTGATGGG	(CA) ₆ CC(AC) ₄ ACC(AC) ₆	9	160-180
RY-3	A: GCGCTCTGACCCCTGAAG B: GTANNCAGNAGTTGTCTTCTGC	(GT) ₄ CT(GT) ₁₀ GC(GT) ₂	8	124-144

All populations were screened using these three primer pairs and genotypes determined (Table 3).

All populations from which multiple individuals were sampled contained one or more polymorphic loci, with the exception of Burro Creek. The average number of alleles per locus was 7.3. The average number of alleles per population, excluding populations from which only a single individual was found (Date Creek and Burro Creek Campground), was 2.25 (RY-1), 4.75 (RY-2) and 3.41 (RY-3). Heterozygosity was 0.266 (RY-1), 0.816 (RY-2) and 0.744 (RY-3). The value for RY-1 is skewed by the fixation of the R allele in all of the Big Sandy River populations. However, when including only Santa Maria River populations the heterozygosity for RY1 was 0.647.

TABLE 3

With the exception of Cottonwood Canyon, all of the Santa Maria River drainage populations (Lower Santa Maria, Upper Santa Maria, Sycamore Spring, Peoples Canyon, Cottonwood Canyon and Date Creek), are in Hardy-Weinberg equilibrium ($p < 0.05$) at all loci. Populations that are fixed at a given locus were also not included in this analysis. None of the Big Sandy River Drainage populations (Upper Knight Creek, Knight Creek, Trout Creek, Burro Creek, Wikiup, Big Sandy River and Groom Spring) were tested for Hardy-Weinberg equilibrium at the RY-1 locus, as they were monomorphic. Neither of the Knight Creek populations were in Hardy-Weinberg equilibrium at locus RY-2, though the rest of the Big Sandy River populations were. Trout Creek was the only Big Sandy River population not in Hardy-Weinberg equilibrium at locus RY-3. Note that though several populations were in Hardy-Weinberg equilibrium, it is not entirely clear that they satisfy the underlying assumptions of this model. Specifically, there likely are overlapping generations (Sartorius and Rosen, 2000; Platz *et al.*, 1997) and though population sizes at times may be large, they undergo periodic, but extreme, fluctuations in size (Weitzel and Panik 1993, Pechman *et al.* 1991). Furthermore, the power of this test tends to be low. This analysis was primarily performed to ensure that there was not a fundamental problem with null alleles at any given locus.

All of the Big Sandy River drainage populations are monomorphic at the RY-1 locus, containing only the R allele. Conversely, all of the Santa Maria River drainage populations, except Date Creek, are polymorphic at this locus, containing as many as four alleles per population. Of the five alleles found in these populations collectively, only the W allele is unique to a single population (Sycamore Spring). At the RY-2 locus, 5 alleles are found in both drainages. The J allele is unique to Knight Creek and Upper Knight Creek, while the Z and F

alleles are found only in the Santa Maria River drainage populations, though they are both shared among two or more Santa Maria River drainage populations. At the RY-3 locus, alleles A, B, and D are unique to the Knight Creek drainage. At this locus, no other alleles are unique to either a population, or drainage.

Genetic distances were evaluated using the Nei (1972), the Nei (1978) minimum distance and the modified Rogers (Wright 1978), distance measures. Given that there were no dramatic differences between the distances generated using these techniques, only the Nei (1972) genetic distances were used for further statistical analyses (Table 4).

Table 4. Genetic Distances among populations surveyed.

Calculated using the technique of Nei (1972).

	A	B	C	D	E	F	G	H	I	J	K
Trout Creek (A)											
Lower Santa Maria (B)	0.169										
Upper Santa Maria (C)	0.177	0.057									
Cottonwood Canyon (D)	0.102	0.143	0.177								
Burro Creek (E)	0.284	0.275	0.170	0.264							
Groom Spring (F)	0.088	0.071	0.138	0.154	0.328						
Big Sandy River (G)	0.065	0.113	0.129	0.125	0.212	0.057					
Sycamore Spring (H)	0.194	0.157	0.272	0.277	0.362	0.119	0.118				
Peoples Canyon (I)	0.117	0.117	0.196	0.166	0.344	0.088	0.076	0.049			
Knight Creek (J)	0.074	0.158	0.187	0.134	0.365	0.967	0.053	0.185	0.098		
Upper Knight Creek (K)	0.133	0.233	0.240	0.246	0.341	0.178	0.131	0.236	0.138	0.053	
Wikiup (L)	0.134	0.203	0.228	0.078	0.305	0.126	0.171	0.366	0.242	0.184	0.305

Populations from which only a single individual was sampled (Burro Creek campground and Date Creek) were not included in these mathematical analyses. Though subtle topological distinctions were apparent between these measures, they exhibited an overall congruence (Figure

3). The *A*, *B*, *C*, *H*, *I* and *J* nodes were consistent using all distance measures. The grouping, or lack thereof, of Knight Creek and Upper Knight Creek was inconsistent when using different distance measures (node *D*). Burro Creek, the only monomorphic population, consistently failed to group with any population (node *J*). Wikiup was the only other Big Sandy River drainage population which failed to group with the other Big Sandy River drainage populations, using all distance matrices (node *A*).

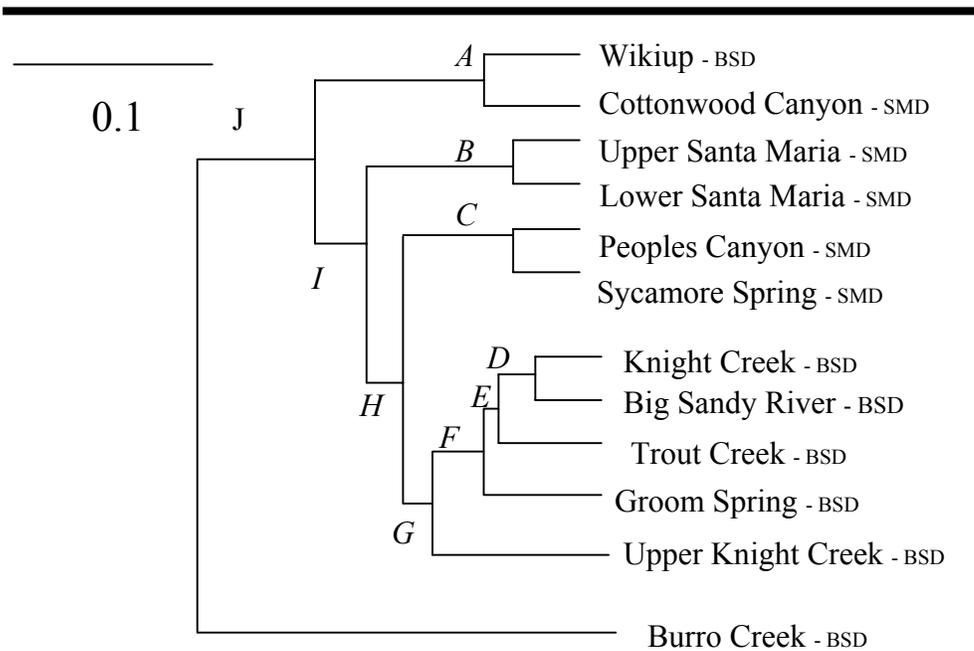


Figure 3. UPGMA tree of microsatellite data.
 Calculated using the Nei (1972) genetic distance measure.
 SMD = Santa Maria River Drainage
 BSD = Big Sandy River Drainage
 Letters *A-J* refer to nodes for discussion purposes.

Both river channel and direct overland geographic distances were calculated among all populations (Table 5).

Table 5. Geographic distances between populations, in kilometers.

River channel distances (a) are in the upper matrix, while direct overland distances (b) are in the lower matrix.

	A	B	C	D	E	F	G	H	I	J	K	L
Trout Creek (A)	0.0	127.5	187.5	187.5	150.9	109.7	56.9	176.0	167.2	68.5	80.5	62.7
Lower Santa Maria (B)	78.1	0.0	60.1	60.1	95.5	53.8	70.6	48.5	39.7	134.5	146.5	64.8
Upper Santa Maria (C)	67.8	38.4	0.0	45.3	155.6	113.9	130.6	32.7	33.4	194.6	206.6	124.8
Cottonwood Canyon (D)	65.9	15.7	24.9	0.0	155.6	113.8	130.6	28.1	35.2	194.6	206.6	124.8
Burro Creek (E)	44.6	37.0	35.7	22.5	0.0	78.0	66.0	116.1	107.2	158.0	170.0	66.0
Groom Spring (F)	53.3	42.7	62.3	41.8	38.9	0.0	52.8	102.3	93.5	116.8	128.8	47.0
Big Sandy River (G)	36.6	50.1	55.3	40.5	28.8	17.3	0.0	144.1	110.3	64.0	76.0	5.8
Sycamore Spring (H)	67.3	17.4	21.9	3.6	23.2	45.5	43.5	0.0	26.5	183.1	195.1	113.3
Peoples Canyon (I)	70.7	18.2	20.2	7.6	26.3	49.5	47.7	4.2	0.0	174.2	186.2	104.5
Knight Creek (J)	15.5	91.2	83.0	80.2	59.3	61.3	45.5	81.9	85.4	0.0	12.0	69.8
Upper Knight Creek (K)	12.9	90.4	78.7	78.5	57.0	64.7	48.8	79.9	83.0	9.2	0.0	81.8
Wikiup (L)	40.0	42.7	51.7	36.1	26.0	16.0	38.9	38.9	42.9	50.9	52.5	0.0

These distances were used to better evaluate a simple “stepping-stone” model of dispersal and connectivity, as discussed by Kimura (1994). Evaluation of the goodness of fit between the direct overland geographic distance and the calculated genetic distances, using the mantel test, resulted in no correlation ($r=0.0207$, $df=11$; Figure 4).

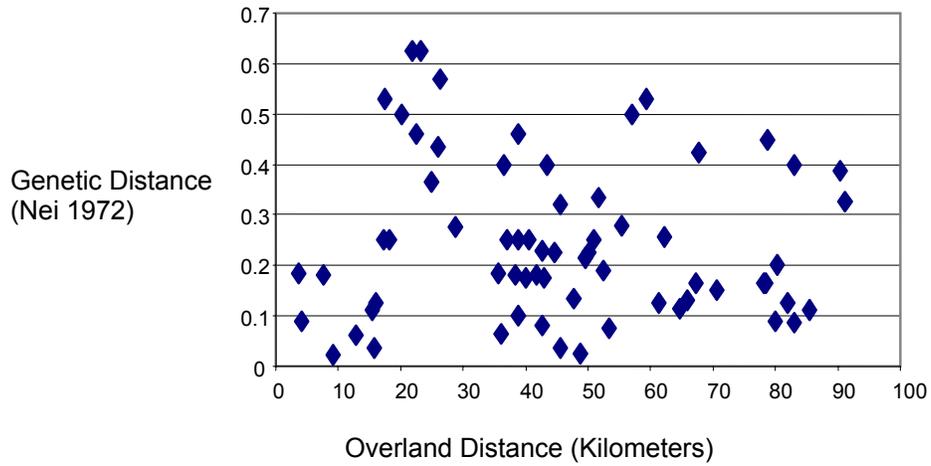


Figure 4. Dot Plot of Mantel Test Results.
Genetic Distance vs. Overland Geographic Distance

Given the inhospitable nature of the habitat over which these frogs would have to migrate in a direct overland route, the same analysis using river channel distances was performed. Though this resulted in a substantially better correlation, it was not statistically significant ($r=0.4808$, $p>0.05$, $df=11$; Figure 5). Nonetheless, these results suggest that there is some type barrier precluding the dispersal of these frogs along a simple distance gradient.

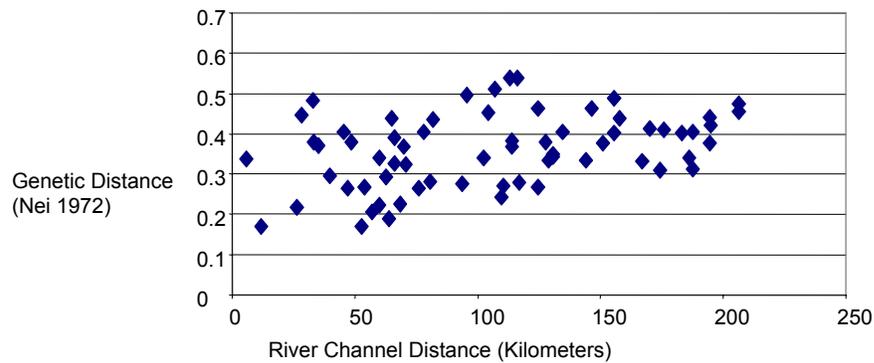


Figure 5. Dot Plot of Mantel Test Results.
Genetic Distance vs. River Channel Distance

Discussion

The number of frogs sampled and the sizes of the populations from which they came, varied dramatically. However, the genetic variability detected does not directly reflect the size of the populations from which they came. Of ultimate importance is the effective population size (N_e), as observed under maximum population contraction. The determination of N_e in species such as amphibians, which undergo population fluctuations in excess of 200-300 fold (Sartorius and Rosen, 2000; Scribner *et al.*, 1997; Beebee, 1996; Weitzell and Panik, 1993; Pechmann *et al.*, 1991), is problematic. Without long-term monitoring it is difficult to evaluate what cycle a particular population is in at any given time.

Mitochondrial cytochrome *b* data have been shown to be informative in addressing other questions of interest regarding members of the *R. pipiens* complex (Benedict, unpublished data). Nonetheless, only a single haplotype was found among these populations, making it uninformative for inferences within these populations.

Big Sandy River Drainage

The largest sample in this study was obtained from Groom Spring, which also was the largest population. Although this spring is found at the headwaters of a tributary to the Big Sandy River, it is relatively low lying (963 m) in relation to other headwater sites. It consists of an unconsolidated alluvium bed and currently receives heavy use as a cattle pond. These unconsolidated alluvial tanks are typically subject to dramatic fluctuations in water levels and often dry up entirely (Sredl, 1997; Meefe and Minckley, 1987). Though the impacts of its use as a cattle pond are evident, a large population (>2,000), composed of all age classes is found. Without long-term monitoring, it is impossible to determine if this large population is a stable feature, or if it cycles dramatically in numbers, as has been observed in other cases (Sartorius and Rosen, 2000; Scribner *et al.*, 1997; Beebee, 1996; Weitzell and Panik, 1993; Pechmann *et al.*, 1991). Future monitoring could both better evaluate population cycling and further elucidate the close interaction between these frogs and the resident cattle. As was the case with every population on the Big Sandy River drainage, this population contained only the R allele at the RY1 locus. A total of 9 alleles were identified in this population, none of which are unique. Conversely, 8 smaller populations contained as many or more alleles. Five of these populations (Knight Creek, Upper Knight Creek, Peoples Canyon, Sycamore

Spring and Cottonwood Canyon) are located in high elevation rockbound tinajas (bedrock pools) that likely are persistent features through all but the most extreme droughts (Sartorius and Rosen, 2000; Collins *et al.*, 1981).

The Knight and Upper Knight Creek populations are in geographically distinct tinajas found on adjacent branches of the same stream. Both branches course over a gently sloped bedrock substrate, consisting of a few widely scattered pools. These pools are likely persistent features and contain well-developed cienegas. Both Knight Creek populations share a number of unique alleles (RY2- J, RY3-D, and RY3-A), suggesting that gene flow between these populations exists. The Knight Creek population likely contained fewer than ten frogs, five of which were sampled. This estimation is based on an extremely thorough search of each square meter of this small (approximately 100 m²) and distinct pool. It is unlikely that more than many individuals went undetected. The Upper Knight Creek population consisted of 80-100 frogs, eleven of which were sampled. Given how few individuals reside in these locations, the genetic diversity retained in these populations is astounding, though not unprecedented in other species (Nichols *et al.*, 2001). These counterintuitive observations might be reconciled if these populations are in a natural low of their population cycle and/ or if they are immigrating from other, currently undetected sites. Given their genetic diversity, persistent habitat, and relatively small population size, continued monitoring would be prudent.

Burro Creek was surveyed on three different occasions, resulting in the capture and sampling of only four individuals. These frogs were monomorphic at both the RY-1 and RY-2 loci. For still undetermined reasons, successful amplification at the RY-3 locus was unsuccessful with these samples. The Burro Creek site is a well-developed

tinaja, which appears to be prime leopard frog habitat. A large upstream area, including a number of higher elevation springs, remain unsurveyed, as access to them is restricted by private inholdings, including an active copper mine. If populations exist in these upper reaches, they would likely be isolated from the lower reaches by the copper mine.

Trout Creek is a perennial, tributary stream to the Big Sandy River, flowing through a wide canyon with walls up to 50m high. It is considered an outstanding aquatic habitat with tremendous diversity of flora and fauna (McLaughlin, 1992). Robust populations of *R. yavapaiensis* were found on the main stem of this tributary, with breeding likely taking place in its upper reaches and backwaters (Sredl, personal communication). These populations were found to be genetically diverse, containing a total of 10 alleles, though none were unique to this site. Given the quality of its habitat, additional sampling from the unsurveyed upper sections of this creek is warranted.

The Wikiup and Big Sandy River populations both reside on the lower reaches of the Big Sandy River (556 and 589 m respectively). Both sites consist of well-developed cienega, with a persistent overland stream flow. Other intervening reaches dry up seasonally, as the water table drops below the alluvial surface. Though these populations contain a number of alleles (6 and 8 respectively), none are unique to these sites. Of additional interest is that every haplotype identified in these populations is found upstream of them.

Santa Maria River Drainage

Sycamore Spring, Cottonwood Canyon and Peoples Canyon are all located in the Arrastra Mountains Wilderness. Separating these from other low-lying, but proximate

populations, are steep canyons which remain dry and inhospitable throughout most of the year. As was the case in Knight Creek, these tinajas function as rare, but persistent aquatic features in an otherwise arid landscape. The bottom of some of the deeper pools (2+ m) contain large mud-filled cracks, in which an amphibian could easily burrow and aestivate during extended periods of extreme drought (Tinsley and Kobel, 1996). These deep pools also allow for better evasion from predators than do shallow habitats, as are found along the lower lying river channels (Degenhart *et al.*, 1996). These populations were intermediate in size (100-200) in that they were somewhat larger than the Knight Creek populations, but contain far fewer individuals than were found in Groom Spring. The three Arrastra Mountain populations (Cottonwood Canyon, Sycamore Spring and Peoples Canyon) contained 18 of the 22 alleles identified in all of the populations surveyed. Only one allele (RY1-W) is unique to a single Arrastra Mountain population, while an additional allele (RY2-F) is restricted to these three sites. An additional 4 alleles are shared only with downstream populations on the Santa Maria River.

The Santa Maria River, like The Big Sandy River, is a small (1-2m wide, 20-40cm deep) and slow moving stream throughout much of the year. Though persistent patches exist, many of its stretches regularly dry up, exposing the barren sandy substrate. Seasonal but extreme flooding, as a consequence of the bimodal sonoran weather patterns, results in brief periods of connectivity between these patches (Meeffe and Minckley, 1987; Sellers, 1985; Collins *et al.*, 1981). During these events, suitable habitat for the unfettered movement of amphibians exists. These relatively regular flash-floods also reshape channels and convey material downstream, in flows which may exceed $1,900 \text{ m}^3/\text{sec}^{-1}$ (Meeffe and Minckley, 1987). The lower Santa Maria River contains more

haplotypes than any other population surveyed (15). Though none of these alleles are unique to these populations, each can be identified in one or more populations upstream and four are restricted to this drainage. Two of the alleles found in both the upper and lower Santa Maria River populations (RY3-W and RY3-E) are found upstream only on the Big Sandy River Drainage. This would suggest that gene flow from the Big Sandy River drainage to the main stem of the Santa Maria River drainage occurs.

Analyses did not include the Date Creek and Burro Creek Campground sites, as they both are represented by a single individual. However, they merit a brief mention, as they are documented resident populations of the Bill Williams River drainage.

The Date Creek site is located on an upper reach of Date Creek, adjacent to the Date Creek Ranch. This site is a well-developed cienega, with deep soils, containing substantial standing water throughout all but the most severe droughts. Only one individual was found in a single survey of this site. Although additional frogs presumably exist, they remained undetected in the thick and occasionally impassable vegetation encountered. It has been reported that other reaches of this stream, located on the privately owned Date Creek Ranch, contain sizeable but unsurveyed populations of *R. yavapaiensis* (Sredl, personal communication).

The Burro Creek Campground contains a number of very deep (>5m) pools underlain by consolidated bedrock. These persistent waters contain a healthy population of canyon treefrogs (*Hyla arenicolor*), located primarily on a rock wall that drops directly into the deepest portions of the pool. This is where the single specimen of *Rana yavapaiensis* was found. More suitable habitats at this site exist, but receive a great deal

of pressure from the adjacent campground. Though other individuals likely exist, they remained undetected after four surveys of this site.

General Patterns of Allele Distribution

Nine alleles were found to be common and widespread (RY1-R, RY2-B, RY2-C, RY2-D, RY2-G, RY2-H, RY3-X, RY3-Z and RY3-F) (Figure 6a).

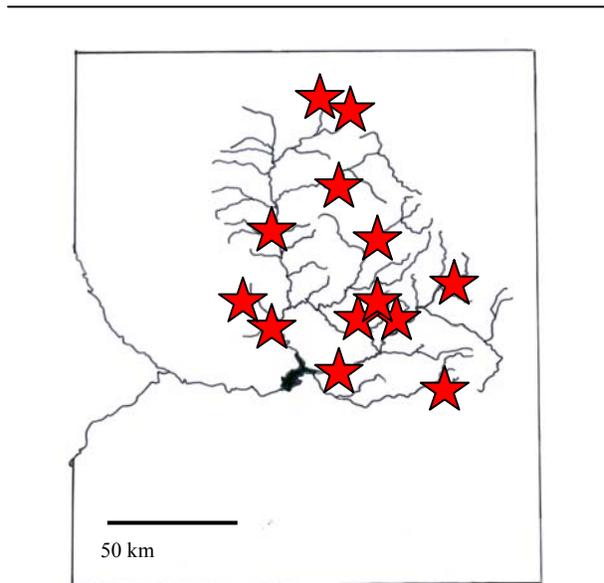


Figure 6(a). Distribution of common and widespread alleles.

Three populations contain unique alleles (RY1-W, RY3-A and RY3-B) (Figure 6b).

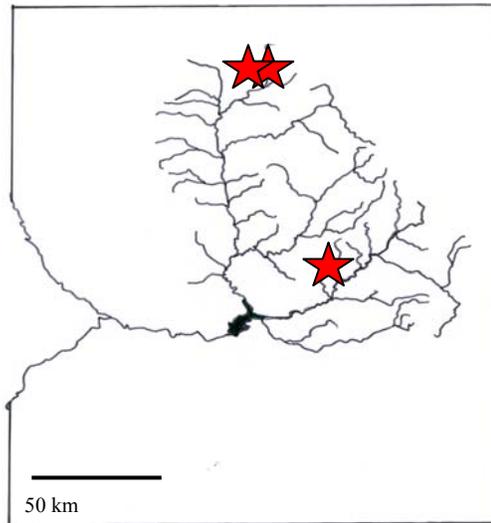


Figure 6(b). Distribution of populations containing unique alleles

Seven alleles (RY1-Q, RY1-S, RY1-V, RY2-Z, RY2-F, RY2-J and RY3-D) are shared among four or fewer populations but are restricted to either the Santa Maria (Figure 7(a)) or the Big Sandy (Figure 7(b)) river drainages.

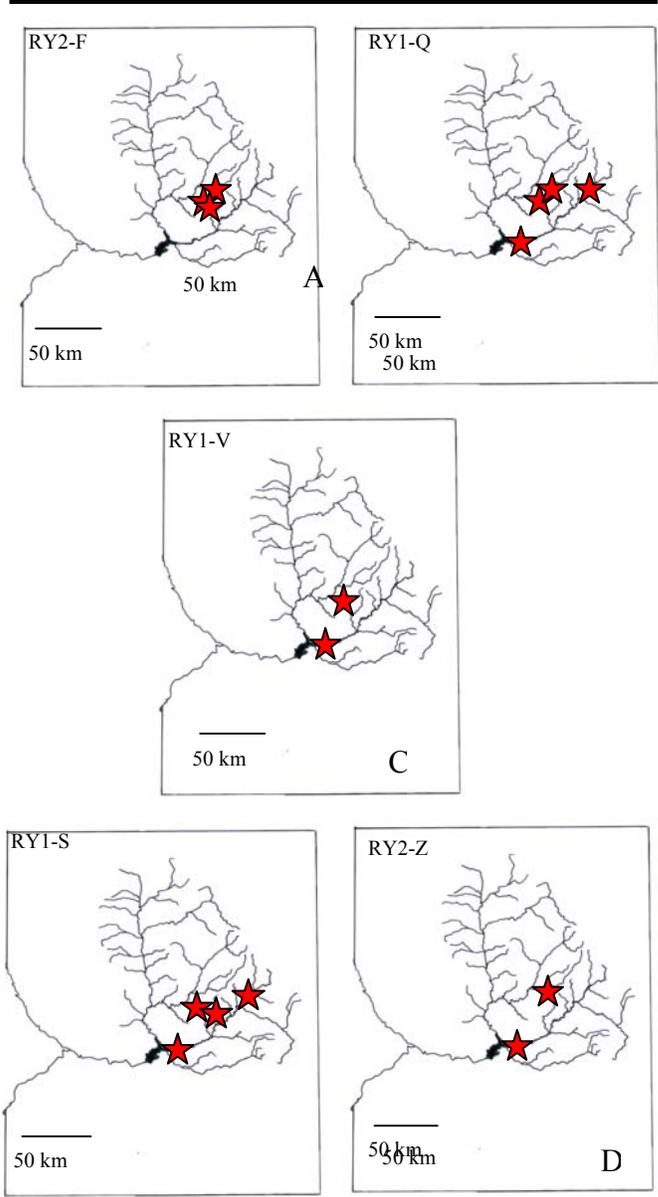


Figure 7(a). Distribution of alleles found among 4 or fewer populations and restricted to the Santa Maria River drainage.

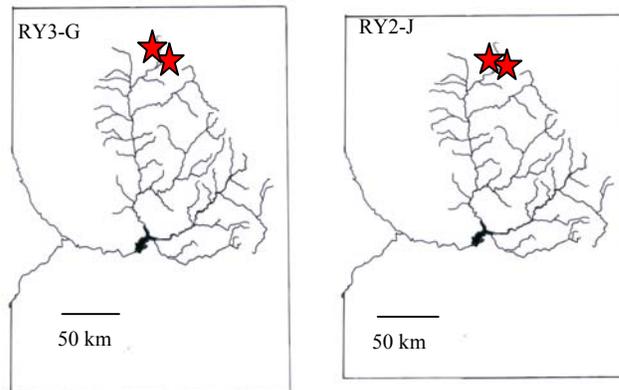


Figure 7(b). Distribution of alleles found among 4 or fewer populations and restricted to the Big Sandy River drainage.

Three alleles are found in a relatively few sites and in both drainages (Ry3-W, Ry3-E and RY3-G) (Figure 8).

Stream flow at the juncture of the Big Sandy River and the Santa Maria River is interrupted, either through drought or high water levels of the heavily stocked Alamo Reservoir, on a seasonal basis. Consequently, connections that exist between these drainages are ephemeral, even in their lower reaches. Although these seasonal connections exist, 10 alleles (45%) are found in only a single drainage. Seven of the nine alleles (78%) that are found in four or fewer sites are restricted to either the Big Sandy (2 alleles) or the Santa Maria (5 alleles) river drainages. Even more striking is the fixation of the RY1-R allele in the Big Sandy drainage, whereas every population on the Santa Maria River contains multiple alleles at this locus.

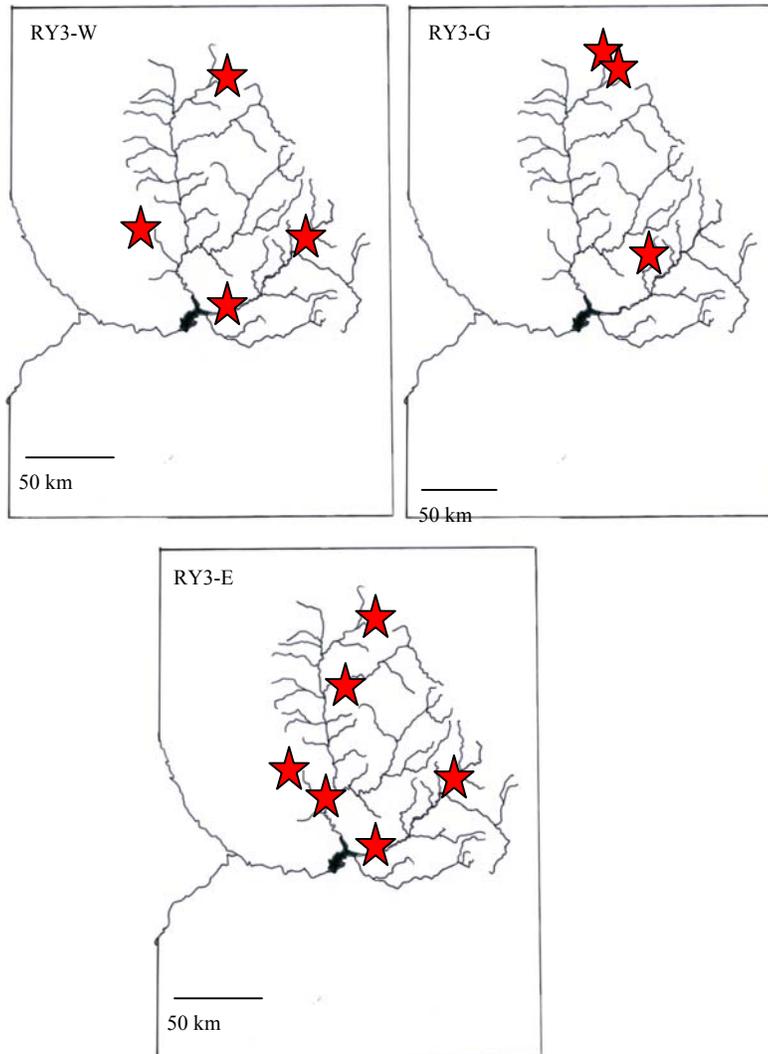


Figure 8. Distribution of alleles found in six or fewer populations and both drainages.

This data would suggest that the metapopulations in both drainages are largely functioning independently of each other. A likely mechanism to explain such a scenario would be the unidirectional (downstream) transport of frogs by seasonal flood events and the subsequent dessication of their intervening corridors.

The shared allele (RY3-G), unique to the most geographically distant sites in our study (Figure 8) can be explained in at least three ways. First, it could be evidence of gene flow between these sites. Secondly, it could be the result of either an accidental or intentional translocation. Finally, it could be a homoplasy (an allele that scores as being the same size, but has obtained that size through different evolutionary pathways). Given the hyper-variable nature of microsatellites, as evidenced by the number of observed alleles at each locus, it is entirely plausible that such homoplasy exists

In apparent discord with these observations is the occurrence of the RY3-W and RY3-E alleles in both drainages, but not in any of the Arrastra Mountain populations. The lower reaches of the Santa Maria River, just above its confluence with Alamo Reservoir, contain well-developed and persistent habitat. Conversely, for approximately the first 12 kilometers upstream of its confluence with Alamo Reservoir, the Big Sandy River consists entirely of unconsolidated alluvium. Individuals who find themselves washed into the lower reaches of the Big Sandy during flood events, would have little time in which to move back up the stream before these reaches desiccate. They would be far more likely to intermingle with individuals in persistent pools along the lower reaches of the Santa Maria River.

Breeding phenology and flood adaptations

Rana yavapaiensis have developed a unique breeding phenology among leopard frogs, which is believed to give them a distinct advantage in the habitat and sonoran weather patterns in which they evolved (Sartorius and Rosen, 2000). *Rana yavapaiensis* egg masses have been observed from January through late April and in October (Ruibal,

1959; Collins and Lewis, 1979; Frost and Platz, 1983). Reproductive activity may decrease between the time temperatures warm in mid-May and prior to the onset of the summer rains in early July (Sredl, unpublished data). Consequently *Rana yavapaiensis* tadpoles and froglets are typically well-developed and robust prior to the most severe flooding in July and August. These regular flood events have been documented to move numerous taxa, including ranid frog tadpoles downstream. Typically, adult frogs are less susceptible to removal by flood and persist in their upper reaches, where they can live as long as three years (Sredl and Fernandez, unpublished data). This unique breeding phenology results in *Rana yavapaiensis* that are more mature and capable of withstanding these extreme flood events (Figure 9). These floods can thus serve as an effective dispersal mechanism for young frogs.

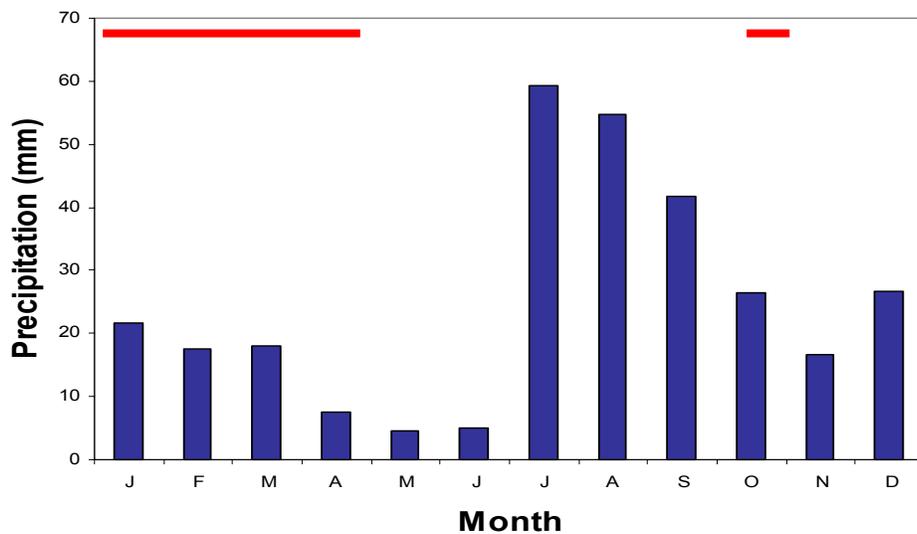


Figure 9. Monthly Precipitation Data for Tucson (Obtained from the National Weather Service Office). Red Bars denote periods of egg deposition for *Rana yavapaiensis*.

In such a flood dominated ecosystem, you would expect to find that the populations along the valley floors contain a mix of haplotypes found upstream of them and few, if any, novel haplotypes. This is precisely what was found among the three loci when considering all valley floor populations (Wikiup, Big Sandy River, Lower Santa Maria River and the Upper Santa Maria River). Every haplotype found in these populations is also found upstream, at one of the higher elevation locations. Downstream sites on the main-stem of both drainages contain more alleles than upstream sites on those same drainages. This is as expected given that downstream sites are expected to be subject to immigration from more source populations than upstream sites. No unique haplotype is found in any valley floor populations of either drainage.

An obvious question that arises is how did these populations initially colonize these upper reaches? Since there are no apparent corridors connecting them to other low-lying riparian reaches, such as the downstream Colorado River, it is likely that they were early invaders into these habitats during a different climatic regime. It has been suggested that southwestern populations of Ranids have persisted for at least the last 11,000 years and likely longer (Holman, 1995). This coincides with the end of the Wisconsin glaciation (15,000-20,000 bp) and beginning of the Holocene period (10,000 bp).

During this period, the desert scrub ecosystems that are found at these elevations today would have been more mesic and contained a vegetational composition more characteristic of higher elevation sites. Vegetational assemblages at the elevations of our study site would have included more grasslands along with pinion, spruce and fir galleries (Elias, 1997). The prevailing weather patterns would have been milder, with

fewer storms of great intensity and short duration. Overall temperatures would have been warmer in the winters and cooler in the summers (Elias, 1997). Under such climatic and vegetational conditions it is plausible that more persistent riparian corridors existed, through which leopard frogs could navigate. The subsequent dessication of these corridors through changes in the floral composition and climatic patterns, climaxing in the late Holocene, would have effectively stranded these populations in high elevation reaches.

Conclusions

Effective conservation would best be accomplished by the preservation of all known populations, their connecting corridors and the seasonal climatic and hydrologic regimes in which these populations evolved. Given the political, financial and social constraints facing wildlife managers, this is unrealistic. Conservation professionals are thus forced to prioritize where to allocate scarce resources to best ensure the long-term persistence of the organisms in their management.

A prudent alternative would be to focus on the conservation of the underlying and historic source populations that periodically serve to repopulate other more ephemeral sites as well as on genetically unique populations. This approach requires the identification and protection of source populations along with the mechanisms enabling the metapopulation to function. By primarily focusing conservation efforts on persistent source populations rather than ephemeral sink populations, the chances of persistence of the metapopulation structure as a whole are enhanced.

These data suggest that the critical source populations are also the most genetically distinct and unique. These populations are found in the high elevation reaches, upstream of the main channels. Their habitats are best characterized as persistent tinajas with well developed cienaga. This is supported not only by the high proportion of unique haplotypes found in those populations, but the complete lack of unique haplotypes found in every low lying sites. These data also suggest that both drainages function as largely independent metapopulations, as evidenced by the dramatic fidelity of alleles to drainages.

These persistent source populations tend to be found in persistent habitats, resistant to seasonal dessication. Based on these limited surveys, site persistence rather than observed population size is a critical factor in the long-term maintenance of overall genetic diversity.

The mechanism that explains this unidirectional movement is the well-documented flood regime of the Sonoran Desert. These flood events are typically followed by the subsequent desiccation of large reaches of habitat along these dispersal corridors. This would preclude the subsequent upstream movement of frogs to the headwaters of these drainages.

In this study, the genetically unique source populations are best exemplified by the Knight Creek and Arrastra Mountain Sites (Cottonwood Canyon, Sycamore Spring and Peoples Canyon). Every haplotype encountered in this study is found in one or more of these sites. Protection of just these five sites would preserve all of the alleles identified in this study area.

The two Knight Creek sites contain 81% of the genetic diversity and 100% of all of the unique alleles found in the Big Sandy River drainage. If Trout creek is included with these two sites 100% of the genetic diversity observed in this drainage is accounted for. Conservation of these three sites could help ensure the long-term persistence of both the metapopulation structure and the genetic diversity of *Rana yavapaiensis* in this drainage. The Knight Creek site is located on public lands and serves as a *de facto* trail for motorized vehicles. While this population was being surveyed a group of approximately 12 motorcyclists rode through the site, potentially crushing frogs in the soft streambed. Though motorized travel through this area does not necessarily need to be precluded, it should be diverted from this small but sensitive site. The Upper Knight Creek site is located on private property adjacent to what appears to be an abandoned mine. Discussions with the owner of this site would be warranted, if they could result in better protection of this population. Additional surveys in the Knight and Trout Creek drainages should focus on identifying additional populations located in persistent tinajas.

The Peoples Canyon, Sycamore Spring and Cottonwood Canyon sites contain 100% of the genetic diversity found in the entire Santa Maria River drainage. Consequently the conservation of these three sites is integral to the development of sound management strategies to both maximize the genetic diversity of these frogs and maintain them as a functional metapopulation. The Peoples Canyon and Sycamore Spring sites already enjoy protection as a function of the Wilderness Area in which they reside. The Cottonwood Canyon population is located on the edge of the Wilderness boundary, just upstream of an active “Cow Camp”. Though some fencing exists to preclude the movement of cattle into this critical habitat, much of it is in need of repair. Further work

to identify other potential source populations throughout this wilderness area is warranted. These surveys should focus on identifying long-term persistent habitats best characterized as tinajas, often with well-developed cienaga.

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References

- Beebee, T.J., 1996. Ecology and Conservation of Amphibians. Chapman and Hall, London.
- Berven, K.A., and T.A. Grudzein, 1990. Dispersal in the Wood Frog (*Rana sylvatica*): Implications for genetic population structure. *Evolution*, 44:2047-2056.
- Brown, D.E., 1982. Biotic Communities of the American Southwest – United States and Mexico. *Desert Plants*, 4:4-413.
- Campbell, J.B., 1970. Hibernacula of a population of *Bufo boreas* in the Colorado Front Range. *Herpetologica*, 26:278-282.
- Carey, C., 1992. Hypothesis concerning the causes of the disappearance of the Boreal Toads from the mountains of Colorado. *Conservation Biology*, 2:355-362.
- Collins, J.P., C. Young, J. Howell and W.L. Minckley, 1981. Impact of flooding in a Sonoran desert stream, including elimination of an endangered fish population (*poeciliopsis o. occidentalis*, *poeciliidae*). *The Southwestern Naturalist*, 26:415-423.
- Collins, J.P. and M.A. Lewis. 1979. Overwintering tadpoles and breeding season variation in the *Rana pipiens* complex in Arizona. *Southwestern Naturalist*. 24:371-373.
- Chronic, H., 1983. Roadside Geology of Arizona. Mountain Press Publishing Company, Missoula.
- Debinski, D.M., 1994. Genetic Diversity Assesment in a Metapopulation of the Butterfly (*Euphydryas gillettii*). *Biological Conservation*, 70:25-31.
- Degenhart, W.G., C.W, Painter and A,H, Price, 1996. Amphibians and Reptiles in New Mexico. University of New Mexico Press, Albuquerque.
- Elias, S.G., 1997. The Ice-Age History of southwestern National Parks. Smithsonian Institution Press, Washington.
- Fellers, G.M., C.A. Drost and R.W. Hayer. 1994. Handling Live Amphibians, in “Measuring and Monitoring Biological Diversity: standard methods for amphibians”, WR Heyer, MA Donnelley, RW McDiarmid, LC Hayek and MS Foster, Editors. Smithsonian Institution Press, Washington.

- Frost, J.S. and J.E. Platz. 1983. Comparative assessment of modes of reproductive isolation among four species of leopard frogs (*Rana pipiens* complex). *Evolution*. 37:66-78.
- Hamilton, M.B., E.L. Pincus, A.D. Fiore and R.C. Fleischer, 1999. Universal Linker and Ligation Procedures for Construction of Genomic DNA Libraries Enriched for Microsatellites. *BioTechniques*, 27:500-507.
- Hedrick, P.W., 1996. Genetics of Metapopulations: Aspects of a comprehensive perspective. In; "Metapopulations and Wildlife Conservation" DR McCullough Editor. Island Press, Washington D.C.
- Hillis D.M. 1988. Systematics of the *rana pipiens* Complex: Puzzle and Paradigm. *Annual Review of Ecology and Systematics*. (19):39-63.
- Hillis D.M., J.S. Frost and D.W. Wright. 1983. Phylogeny and Biogeography of the *Rana pipiens* Complex: A Biochemical Evaluation. *Systematic Zoology*. (2):132-143.
- Holman, J.A., 1995. Pleistocene Amphibians and Reptiles in North America. Oxford University Press, Oxford.
- Kahn, N.W., C.E. Braun, J.R. Young, S. Wood, D.R. Mata and T.W. Quinn, 1999. Molecular analysis of genetic variation among large- and small-bodied Sage-Grouse using mitochondrial control region sequences. *Auk*, 116:819-824.
- Kimura, M. and G.H. Weiss, 1994. The stepping-stone model of population structure and the decrease of genetic correlation with distance. In; "Population Genetics, Molecular Evolution and the Neutral Theory", N Takahata, Editor. The University of Chicago Press, Chicago.
- Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca and A.C. Wilson, 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, 86:6196-6200.
- Marsh, D.M. 2001. Fluctuations in amphibian populations: a meta-analysis. *Biological Conservation*. 101:327-335.
- Marsh, D.M. and P.C. Trenham. 2001. Metapopulation Dynamics and Amphibian Conservation. *Conservation Biology*. 15:40-49.
- MacMahon. 1992. "Deserts". Published by Alfred A. Knopf, Inc. New York, NY.

- Meefe, G.K. and W.L. Minckley, 1987. Persistence and stability of fish and invertebrate assemblages in a repeatedly disturbed Sonoran desert stream. *The American Midland Naturalist*, 1:177-191.
- McLaughlin, S.P., 1992. Upper Trout Creek Inventory. Arizona Department of Game and Fish – Habitat Branch.
- Miller, M.P., 1997. Tools For Population Genetic Analysis (TFPGA): A windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by the Author.
- Nei, M., 1972. Genetic distance between populations. *American Naturalist*, 106:283-292.
- Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89:583-590.
- Nichols, R.A., M.W. Brufford and J.J. Groombridge, 2001. Sustaining genetic variation in a small population: evidence from the Mauritius kestrel. *Molecular Ecology*, 10:593-602.
- Pechman, J.H., D.E. Scott, R.D. Semlitsch, J.P. Caldwell, L.J. Vitt and J.W. Gibbons, 1991. Declining Amphibian Populations: The problem of separating human impacts from natural fluctuations. *Science*, 253:892-895.
- Platz, J.E., A. Lathrop, L. Hofbauer and M. Vrandenburg, 1997. Age distribution and longevity in the Ramsey Canyon Leopard Frog, *Rana subaquavocalis*. *Journal of Herpetology*, 31:552-557.
- Platz, J.E. and J.S. Frost. 1984. *Rana yavapaiensis*, a new species of leopard frog (*Rana pipiens complex*). *Copeia*, 940-948.
- Reh, W. and A. Seitz. 1990. The influence of land use on the genetic structure of populations of the common frog *Rana temporaria*. *Biological Conservation*. 54:239-249.
- Ruibal, R. 1959. Ecology of a brackish water population of *Rana pipiens*. *Copeia*. 1959:315-322.
- Sartorius, S.S. and P.C. Rosen, 2000. Breeding phenology of the Lowland Leopard Frog (*Rana yavapaiensis*): Implications for conservation and ecology. *The Southwestern Naturalist*, 45:267-273.
- Scribner, K.T., J.W. Arntzen and T. Burke, 1997. Effective number of breeding adults in *Bufo bufo* estimated from age-specific variation at minisatellite loci. *Molecular Ecology*, 6:701-712.

- Sellers, W.D., 1985. Arizona Climate. University of Arizona Press, Tucson.
- Seutin, G., B. White and P. Boag, 1991. Preservation of avian blood and tissue samples for DNA analysis. *Canadian Journal of Zoology*, 69:82-90.
- Shaffer, M., 1987. Minimum Viable Population: coping with uncertainty, in "Viable Populations For Conservation". ME Soule, Editor. Cambridge University Press, Cambridge.
- Sjoren, P. 1991. Extinction and isolation gradients in metapopulations: the case of the pool frog (*Rana Lessonae*). *Biological Journal of the Linnean Society*. 42:135-147.
- Sredl, M.J. In Press. *Rana yavapaiensis* (Platz 1984) Lowland Leopard Frogs. In Lanoo, M.J. (Ed), Status and Conservation of U.S. Amphibians. Volume 2: Species Accounts. University of California Press, Berkeley, CA.
- Sredl, M.J. Editor, 1997. Ranid frog conservation and management, Nongame and Endangered Wildlife Program Technical Report 121. Arizona Game and Fish department, Phoenix, Arizona.
- Tanaka, T., M. Matsui and O. Takenaka. 1994. Estimation of Phylogenetic Relationships among Japanese Brown Frogs from Mitochondrial Cytochrome *b* Gene (Amphibia:Anura). *Zoological Science*. (11):753-757.
- Tinsley, R.C. and H.R. Kobel, 1996. The Biology of *Xenopus*. Oxford University Press, Oxford.
- Weitzel, N.H. and H.R. Panik, 1993. Long-term fluctuations of an isolated population of the Pacific Chorus Frog (*Pseudacris regilla*) in northwestern Nevada. *Great Basin Naturalist*, 53:379-384.
- Wright, S., 1978. Evolution and the Genetics of populations, vol. 4. Variability within and among natural populations. University of Chicago press, Chicago.