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Phylogeography of two closely related anurans, the Relict Leopard Frog (Rana onca) and Lowland Leopard Frog (Rana yavapaiensis)

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PHYLOGEOGRAPHY OF TWO CLOSELY RELATED ANURANS, THE RELICT

LEOPARD FROG (*RANA ONCA*) AND LOWLAND

LEOPARD FROG (*RANA YAVAPAIENSIS*)

by

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A thesis submitted in partial fulfillment
of the requirements for the

Master of Science in Biological Sciences
School of Life Sciences
College of Science

Graduate College
University of Nevada, Las Vegas
May 2010

We recommend the thesis prepared under our supervision by

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entitled

Phylogeography of Two Closely Related Anurans, the Relict Leopard Frog (*Rana onca*) and Lowland Leopard Frog (*Rana yavapaiensis*)

be accepted in partial fulfillment of the requirements for the degree of

Master of Science in Biological Sciences
School of Life Sciences

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May 2010

ABSTRACT

Phylogeography of Two Closely Related Anurans, the Relict Leopard Frog
(*Rana onca*) and Lowland Leopard Frog (*Rana yavapaiensis*)

by

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Climate changes during the Quaternary have had dramatic effects on the distributions

of organisms and communities in the Mojave and Sonoran deserts of North America.

Herein, I evaluate the phylogeography of two amphibian species inhabiting these regions

that have undergone substantial population declines in recent decades, the relict leopard

frog, *Rana onca* (= *Lithobates onca*) and the lowland leopard frog, *R. yavapaiensis* (= *L.

yavapaiensis*). This thesis comprises two chapters. In the first chapter, I summarize

literature describing taxonomy, phylogenetics, and conservation status for these two

species. I then explore the possible effects of Quaternary climate changes on distribution

of these frogs within regions. I also provide examples of phylogeographic patterns from

other generally co-distributed organisms to develop background for interpreting the

phylogeographic structure of *R. onca* and *R. yavapaiensis* that I describe in Chapter 2.

This chapter investigates the phylogeography of *R. onca* and *R. yavapaiensis* using

sequence data from mitochondrial DNA (mtDNA) to assess 276 individuals representing

30 sites from across current distributions. Chapter 2 represents an article (Oláh-

Hemmings et al. 2010) in press (Journal of Zoology), co-authored with Jef R. Jaeger,

Michael J. Sredl, Martin A. Schlaepfer, Randy D. Jennings, Charles A. Drost, David F. Bradford, and Brett R. Riddle. Therefore, I use plural pronouns throughout this chapter to be consistent with the article. The analysis supported a previously determined phylogenetic break between taxa, with no admixing of *R. onca* and *R. yavapaiensis* haplotypes within sites. The phylogeographic assessment, however, further divided *R. yavapaiensis* into two distinct mtDNA lineages, one representing populations across Arizona and northern Mexico and the other a newly discovered population within the western Grand Canyon (Arizona). Estimates of sequence evolution indicate a possible Early Pleistocene divergence of *R. onca* and *R. yavapaiensis*, followed by a Middle Pleistocene separation of the western Grand Canyon population of *R. yavapaiensis* from the main *R. yavapaiensis* clade. Phylogeographic and demographic analyses indicate population or range expansion for *R. yavapaiensis* within its core distribution that appears to predate the latest glacial maximum. Species distribution models under current and latest glacial climatic conditions suggest that *R. onca* and *R. yavapaiensis* may not have greatly shifted ranges since the last glacial maximum.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

A REVIEW OF THE PHYLOGENY OF RELICT AND LOWLAND LEOPARD

FROGS AND THE POSSIBLE EFFECTS OF QUATERNARY

CLIMATE CHANGE ON SPECIES DISTRIBUTIONS

Taxonomy, Population Status, and Evolutionary Relationships of

Rana onca and *R. yavapaiensis*

Attempts to define the taxonomy and evolutionary relationships among North

American true frogs (*Rana*) have used a variety of data (albumin, Wallace et al. 1973;

albumin and electrophoretic, Case 1978; allozyme, Hillis et al. 1983, Hillis 1988; nuclear

ribosomal DNA, Hillis & Davis 1986), with the most recent study (Hillis & Wilcox 2005)

based on mitochondrial DNA (mtDNA) including most of the known taxa (~ 60). One of

five groups of North American true frogs, the *Rana pipiens* complex (recognized in the

Hillis and Wilcox 2005 revision as the *Pantherana* clade), comprises several taxa that

occur in North, Central and South America. My study taxa, the relict leopard frog (*Rana*

onca) and the lowland leopard frog (*R. yavapaiensis*) belong to the *R. berlandieri*

subgroup (*Scurrilirana* subclade; Hillis & Wilcox 2005) within the *R. pipiens* complex.

A current debate exists about the nomenclature of several North American ranid frogs

(e.g. Dubois 2006; Frost et al. 2006, 2008; Hillis 2007; Wiens 2007, Pauly et al. 2009),

and there are some scientists who recognize these frogs as *Lithobates onca* and *L.*

yavapaiensis, respectively; however, I retain the historical nomenclature throughout this

document.

Both lowland and relict leopard frogs suffer from recent population declines

attributed to a number of potential causes including: the introduction of exotic predators

and competitors, habitat modification and fragmentation (Jennings 1985; Hayes & Jennings 1986; Jennings 1988; Clarkson & Rorabaugh 1989; Jennings & Hayes 1994; Bradford et al. 2004; Sredl 2005); and the emerging fungal disease, chytridiomycosis, that has negatively impacted *R. yavapaiensis* (Bradley et al. 2002; Sredl et al. 2003; Schlaepfer et al. 2007; Witte et al. 2008). *Rana onca* appears to have suffered the worst, and it is currently listed as a candidate species under the U.S. Endangered Species Act and managed under a voluntary conservation agreement and strategy by the Relict Leopard Frog Conservation Team (RLFCT 2005). *Rana yavapaiensis* was once listed as a 'Category 2' candidate species (a designation no longer in use, however implying conservation concern) by the U.S. Fish and Wildlife Service (1991), but was dropped from consideration as a candidate from federal listing in 1996 (Sredl 2005). Both species are covered under various state (Arizona, California, New Mexico, Nevada, and Utah) laws.

Rana onca once occupied springs, streams, and wetlands along the drainages of the Virgin and Muddy rivers, and portions of the Colorado River within the region of southwestern Utah, southern Nevada, and northwestern Arizona (Bradford et al. 2004; Fig.1.1). This frog currently occurs naturally at only a few spring sites within two general areas of southern Nevada near the north shore of the Overton Arm of Lake Mead and in the Black Canyon below Hoover Dam (Jaeger et al. 2001; Bradford et al. 2004; Fig.1.1); however, conservation efforts have established several additional sites within the region (RLFCT 2005). The historical distribution of *R. onca* along the Lower Colorado River (i.e., downriver from Hoover Dam, Nevada) has not been determined (Bradford et al. 2004), but populations downriver from Black Canyon within the Bill

2

Williams River drainage (Arizona) and in the vicinity of the Imperial Valley (California) have been identified as *R. yavapaiensis* (Vitt & Ohmart 1978; Platz & Frost 1984; Platz 1988; Clarkson & Rorabaugh 1989; Jennings & Hayes 1994; Jaeger et al. 2001; Benedict 2002; Fig.1.1).

The historical distribution of *R. yavapaiensis* was considered to include most of Arizona, southeastern California, southwestern New Mexico, and the Sonoran region of northern Mexico (Vitt & Ohmart 1978; Platz & Frost 1984; Platz 1988; Clarkson & Rorabaugh 1989; Jennings & Hayes 1994; Sredl 2005). This species has suffered regional declines, except in central Arizona and possibly Mexico, although little is known about the status of populations in the latter (Sredl 2005). Populations of purported *R. yavapaiensis* in California from along the Lower Colorado River and areas in the vicinity of the Imperial Valley (Fig. 1.1) are believed to be extinct (e.g. Vitt & Ohmart 1978; Clarkson & Rorabaugh 1989; Jennings & Hayes 1994), and populations in New Mexico are nearly extirpated (Jennings et al. 1985; Jennings & Hayes 1994).

All leopard frog species are physically very similar (e.g. Jennings & Hayes 1994), which has led to much taxonomic confusion. *Rana onca* was initially described from a specimen collected along the Virgin River in Washington County, Utah (Cope 1875). Later, the Vegas Valley leopard frog (*R. fisheri*, Stejneger 1893) was described from springs within the Las Vegas Valley in Nevada, but these populations are now extinct (Stebbins 1951). Taxonomic confusion pervades the early literature (described in Jaeger et al. 2001); however, preliminary unpublished research based on morphology indicated that these two species are not closely related (Jennings et al. 1995).

3

Rana yavapaiensis was first described from a specimen collected at Tule Creek, Yavapai County, Arizona (Platz & Frost 1984), and at that time a population along the Virgin River in northwestern Arizona (Littlefield, Mohave County) was also identified as *R. yavapaiensis* by the same authors and reiterated in several publications (Jennings 1988; Platz 1988; Clarkson & Rorabaugh 1989; Jennings & Hayes 1994; Stebbins 2003). Ranid frogs from this site were later defined as *R. onca* by Jaeger et al. (2001) in a phylogenetic analysis based on mtDNA restriction fragment length polymorphism (COI through ND2) and control region (CR) sequence data. In that analysis, no variation was detected among individuals from extant northern populations (defined as *R. onca*) including samples from the Littlefield area however, these frogs were genetically distinct from *R. yavapaiensis* samples representing four populations from the core distribution of that species in Arizona and northern Mexico (0.042 *p*-distance, CR sequence data). The genetic break between *R. onca* and *R. yavapaiensis* was also confirmed by the same authors based on nuclear randomly amplified polymorphic DNA. Multivariate analysis of morphological characters from museum specimens, although less definitive, also revealed differences among samples representing these two frogs, and placing the Imperial Valley frogs generally intermediate between *R. onca* and *R. yavapaiensis* (Jaeger et al. 2001). Genetic data would be necessary to further evaluate the taxonomic status of the extinct frog populations in California; however, currently such data are not available from museum specimens.

Based on these genetic and morphological differences, Jaeger et al. (2001) argued for recognizing *R. onca* as an Evolutionarily Significant Unit (ESU; Moritz, 1994a,b) and retention of the existing taxonomy. These authors, however, suggested that the very

recent divergence between *R. onca* and *R. yavapaiensis* "probably" occurred during the Late Pleistocene-Holocene. Later, in a broad phylogenetic analysis of North American ranid frogs, Hillis and Wilcox (2005) showed only low levels of sequence divergence (0.012 *p*-distance) between these species based on slowly evolving 12S-16S mtDNA and suggested that this genetic difference was less than that observed for most recognized ranid species.

Recently a population of frogs physically similar to *R. onca* was found in Surprise Canyon within the western Grand Canyon (Arizona) upriver of existing *R. onca* sites along the Colorado River (Gelczis & Drost 2004; Fig.1.1). A preliminary analysis of mtDNA sequence data from one specimen showed this frog to be more closely related to *R. yavapaiensis* than to *R. onca*. That observation raised questions regarding the conclusion from Jaeger et al. (2001) that *R. yavapaiensis* did not occur within the region occupied by *R. onca*. Further, it generally put on hold options for potential translocations of *R. onca* into the western Grand Canyon until further information could be gathered. Introduction of *R. onca* to the canyon and its tributaries should be treated with care as hybridization between *R. onca* and *R. yavapaiensis* is a good possibility and such an event would likely cause the degradation of these genetically different lineages.

The Effects of Quaternary Climate Change on the Distribution of Species

The genetic and phylogeographic structure of *R. onca* and *R. yavapaiensis* appears to have been formed, to a great extent, under different climatic and hydrological conditions than today. Previously, Jaeger et al. (2001) suggested a possible Late Pleistocene-Holocene divergence between *R. onca* and *R. yavapaiensis*, but the genetic patterns I

5

describe in Chapter 2 indicate a possible divergence for these frogs dating to the Early

Pleistocene. Ranid frogs, like many other amphibians, are restricted by water and

temperature; therefore the separation of ancestral *R. onca* and *R. yavapaiensis* lineages

and diversification of *R. yavapaiensis* may have been induced by habitat shifts arising

from Quaternary glacial-interglacial cycles – a process implicated in the diversification of

numerous taxa occupying the arid Southwest (e.g. Hafner & Riddle 2005; Jaeger et al.

2005; Riddle & Hafner 2006, Bell et al. 2010).

Reconstructions of very early climatic patterns (pre-Late Wisconsin) and associated

ecological transformations have mostly been described in generalized terms (see Hafner

& Riddle 2005; Riddle & Hafner 2006), as early paleorecords from the Southwest are

limited (Betancourt et al. 1990a), thus reducing the detail of climate and environmental

reconstructions from pre-last glacial maximum (LGM) times. Better developed are

reconstructions dating back to the LGM, some 21,000 years ago (Kya). Many of the

biotic patterns within the Southwest are from pre-Late Wisconsin times; nevertheless,

models dating to the LGM often are used as surrogates for the older patterns of glacial-

interglacial changes at least through the most recent major cycles. Furthermore, the

climates of the LGM through the Holocene likely represent conditions close to the

coldest and warmest extremes of the Quaternary and therefore could imply extreme

distributional shifts in *R. onca* and *R. yavapaiensis* throughout this time period. I

recognize that these are large assumptions, but consider that such an exercise could

provide insights to potential distributional responses of these ranids to earlier climatic

oscillations.

Within the regions occupied by *R. onca* and *R. yavapaiensis*, paleovegetation data have been predominately derived from pollen and plant macrofossil records mostly obtained from packrat (*Neotoma* spp.) middens (e.g. Betancourt et al. 1990a; Thompson & Anderson 2000). Paleoclimate can be reconstructed by comparing ancient and modern plant distributions at particular sites, given species-specific climatic requirements (Betancourt et al. 1990a; Thompson & Anderson 2000). These reconstructions provide evidence that climate within the Mojave and Sonoran deserts has changed throughout the Quaternary (e.g. Van Devender & Spaulding 1979; Spaulding 1990; Van Devender 1990; Thompson & Anderson 2000). In Chapter 2, I provide evidence based on mtDNA data and species distribution modeling (SDM) of the possible impacts of Quaternary climate change on the population structure of *R. onca* and *R. yavapaiensis*. Below, I explore possible scenarios that could have influenced the distribution of these frogs within regions to better inform the research I present in the following chapter. I also provide examples of phylogeographic patterns from other generally co-distributed organisms to develop background for interpreting the phylogeographic structure of *R. onca* and *R. yavapaiensis*.

The Virgin, Muddy and Adjacent Colorado Rivers

Rana onca appears to be endemic to the eastern Mojave Desert, and restricted to areas downriver from the Grand Canyon along the Colorado River, and adjacent Virgin and Muddy rivers (Bradford et al. 2004; Fig.1.1). At present, this region is dominated by Mojave desertscrub (Shreve 1942), and the climate is characterized by hot summers with monsoons coming from the Gulfs of Mexico and California; winters are cool with occasional freezes, and storms arriving from the Pacific Ocean (Lowe 1964).

Paleoclimate reconstruction suggests that in southern Nevada, during the Late Wisconsin, the climate was relatively cooler with winter precipitation higher than today (Spaulding 1985). This is supported by lake sediment data, which indicate the presence of shallow pluvial lakes in the northern Las Vegas Valley (Haynes 1967; Quade 1986). Sites once likely occupied by *R. onca* along the Colorado River (now under the reservoirs of Lakes Mead and Mohave) are quite low in elevation.

A full-glacial vegetation map based on pollen data (Martin & Mehringer 1965, in Betancourt et al. 1990b) portrays the region currently occupied by *R. onca* as sagebrush and chaparral. Just south of this region, areas along the Lower Colorado River are depicted as desertscrub, indicating relatively warm climatic conditions. Similarly, another paleomap (Latest Wisconsin, 11 Kya) derived from macrofossil data (Betancourt et al. 1990c) depicts the region along the Lower Colorado River south of current *R. onca* locations as occupied by desertscrub, also indicating a warm climate. This map portrays areas currently occupied by *R. onca* as covered by pygmy conifer woodland (Betancourt et al. 1990c) implying that the paleoclimate was somewhat cooler than at present. Warmer microhabitats, however, could have existed within the region as Van Devender and Spaulding (1979) speculated that desertscrub most likely persisted below 400 m during the Late Wisconsin (~ 25-11 Kya), even though no lower elevation midden macrofossil data exist older than 11 Kya. Retention of warm paleomicrohabitats within some lower elevation areas of the Mojave Desert have also been suggested (Spaulding 1990). The eastern Mojave Desert along the Colorado River and its tributaries contains numerous thermal springs that have persisted through time, and their microhabitats could have served as refugia for these frogs despite the Quaternary climate and habitat change.

Based on these interpretations, it seems reasonable to assume that suitable habitats may have been retained though the LGM at low elevation sites within the region currently occupied by *R. onca*. A scenario of long-term stability of suitable habitat for this species is supported by genetic and modeling data that I present in Chapter 2.

A north-south genetic break between *R. onca* and its close relative *R. yavapaiensis* along the Lower Colorado River (suggested by Jaeger et al. 2001) may seem unlikely. Similar phylogeographic breaks within this region, however, have been described for some taxa (summarized in Bell et al. 2010). The north-south phylogenetic break is also represented by the speckled dace (*Rhinichthys osculus*). This species shows high mtDNA haplotype diversity in lower-elevation drainages of the Lower Colorado River (below Grand Canyon) compared to high-elevation drainages along the Upper Colorado River, implying that hydrographic events induced by climatic oscillations could have isolated the populations for periods of time along the upper and lower portions of the Colorado River (Oakey et al. 2004; Smith & Dowling 2008). It is speculated that cold temperatures during glacial periods forced *Rhinichthys* populations to lower elevation areas within the lower portions of the Colorado River and subsequently, as climate warmed, populations colonized or re-colonized areas within the upper portion of the river. Further, a north-south divergence in mtDNA has been described between populations of the desert pocket mouse (*Chaetodipus penicillatus*) within the Mojave Desert and those in the Sonoran Desert, with a broad secondary contact zone along the upper reaches of the Lower Colorado River valley (Jezkova et al. 2009). This phylogenetic break probably dates to the Middle Pleistocene, but populations of *C. penicillatus* in the northern Mojave Desert, including the general area occupied by *R.*

onca, show low levels of genetic diversity and clade structuring suggested by the authors to be associated with the most recent glacial period. Given these observed phylogeographic patterns in co-distributed species, a substantial genetic break along the Colorado River between *R. onca* and *R. yavapaiensis* seems quite possible, although the mechanism that specifically caused the divergence is not entirely clear.

Western Grand Canyon

As described above, the western Grand Canyon contains a disjunct population of *R. yavapaiensis* (Gelczis & Drost 2004), and I provide evidence in Chapter 2 that this population has been isolated for some time, rather than representing a recent, Late Pleistocene-Holocene colonization into the canyon. My estimate of the divergence time (based on genetic data) of the Surprise Canyon population from core populations of *R. yavapaiensis* to the south supports the idea of persistence of this taxon in the western Grand Canyon at least through the latest glacial period.

Lower elevations within the western Grand Canyon represent essentially eastern Mojave Desert climate and vegetation extending east into the Colorado Plateau (e.g. Van Devender & Mead 1976). The current climate within the canyon is characterized by hot summers, relatively cold winters with occasional freezes, and dominated by summer and winter rainfall (e.g. Spaulding 1990; Koehler et al. 2005). Paleovegetation reconstructions indicate that during the Late Pleistocene a mixture of open-juniper woodland and desert plant species, such as desert mallow (*Sphaeralcea ambigua*), ragged rockflower (*Crossosoma bigelovii*), and beavertail cactus (*Opuntia basilaris*) existed in the lower elevation reaches of the canyon (Phillips 1977; Mead & Phillips 1981). Climate reconstructions based on vegetation data indicate that the Late Pleistocene was

10

likely wetter, with cooler summers, and with winters possibly only mildly colder than today (Phillips 1977). As such, microhabitats within the lower reaches of the canyon might have served as glacial refugia for *R. yavapaiensis*.

Other researchers (Cole & Arundel 2005), however, have deduced from examinations of agave fossils that winters in the canyon during the LGM and during the subsequent cooling period called Younger Dryas (12.9-11.6 Kya) were approximately 8 ^{o}C colder than those at present. Considering the current average winter temperature (December-February) within the canyon (about 5 ^{o}C measured at Pierce Ferry, Truxton Canyon, and Tuweep in Arizona, the closest weather stations from which data are available; www.wrcc.dri.edu), an 8 ^{o}C drop in temperature might have resulted in unsuitably cold conditions for *R. yavapaiensis*, which currently exists along the fringes of the Sonoran Desert where freezing temperature are uncommon (Lowe 1964). If this scenario is correct, then the population within the canyon must have subsequently colonized from some unidentified region. *Rana yavapaiensis* could have colonized the western Grand Canyon from a source population located to the south within the core distribution of the species, which seems likely as a population of *R. yavapaiensis* currently exists in a stream system only about 85 km away from a sampled location (see Chapter 2). The other possibility is that *R. yavapaiensis* in the canyon may represent the remnant of a much broader distributed population that extended west and south along the Colorado River (Jaeger et al. 2001). This possibility, however, seems less likely given that populations of the sister-taxon, *R. onca* occupy the Colorado River corridor between the western Grand Canyon and purported historical populations of *R. yavapaiensis* further south along the Lower Colorado River. Nevertheless, recent, possible post-Pleistocene range expansion

into the western Grand Canyon from more stable populations to the south has been suggested for other Sonoran Desert taxa, including brittlebush (*Encelia farinosa*; Fehlberg & Ranker 2009) and the southwestern speckled rattlesnake (*Crotalus michellii pyrrhus*; Douglas et al. 2006). Interestingly, post-Pleistocene range expansion of the arid-dwelling red-spotted toad (*Bufo punctatus*) into the western Grand Canyon has also been implicated, but from populations within the Chihuahuan Desert (Jaeger et al. 2005). These authors speculated that *B. punctatus* may have entered the Colorado River system from the east by crossing over the continental divide at low elevation areas, possibly into the headwaters of the Little Colorado River. *Rana yavapaiensis* is known from the upper Gila and San Francisco rivers (although currently in areas further south) and it seems reasonable to speculate that this frog could have entered the Colorado River system following a similar path.

The phylogeographic patterns indicate that populations of several warm-adapted taxa are fairly new to the Grand Canyon, apparently arriving during post-Pleistocene times. This creates some perplexity, as the genetic data I present in Chapter 2 indicate long-term isolation of the population of *R. yavapaiensis* currently found in the western Grand Canyon, and yet there is some evidence to suggest that conditions in the canyon may not have been favorable to these frogs during the last glacial period. If the latter scenario is true, than *R. yavapaiensis* must have migrated into the canyon after the LGM from some unknown area, and from some already divergent population.

Sonoran Desert and the Apache Highlands

The core distribution of *R. yavapaiensis* appears to exist within areas of the Arizona Upland subdivision of the Sonoran Desert and the adjacent Apache Highlands in Arizona

and northern Sonora, Mexico (Fig.1.1). In general, the Sonoran Desert is characterized

by hot summers and mild, virtually freeze-free winters (Lowe 1964; Van Devender 1990;

Sheppard et al. 2002). Rainfall is bimodal, dominated by summer monsoons originating

from the Gulfs of Mexico and California, as well as the eastern tropical Pacific Ocean,

and less severe winter storms coming from the Pacific Ocean (Sheppard et al. 2002).

To compare the climate within the Sonoran Desert and adjacent highlands occupied

by *R. yavapaiensis*, I obtained climatic information from the WorldClim database v. 1.4

with resolution of 2.5 minutes (~ 5 km; www.worldclim.org, Hijmans et al. 2005) for 425

R. yavapaiensis locations that were used for modeling in Chapter 2. The climate data

indicate that occupied sites in the uplands and highlands are relatively cooler and receive

more precipitation than sites at lower elevations in the Lower Colorado Valley where

fewer sites are known (Table 1.1). The hot and dry lower elevation areas of the Lower

Colorado Valley are predominantly inhabited by desertscrub communities (Shreve 1942).

Upland areas are dominated by palo verde communities mixed with succulents and

shrubs (Lowe 1964; Van Devender 1990), but populations of *R. yavapaiensis* also extend

from the margins of the Arizona Upland into various biotic communities within the

Apache Highlands (i.e., Chihuahuan desertscrub, interior chaparral, semi-desert

grassland, Madrean evergreen woodland, Great Basin conifer woodland). Only a few

known *R. yavapaiensis* exist at the edges of the Colorado Plateau (Petran montane conifer

forest), Transitional dry forest (Sinaloan thornscrub), and Sierra Madre Occidental

(Madrean evergreen woodland) ecoregions (Brown & Lowe 1980; Brennan & Holycross

2006).

Pollen records indicate that during the full-glacial (~21-15 Kya) the Arizona Upland was occupied by sagebrush, chaparral, and at higher elevations by pinyon-juniper woodland, while the Apache Highlands were dominated by the latter vegetation and ponderosa pine woodland (Martin & Mehringer 1965). Macrofossil data show that during the latest Wisconsin (~11 Kya) the Arizona Upland and lower elevation areas of the Apache Highlands supported pygmy conifer woodlands (Betancourt et al. 1990c); while higher elevations were predominantly inhabited by montane conifer forests (Betancourt et al. 1990c). Within these regions, both reconstructions suggest a colder and wetter paleoclimate than present (Table 1.1) and support a hypothesis (detailed in Chapter 2) that during the last glacial period colder climate induced a range shift of *R. yavapaiensis* from upland areas towards lower elevations generally within the Sonoran Desert.

Paleoreconstructions suggest that the lowlands of the Sonoran Desert were dominated by Mojave desertscrub during the Late Wisconsin (e.g. Van Devender 1977; Cole 1986; Van Devender et al. 1987), indicating the persistence of relatively warm climatic conditions (Cole 1986, 1990; Van Devender 1990; Table 1.1). The paleoclimate appears to have been somewhat cooler during summers, but with winter temperatures similar to current conditions and greater winter precipitation (Van Devender 1977; Table 1.1). The generally warm and potentially wetter paleoconditions within the lowlands suggest the possibility of more widespread wetland habitats and riparian corridors during glacial periods. These conditions may have been more favorable to *R. yavapaiensis,* allowing extensive dispersal within the region.

Numerous warm desert taxa show Pleistocene persistence within lower elevation areas within the Lower Colorado Valley , such as flat-tailed horned lizard (*Phrynosoma mcallii*, Mulcahy et al. 2006), western diamondback rattlesnake (*C. atrox*, Castoe et al. 2007), creosote bush (*Larrea tridentata*, Hunter et al. 2001), *E. farinosa* (Fehlberg & Ranker 2009), and *C. penicillatus* (Jezkova et al. 2009). Several other taxa show recent (Late Pleistocene-Holocene) range expansion into, or within, areas occupied by *R. yavapaiensis* potentially following habitat changes (e.g. Barber 1999; Jaeger et al. 2005; Douglas et al. 2006). For instance, *B. punctatus* shows low mtDNA diversity within the northeastern Sonoran Desert (south of the Gila River), which was interpreted as probable expansion into this region from the Lower Colorado Valley following the development of warmer climatic conditions in the Middle to Late Holocene (Jaeger et al. 2005). Similarly, the canyon treefrog (*Hyla arenicolor*) demonstrates low genetic variation within the highlands of Arizona and New Mexico, indicative of recent range expansion suspected to have occurred from persistent populations in the Chihuahuan Desert (Barber 1999). A shallow phylogenetic pattern and recent range expansion has also been indicated for the tiger rattlesnake (*Crotalus tigris*) within the Arizona Upland, probably from populations that persisted in low lying areas east of the Sea of Cortez (Douglas et al. 2006). The range shift of plant and animal taxa from higher elevation areas to adjacent lower regions during the LGM is consistent with patterns I predict from modeling of *R. yavapaiensis* presented in the following chapter. An important point to make here, however, is that the range expansion for *R. yavapaiensis*, that I describe in the next chapter from genetic interpretation, appears to pre-date the LGM.

Conclusions

Even though Pleistocene glacial ice sheets never covered the present area of the desert southwest, climate within the region was greatly affected (e.g. Axelrod 1979; Betancourt et al. 1990a; Thompson & Anderson 2000), as were the histories of species (Avise 2000) and regional biota (Hafner & Riddle 2005; Riddle & Hafner 2006). Climatic oscillations could have induced habitat shifts and regional isolation of the ancestor of *R. onca* and *R. yavapaiensis*, ultimately causing ancestral lineages to diverge and evolve on separate trajectories within different ecoregions. *Rana onca* appears to be a local endemic of the eastern Mojave Desert (Bradford et al. 2004) restricted to a very narrow portion of the Colorado River and adjacent tributaries. Paleoreconstructions suggest that climatic conditions may have been suitable for *R. onca* to allow persistence generally in place through at least the most recent glacial period. On the other hand, distributions of *R. yavapaiensis,* predominately along the higher elevation edges of the Sonoran Desert, may not have been stable as the colder paleoclimate likely resulted in unfavorable transitions of upland habitats in the region. A range shift towards lower elevations within the Lower Colorado Valley seems reasonable as this region probably retained warmer climate. It is even possible that conditions within this region were more favorable to this species allowing broader distributions and interconnectivity during glacial times. Such patterns may also explain the historical distribution of this species along the southern portion of the Lower Colorado River. Occupation and isolation of *R. yavapaiensis* within the lower elevation reaches of the western Grand Canyon through the latest glacial period is less convincing; nevertheless, the genetic data I present in the next

chapter clearly implicate some type of interesting and unusual history for this remnant

population.

Table 1.1. Current and last glacial maximum (CCSM and MIROC) temperature and precipitation variables of *Rana onca* and *R. yavapaiensis* locations (*n*) extracted from WorldClim bioclimatic layers BIO1 = annual mean temperature (^{o}C), and BIO12 = annual mean precipitation (mm). *Rana onca* sites are located within the area of the Virgin and Muddy rivers, and Black Canyon. *Rana yavapaiensis* sites are located within the rest of the areas listed.

Area	BIO1	Current	Mean	CCSM	Mean	MIROC	Mean	*n*
Virgin and Muddy rivers / Black Canyon		13.9 - 21.0	18.7	2.5 - 11.8	8.0	6.2 - 14.8	11.5	47
Apache Highlands		10.3 - 20.4	15.6	-2.1 - 13.6	4.9	4.1 - 16.6	10.4	204
Arizona Upland		14.4 - 21.6	18.6	3.1 - 11.6	8.0	8.3 - 16.7	13.2	118
Lower Colorado Valley-Arizona		17.5 - 21.9	20.3	8.7 - 13.9	11.9	12.4 - 16.9	15.2	7
Lower Colorado Valley-California		22.2 - 23.2	22.6	15.7 - 18.1	17.5	18.0 - 19.3	18.7	20
Lower Colorado Valley-Arizona / California		17.5 - 23.2	22.0	8.7 - 18.1	16.0	12.4 - 19.3	17.8	27

Area	BIO12	Current	Mean	CCSM	Mean	MIROC	Mean	*n*
Virgin and Muddy rivers / Black Canyon		101 - 287	161.3	182 - 429	261.8	275 - 400	399.2	47
Apache Highlands		208 - 617	464.8	248 - 874	584.9	531 - 1856	1142.3	204
Arizona Upland		212 - 549	369.3	285 - 736	512.3	497 - 1641	878.6	118
Lower Colorado Valley-Arizona		146 - 320	204.7	243 - 488	325.7	375 - 788	513.7	7
Lower Colorado Valley-California		60 - 101	79.4	104 - 168	130.2	179 - 268	222.1	20
Lower Colorado Valley-Arizona / California		60 - 320	111.9	104 - 488	180.9	179 - 788	297.7	27
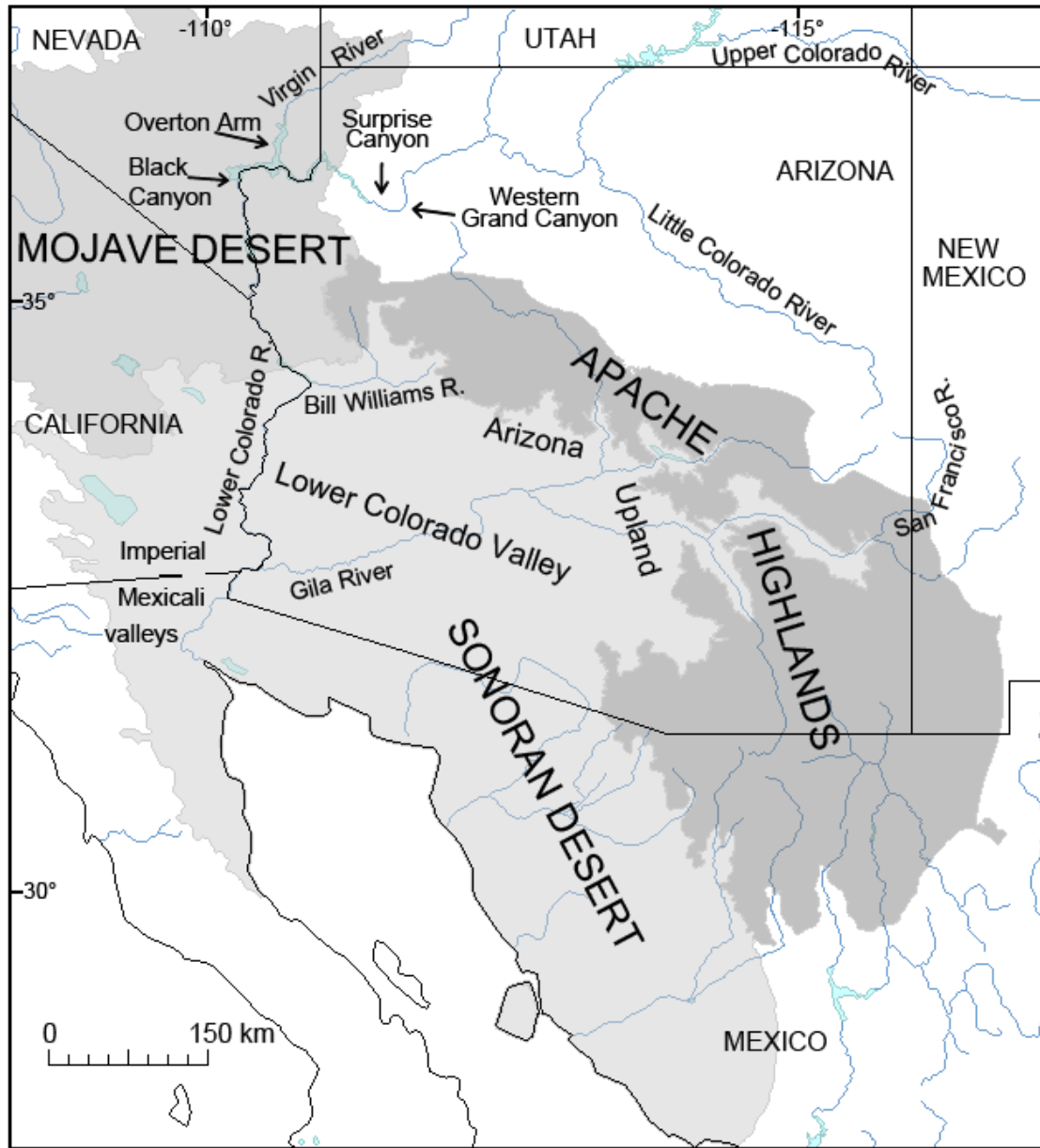
Figure 1.1. Map showing areas of the eastern Mojave Desert, the Sonoran Desert, and the Apache Highlands discussed in text.

CHAPTER 2

PHYLOGEOGRAPHY OF DECLINING RELICT AND LOWLAND LEOPARD

FROGS IN THE DESERT SOUTHWEST OF NORTH AMERICA

Introduction

The relict leopard frog, *Rana onca* (= *Lithobates onca*) and the lowland leopard frog,

R. yavapaiensis (= *L. yavapaiensis*), occupy springs, streams, and wetlands within warm-

desert regions of southwestern North America. In recent years, both of these closely

related frogs have experienced population declines and broad range contractions

(Clarkson & Rorabaugh 1989; Bradford, Jaeger & Jennings 2004; Sredl 2005). As an

apparent regional endemic, *R. onca* has suffered the worst and is currently managed

under a federally reviewed conservation agreement and strategy. Previous phylogenetic

analysis based on mitochondrial DNA (mtDNA), nuclear DNA markers, and morphology

revealed that these frogs were distinct taxa but at a shallow level of divergence, which led

to the speculation that this level of difference "probably" represents relatively recent,

Late Pleistocene-Holocene isolation (Jaeger et al. 2001). Further evidence that these taxa

are closely related was subsequently provided in a broader phylogenetic analysis of ranid

frogs in which a lower than species-level distinction was implied (Hillis & Wilcox 2005).

The "minimum historical range" of *R. onca* included the eastern fringe of the Mojave

Desert within the drainages of the Virgin and Muddy rivers and adjacent portions of the

Colorado River in the region of southwestern Utah, northwestern Arizona, and southern

Nevada (Bradford et al. 2004). It now occurs naturally only at a few sites along the

Colorado River in Nevada (Jaeger et al. 2001; Bradford et al. 2004). Whether *R. onca*

once occurred further south on the Lower Colorado River is not clear (Bradford et al.

2004), but the Bill Williams drainage which joins the Lower Colorado River below sites

occupied by *R. onca* (Fig. 2.1a) contains *R. yavapaiensis* populations (Jaeger et al. 2001).

Rana yavapaiensis is more widespread and primarily occurs in the higher elevation

uplands of the Sonoran Desert in Arizona extending south into northern Sonora, Mexico

and east into New Mexico where this frog is nearly extirpated (Platz & Frost 1984;

Jennings & Hayes 1994; Jennings 1995; Sredl 2005). Populations of purported *R.*

yavapaiensis from more southern reaches of the Lower Colorado River and the adjacent

Imperial and Mexicali valleys of southern California and northern Baja are believed to be

extinct (Vitt & Ohmart 1978; Clarkson & Rorabaugh 1989, Jennings & Hayes 1994).

Previously, Jaeger et al. (2001) had rejected the hypothesis that *R. yavapaiensis*

occurred within the current range of *R. onca*, including in their analysis samples from a

now extinct population on the Virgin River (site LF in Fig. 2.1a) formerly identified as

containing *R. yavapaiensis* (Platz & Frost 1984). Provokingly, a recent discovery of an

isolated population of related leopard frogs from a tributary to the Colorado River

(Surprise Canyon; site SU in Fig. 2.1a) in the western Grand Canyon has raised further

questions about the history of the *R. onca-yavapaiensis* group in that a tentative mtDNA

assessment of a single sample from this newly discovered population showed that it

grouped more closely with *R. yavapaiensis* (Gelczis & Drost 2004).

The Southwest deserts have complex biogeographic histories, and desert biotas show

the genetic influence of major historical events, some of which implicate pre-Pleistocene

vicariance (Hafner & Riddle 2005). Quaternary climatic oscillations, however, have

greatly affected environmental conditions in these deserts (e.g. Betancourt et al. 1990;

Thompson & Anderson 2000), and several warm-desert taxa with distributions in the

regions occupied by *R. onca* and *R. yavapaiensis* display genetic structures impacted by the most recent (Late Pleistocene - Holocene) climatic changes (e.g. Riddle et al. 2000; Douglas et al. 2006; Fehlberg & Ranker 2009). For example, low mtDNA diversity in populations of the red-spotted toad (*Bufo punctatus*) within the northeastern Sonoran Desert was interpreted as evidence of range expansion into this region following the development of warmer climatic conditions in the Middle to Late Holocene (Jaeger et al. 2005). Anurans, in general, may be especially susceptible to changes in climatic factors because they are exothermic, have permeable skins, and many lay unshelled eggs dependent on surface waters (Blaustein et al. 2001).

Both *R. onca* and *R. yavapaiensis* show affinities for warmer climatic conditions, although *R. yavapaiensis* does not generally occur in the warm lowlands of the Sonoran Desert. The stream and wetland habitats occupied by these frogs have undergone substantial changes throughout modern times (Bradford et al. 2004; Sredl 2005) and presumably dramatic changes have occurred during Quaternary climatic oscillations. These fluctuations likely caused periods when aquatic habitats were broader and better connected allowing dispersal among populations and regions, and periods of isolation when habitats were reduced and fragmented. The climatic conditions that favor these frogs, however, may be more subtle than glacial-interglacial (pluvial-interpluvial) patterns.

The purpose of our study was to gain further insight into the evolutionary history of *R. onca* and *R. yavapaiensis* in light of the recent discovery of the purported population of *R. yavapaiensis* in the western Grand Canyon. We expand on the analysis of Jaeger et al. (2001) by obtaining samples from numerous sites across the extant ranges of these

species, and define lineages of mtDNA genes through phylogeographic analyses. To corroborate genetic signals, we evaluate sequence data using demographic analyses (i.e. mismatch distribution and neutrality tests). We also explore independent scenarios of late Quaternary population histories using species distribution models (SDMs, e.g. Peterson 2001; also known as ecological niche models) and project these models onto reconstructions of climatic conditions during the latest glacial maximum (e.g. Carstens & Richards 2007; Waltari et al. 2007).

Materials and Methods

Sampling

We collected or acquired tissue samples predominantly from animals captured and released, and assessed 276 samples of our target species from 30 sites (Fig. 2.1a; Table 2.1, Table 2.2). These samples included: 51 *R. onca* from five sites in southern Nevada and one site in northwestern Arizona (the LF site in Fig. 2.1a); 202 *R. yavapaiensis* samples from 23 sites in Arizona and northern Mexico; and 23 samples from the population in Surprise Canyon, Arizona. We included an additional 36 samples from four sites in southern Sonora collected at locations thought to represent *R. yavapaiensis* sites but that revealed divergent mtDNA we interpret tentatively as representing *R. magnaocularis* (Frost & Bagnara 1976; see below). We used samples of *R. forreri* and an undescribed ranid species (*Rana* 'species 8') as outgroups based on their close phylogenetic relationship to our target taxa (Hillis & Wilcox 2005).

23

Laboratory Methods

We isolated total genomic DNA using phenol-chloroform extraction, and assessed the entire 1035 base pairs (bp) of NADH dehydrogenase subunit 2 (ND2) for all samples. For phylogenetic analysis we sequenced exemplars of each ND2 haplotype ($n = 23$) for an additional 916 bp segment of cytochrome *b* (Cyt*b*). We used primers L3880 and H6033 (Riddle et al. 1993) to amplify the ND2 gene, and for sequencing replaced the reverse primer with two internal primers, H5532 (Macey et al. 2001) and H23C (designed for this study; 5`- GAAATTCCTTGA AGGACCTCAGG - 3`). To amplify and sequence Cyt*b,* we used modified primers of MVZ15-L and CytbAR-H (Vences et al. 2004).

We conducted amplifications by polymerase chain reaction at annealing temperatures between 53-57 °C using *Ex Taq* Polymerase Premix (Takara Mirus Bio, Inc., Madison, WI, USA), and purified products with ExoSAP-IT (USB Corp., Cleveland, OH, USA). We conducted fluorescence-based cycle sequencing using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1, with electrophoresis on an ABI 3130 automated sequencer (Applied Biosystems, Inc., Foster City, CA, USA). We aligned sequences using SEQUENCHER v. 4.6 (Gene Codes Corp., Inc., Ann Arbor, MI, USA), and verified alignments against those of other ranids accessed from GenBank (Lee et al. 1999; Macey et al. 2001).

Phylogeographic Analyses

We calculated haplotype and nucleotide diversity using ARLEQUIN v. 3.11 (Excoffier et al. 2005) and mean pairwise sequence divergences (uncorrected *p*-distances) using MEGA v. 4 (Tamura et al. 2007). Prior to phylogenetic analysis of the concatenated (ND2

+ Cyt*b*) sequence data of the haplotype exemplars, we applied the partition homogeneity test (Farris et al. 1995) in PAUP *v. 4.0b10 (Swofford 2002) which indicated that the two genes were congruent ($P = 1.00$). We assessed phylogenetic patterns using the concatenated data under the criteria of Maximum Parsimony (MP) in PAUP* and Bayesian inference (BI) in MRBAYES v. 3.1.2 (Ronquist & Huelsenbeck 2003).

We generated unweighted MP trees employing 1000 non-parametric bootstrap replicates, heuristic search with 10 random stepwise additions, and tree-bisection-reconnection branch-swapping. To select appropriate models for BI, we used MRMODELTEST v. 2.2 (Nylander 2004) under the Akaike Information Criterion (AIC; Posada & Buckley 2004). We evaluated preliminary runs for best fit partitioning schemes using Bayes factors on the harmonic mean marginal likelihood values (Nylander et al. 2004). Final analyses were run with the Hasegawa-Kishino-Yano (HKY) model for the combined $1^{st} + 2^{nd}$ codon positions and the General Time Reversible (GTR) model for the 3rd codon position for both genes, with equal rates of substitution between nucleotide positions.

For BI runs, we unlinked model parameters across character partitions and left the Metropolis-coupled Markov chain Monte Carlo on default, except we set the heating parameter to 0.1 in order to keep state swap frequencies between 10% and 70%. The 50% majority-rule consensus tree and associated posterior probabilities used for final interpretations were based on 3 runs of 4 million generations each. Trees were sampled every 100 generations with the first 25% of sampled trees discarded as burn-in after confirming chain stationarity using the program TRACER v. 1.4 (Rambaut & Drummond 2007).

To assess divergence times, we employed a molecular clock approach, while recognizing the potential limitations with these interpretations (e.g. Edwards & Beerli 2000; Arbogast et al. 2002). Molecular clock evaluations in anurans have often been based on a rate estimated by Macey et al. (1998) for the separation of European and Asian bufonids. This rate of 1.38% sequence divergence between lineages per million years, or $\mu = 6.9 \times 10^{-9}$ substitutions/site/year (s/s/y), was based on partial ND1, ND2, and the intervening tRNAs, but it has been applied widely as an estimate, although probably a conservative one, for both Cyt*b* and ND2 (e.g. Jaeger et al. 2005; Austin & Zamudio 2008). This clock has been recalculated for only the ND2 gene in the genus *Eleutherodactylus* (Crawford 2003) which resulted in a mutation rate of 1.91% ($\mu = 9.57 \times 10^{-9}$ s/s/y). A much faster rate of 3.6% ($\mu = 1.8 \times 10^{-8}$ s/s/y) has been applied to Cyt*b* in European ranid species (e.g. Babik et al. 2004).

To estimate the time to the most recent common ancestor, we applied the slower and faster substitution rates in the coalescence-based program BEAST v. 1.4.8 (Drummond & Rambaut 2007). Prior to estimation, we tested the concatenated (haplotype) data set without outgroups for rate heterogeneity using a likelihood ratio test (Huelsenbeck & Crandall 1997) in PAUP*, which failed to reject the molecular clock assumption ($\chi^2 = 14.88$, d.f. $= 21$, $P = 0.83$). We evaluated partitioning of the concatenated sequence data using Bayes factors, and for analysis, we used a strict clock and partitioned using models HKY for the combined $1^{st} + 2^{nd}$ codon positions and GTR for the 3rd codon position obtained from MRMODELTEST. We also assessed coalescent models of constant population size, exponential growth, expansion growth, and Bayesian skyline using Bayes factors, and selected constant population size. For final analysis, we conducted

two Markov Chain Monte Carlo (MCMC) runs of 20 million generations each, sampling every 2000 generations, with the first 10% discarded as burn-in. For interpretation, we combined runs and used TRACER to examine the estimated sample sizes (ESS) to avoid poor estimates of the parameters (ESS < 200) and to depict means and credibility intervals (CI).

Population Analyses

Given the expected shallow intraspecific genetic structure (Jaeger et al. 2001), we evaluated the complete ND2 data set of our taxa using a median-joining network (Bandelt et al. 1999) constructed in NETWORK v. 4.2.0.1 (www.fluxus-engineering.com). We evaluated isolation by distance among sites (pairwise F_{st}-values versus Euclidean geographic distances) using a Mantel test in the program AIS (Miller 2005). We also applied a series of demographic genetic approaches to assess the ND2 data of *R. yavapaiensis*, but do not present these analyses for *R. onca* and the Surprise Canyon population as these taxa were limited in geographic scope and genetic variation (see Results).

We used mismatch distributions to test for sudden demographic expansion (Rogers & Harpending 1992; Schneider & Excoffier 1999) in *R. yavapaiensis* using ARLEQUIN, and estimated population expansion parameters τ (time since expansion expressed in units of mutational time), $\theta_0 = 2\mu N_0$, and $\theta_1 = 2\mu N_1$ (where N_0 and N_1 are the estimated number of females before and after the expansion). For sudden expansion, we approximated the beginning of the time of expansion using the formula $t = \tau/2\mu$, where t is the time measured in years since expansion and μ is the per-sequence mutation rate per generation (Rogers & Harpending 1992). We assumed ND2 rates of both 7.1×10^{-6} and 9.9×10^{-6}

substitutions/locus/year (from above) and a two-year generation time for female *R. yavapaiensis* (Sredl et al. 1997). For comparison, we conducted neutrality tests of Fu's F_s (Fu 1997) in Arlequin and R_2 (Ramos-Onsins & Rozas 2002) in DNASP v. 4 (Rozas et al. 2003).

Species Distribution Modeling

We used the program MAXENT v. 3.3.1 (Phillips et al. 2006) to develop SDMs based on recent occurrence records and 19 bioclimatic layers representing trends, seasonality, and extremes of temperature and precipitation. We assumed in these SDMs that species distributions were determined by climate, thus ignoring potentially important features limiting frog distributions such as surface hydrology and biotic interactions (other than those driven by climate). Our emphasis, however, was on exploring broad geographic shifts in potential habitat based on changes in climate. We also made the simplifying assumption that these frogs did not shift ecological niches in response to climatic changes (niche conservatism; Wiens & Graham 2005).

We used bioclimatic data from the WorldClim database v. 1.4 with resolution of 2.5 minutes (~ 5 km; www.worldclim.org; Hijmans et al. 2005) and obtained occurrence records of *R. onca* and *R. yavapaiensis* from museum collections, literature references, and a regional database (Table 2.3). Our genetic sampling, however, revealed frogs with divergent mtDNA at four locations purported to be *R. yavapaiensis* sites in southern Sonora (Fig. 2.1a), within the Plains of Sonora and Sinaloan thornscrub biomes. Because of this taxonomic uncertainty, we excluded these four sites, as well as seven other records within the boundaries of the same lower elevation biomes within Sonora. For occurrence records that lacked coordinates or associated uncertainty, we derived estimates using the

'Georeferencing Calculator' (http://herpnet.org). We also excluded occurrence records that lacked acceptable geographic description or had an uncertainty greater than 5 km. The final data set included 27 locations of *R. onca* within its historical distribution (Bradford et al. 2004), 270 locations of *R. yavapaiensis*, and 17 locations of purported *R. yavapaiensis* from southern California.

For MAXENT runs we used logistic regression under default settings (except for random seed) and averaged 20 replicate bootstrap models per species. We assigned 85% of occurrence records for model training and 15% for model testing. The SDMs were then projected onto simulated past climate data (Thompson & Anderson 2000) representing the latest glacial maximum (approximately 21,000 years before present) derived from two climatic models – Community Climate System Model (CCSM; Collins et al. 2006) and Model for Interdisciplinary Research on Climate (MIROC; Hasumi & Emori 2004). We explored the impact of various masks on SDMs, including generating models using masks based on appropriate ecoregions for each species. The various approaches generally converged on similar overall patterns, and we present models developed using restricted rectangular masks for *R. onca* (NW corner 38.25^{o}, -118.67^{o}; SE corner 31.46^{o}, -111.50^{o}) and *R. yavapaiensis* (NW corner 38.04^{o}, -118.63^{o}; SE corner 25.50^{o}, -105.63^{o}). Habitat suitability was displayed as two categories in ARCGIS v.9.2. (ESRI, Inc., Redlands, CA 2007) with the lowest probability habitat defined as the lowest training presence threshold. This threshold presents suitable habitat as having values at least as high as that of all the occurrence records (Pearson et al. 2007).

Results

Phylogeographic Analyses

Our assessment of ND2 resulted in the identification of 2 *R. onca* and 21 *R. yavapaiensis* haplotypes for which we generated additional Cyt*b* data on exemplars (Table 2.1). The pairwise number of nucleotide differences among the concatenated haplotypes was at least 45 (out of 1951) between *R. onca* and *R. yavapaiensis*, with an uncorrected *p*-distance of 0.022. We identified six divergent haplotypes (based on ND2) from four locations in Sonora (Fig. 2.1a), and sequenced representative samples for Cyt*b* to include in the phylogenetic analysis. These divergent samples differed from *R. onca* and *R. yavapaiensis* by a minimum of 142 nucleotides resulting in an uncorrected *p*-distance of 0.07 to the nearest ingroup taxa (*R. onca*). We tentatively identify these samples as representing *R. magnaocularis* as our sequences were little different from that we derived for an adult specimen of *R. magnaocularis* (data not shown) collected from the Río Urique in Chihuahua (number MSB 75171, Museum of Southwestern Biology, University of New Mexico). We also sequenced three of our samples for a partial segment of mtDNA 12S and compared these with published sequences (see Pfeiler & Markow 2008) for species in the *R. berlandieri* subgroup (*Scurrilirana* clade of Hillis & Wilcox 2005). Our samples were identical (403 bp) to a sample from Sierra El Aguaje in southern Sonora (GenBank: EU728669) and closely related to a *R. magnaocularis* sample from Nayarit (GenBank: AY115131). As previously noted by Pfeiler and Markow (2008), this haplotype was not closely related to a purported *R. magnaocularis* sample from near Nuri, Sonora (GenBank: AY779239). Within the region of the Río Yaqui and Río Moctezuma, where our samples were acquired, considerable genetic variation among

topminnows, genus *Poeciliopsis*, has been associated with river drainages (Quattro et al. 1996), and it is possible that leopard frogs may also demonstrate similar phylogeographic structure. As previously suggested by Pfeiler and Markow (2008), further assessments are necessary clarifying the phylogenetic and taxonomic relationships among leopard frogs in the region.

Maximum parsimony analysis of the concatenated data set resulted a single tree (length = 644, CI = 0.885, RI = 0.929) which showed the same general topology as that from BI (Fig. 2.1b). All major clades were strongly supported (Wilcox et al. 2002) based on bootstrap values (= 100) and posterior probabilities (= 1.00; Fig. 2.1b). These analyses supported the phylogenetic break between *R. onca* and *R. yavapaiensis* (Jaeger et al. 2001), and further divided *R. yavapaiensis* into two monophyletic clades (with uncorrected *p*-distance = 0.008). One of these clades (herein called the 'main *R. yavapaiensis* clade') represents populations from Arizona and Mexico typically within the uplands of the Sonoran Desert. The other clade represents the single population from Surprise Canyon in the western Grand Canyon (herein called the 'Surprise Canyon population').

Application of substitution rates in BEAST indicate divergence for *R. onca* and *R. yavapaiensis* that most likely occurred around the Early Pleistocene; although the array of molecular rates for the ND2 and Cyt*b* genes results in a broad range for the potential timing of this event (slower rate = 1.95 Mya, 95% CI = 1.42-2.47; faster rate = 0.75 Mya, 95% CI = 0.56-0.96). Divergence of the Surprise Canyon population from the main *R. yavapaiensis* clade appears to have followed around the Middle Pleistocene (slower rate = 0.74 Mya, 95% CI = 0.46-1.05; faster rate = 0.29 Mya, 95% CI = 0.18-0.40).

Population Analyses

The haplotype network for *R. onca* and *R. yavapaiensis* (Fig. 2.2a) depicted three main groups consistent with the major clades inferred from the MP and BI trees. The two haplotypes of *R. onca* were a minimum of 28 mutational steps within the network from the nearest *R. yavapaiensis* sample from the Surprise Canyon population, and the two haplotypes from the Surprise Canyon population were separated from the main *R. yavapaiensis* group by an additional seven to eight steps. Our ND2 data showed low haplotype and nucleotide diversity within *R. onca* (Table 2.4), consistent with the current population bottleneck.

The main *R. yavapaiensis* clade showed relatively high haplotype diversity (Table 2.4), but the majority of these haplotypes were only a single bp from the common haplotype resulting in a shallow star-shaped pattern (Fig. 2.2a). The most common haplotype (H6) was present at 78% (18/23) of sites (Fig. 2.2b), which affected the assessment of isolation by distance (Mantel test) with only a weak correlation determined between geographic and genetic distances (r = 0.17, *P* = 0.001). Many of the *R. yavapaiensis* sites (9/23) were fixed for particular haplotypes, with most of these fixed for the most common haplotype. Visual inspection of haplotype diversities among *R. yavapaiensis* sites showed nearly equal levels across latitudes and elevations indicating no strong correlations with these variables, but this was not surprising given the low genetic diversity within sites (the maximum number of haplotypes at any one site was only three). River basins also appeared to explain only low amounts of genetic variation (Appendix).

The moderately high haplotype diversity coupled with low nucleotide diversity

observed within the main *R. yavapaiensis* clade (Table 2.4) indicates the possibility of

rapid population growth (Grant & Bowen 1998; Avise 2000). A signature of growth was

also detected from the mismatch distribution assessment which showed a smooth

unimodal curve (Fig. 2.3) under the sudden expansion model ($SSD = 0.0001$, $P = 0.949$; r

$= 0.0394$, $P = 0.828$) indicating no significant difference between the observed and

simulated pairwise differences. The estimated demographic parameters from the

mismatch distribution all indicated sudden expansion (Excoffier & Schneider 1999) since

τ was greater than 0 and $\theta_1 > \theta_0$ ($\tau = 1.25$, 95% CI = 0.28-2.33; $\theta_1 = 10.93$, 95% CI =

1.45-99,999; $\theta_0 = 0.035$, 95% CI = 0.00-0.55). The time of expansion was indicated to

occur around the transition between Middle and Late Pleistocene but with a wide level of

uncertainty (slower rate = 0.18 Mya, 95% CI = 0.04-0.33; faster rate = 0.13 Mya, 95% CI

= 0.03-0.24). Expansion was also detected in the main *R. yavapaiensis* clade from the

significantly negative Fu's F_S (-12.0855; $P = 0.001$) value and low R_2 value (0.0316; $P =$

0.014) expected from population growth.

<p align="center">*Species Distribution Modeling*</p>

The SDMs for both species produced high training and testing AUC values (Area

Under the Curve parameter of the Receiver Operating Characteristic plot; all values $\geq$

0.970), indicating that all models performed better than random (Raes & Ter Steege

2007). The SDM for *R. onca* under current climate conditions (Fig. 2.4a) generally

represented a reasonable prediction of the known historical distribution as defined by

Bradford et al. (2004). The projection of this SDM onto the two Pleistocene climate

simulations of the latest glacial maximum produced very different results. The CCSM

<p align="center">33</p>

model (Fig. 2.4b) predicted persistence of potential habitat essentially within the area predicted under current climate along with an unlikely distribution within Death Valley, California. The MIROC model (Fig. 2.4c), however, predicted an expansion of suitable habitat (along with some overpredictions in areas not likely occupied by these frogs), but importantly this did not extend very far south along the Lower Colorado River or into the Imperial and Mexicali valleys – areas historically occupied by purported *R. yavapaiensis*. Potential habitat was also identified in areas of central Arizona, but this prediction was not always stable under alternative masks used for modeling (data not shown).

For *R. yavapaiensis*, the SDM under current climatic conditions also depicted a reasonable representation of current distribution, but with substantial overprediction of lower probability habitat (Fig. 2.4d). Even with the overprediction, this model did not show substantial overlap with areas occupied by *R. onca*. The projection of the current SDM for *R. yavapaiensis* onto the two Pleistocene climate simulations also produced very different results, although both models predicted a geographic shift towards lower elevation areas of the Sonoran Desert. The model based on CCSM (Fig. 2.4e) predicted a reduction of suitable habitat (particularly higher probability habitat) from that depicted under current conditions, as well as a possible north-south vicariance. The model based on MIROC (Fig. 2.4f) predicted moderate expansion, mostly of lower probability habitat. Importantly, both paleo-SDMs for *R. yavapaiensis* indicated persistence of habitat along the Lower Colorado River extending into the region around the Imperial and Mexicali valleys. Habitat also was predicted in these valleys by SDMs generated for *R. yavapaiensis* that did not include occurrence records from southern California (data not shown).

Discussion

Comparison to Previous Assessments

Our assessment corroborates the previously determined phylogenetic break between

R. onca and *R. yavapaiensis* (Jaeger et al. 2001), as we found no admixing of *R. onca* and

R. yavapaiensis haplotypes within sites after extensive sampling. However, our analyses

indicate a more complex history for these frogs than previously supposed (Jaeger et al.

2001), and our phylogeographic assessment further divided *R. yavapaiensis* into two

distinct mtDNA lineages – one representing populations across the main range in Arizona

and northern Mexico, and the other representing the disjunct population in the western

Grand Canyon.

Jaeger et al. (2001) suggested that the level of mtDNA divergence between *R. onca*

and *R. yavapaiensis* represented Late Pleistocene-Holocene isolation, but our divergence

estimates indicate the possibility of an older timing for this separation, possibly dating to

around the Early Pleistocene. Further, under the assumption that our molecular clocks

are moderately accurate, the shallow divergence of the Surprise Canyon population from

the main clade of *R. yavapaiensis* appears to date to the Middle Pleistocene. These

molecular clock interpretations, however, must be viewed speculatively, as demographic

and selective processes can greatly influence the coalescence of mtDNA, resulting in

deeper phylogenetic separation than warranted by actual divergence time (Avise 2000).

One possibility is that the observed patterns could have been caused by an overall decline

in a highly diverse ancestral (*R. onca-yavapaiensis*) species that left behind small

regional populations that retained, and then fixed divergent ancestral polymorphisms.

This may be more common in organisms, such as these frogs, in which regional dispersal

is perhaps limited, population size fluctuates greatly (lowering N_e), and selective sweeps may be an important evolutionary factor; for example in anurans (and other ectotherms) temperature directly impacts the mitochondria and changes in this climatic feature may lead to selection favoring particular genotypes (Ballard & Whitlock 2004).

Demographic patterns that could have affected interpretations of divergence timing are clearly evident in these species. The Surprise Canyon population of *R. yavapaiensis* currently appears to be isolated in one drainage within the western Grand Canyon (CAD, JRJ, and DFB unpublished data), and *R. onca* has suffered a dramatic, recent decline (Bradford et al. 2004). The low genetic diversity observed in *R. onca* was expected given its overall decline, and was consistent with a previous assessment of nuclear genetic diversity based on randomly amplified polymorphic DNA (RAPD) data (Jaeger et al. 2001). It is also possible that *R. onca* may have always been geographically limited (as depicted in one paleo-SDM; Fig. 2.4b), and even if it was more broadly distributed our genetic sampling represents only the few remaining, closely situated populations.

For *R. yavapaiensis,* the genetic data indicate that the main clade has historically undergone population expansion. Moderately high haplotype diversity coupled with low nucleotide diversity within the *R. yavapaiensis* clade indicates the possibility of a population bottleneck followed by rapid growth (Grant & Bowen 1998; Avise 2000). Support for an interpretation of population expansion comes from the mismatch distribution assessment and from the neutrality test results. This signal of expansion in *R. yavapaiensis* might be attributable to population or range expansion following the latest glacial period, as depicted by the difference between the current SDM (Fig. 2.4d) and one of the paleo-SDMs (Fig. 2.4e). However, a rough estimate of the time of this expansion,

36

derived from the assessment of mismatch distribution, suggests a time frame that likely predates the recent glacial maximum. Importantly, genetic diversity across the core *R. yavapaiensis* distribution shows no strong correlation with latitude, thus providing no evidence for the commonly envisioned pattern of northward expansions of warm-adapted species from glacial refugia in more southern areas of the Sonoran Desert. Instead, the genetic pattern is consistent with an interpretation that *R. yavapaiensis* responded with only moderate shifts in distributions during the last glacial period mostly to adjacent areas of lower elevation (Fig. 2.4e, 2.4f).

Biogeographic Patterns

A likely scenario for the phylogeographic patterns observed for *R. onca* and *R. yavapaiensis*, particularly along the Colorado River, is that the ancestral lineage to these frogs expanded and contracted multiple times (at least twice) during the Quaternary, probably from the core areas identified for *R. yavapaiensis* within the northern Sonoran Desert, essentially allowing connections to the Colorado River. This was followed by contractions of the main population and subsequent isolation and divergence of remnant populations within northern, or possibly western, refugia. *Rana onca* may have subsequently evolved as a local endemic, restricted to a narrow area along the Colorado River and its tributaries within the eastern Mojave Desert (Fig. 2.4a). *Rana yavapaiensis*, on the other hand, is associated with areas identified as Sonoran Desert, including areas along the Lower Colorado River and the Imperial and Mexicali valleys (Fig. 2.4d). Assuming local adaptation, differences in the climates between these desert regions may have contributed to limiting long term contact between these taxa.

The disjunct location of the Surprise Canyon population of *R. yavapaiensis* may seem hard to explain, given that *R. onca* populations occupy the Colorado River corridor between Surprise Canyon and populations of *R. yavapaiensis* along the Lower Colorado River. However, the nearest population of *R. yavapaiensis* to Surprise Canyon is in Willow Creek, about 85 km due south (site WC in Fig. 2.1a), and there is a relatively low divide between the headwaters of this drainage and the north-flowing tributaries that feed into the Colorado River in the vicinity of Surprise Canyon. Much of the upper parts of these drainages are dry under current climatic conditions, but we suspect that this was a likely pathway that once connected the main distribution of *R. yavapaiensis* with Surprise Canyon under a cooler or wetter climate. What is striking is that the Surprise Canyon population shows a level of divergence that indicates longevity to its isolation. There is, however, evidence from paleo-reconstructions that lower elevations of the western Grand Canyon retained warmer conditions through the last glacial maximum (e.g. Phillips 1977). This could have allowed persistence of these frogs through time within an isolated northern refugium in the canyon region (one not depicted by our coarse-scale paleo-SDMs).

Conclusions

The main phylogeographic patterns observed for *R. onca* and *R. yavapaiensis* are likely robust at the organismal level and expand our understanding of the evolutionary history of this group. Given the observed levels of mtDNA divergence and previous research that included nuclear (RAPD) and morphological assessments which supported the main divergence (Jaeger et al. 2001), the further application of nuclear genes are not

likely to change the interpretation of these patterns, as many of these genes would not be expected to track this more recent evolutionary history (e.g. Zink & Barrowclough 2008). Of more importance to interpretations of the phylogeography of *R. onca* and *R. yavapaiensis* would be a genetic assessment of historical (museum) specimens from extirpated populations in southern California.

Our data point to the uniqueness of the northernmost population of *R. yavapaiensis* within Surprise Canyon. While the level of difference from other *R. yavapaiensis* populations based on mtDNA may not warrant taxonomic recognition at this time, this disjunct population merits conservation consideration and further study. Finally, the tentative identification of *R. magnaocularis* haplotypes at sites in Sonora thought to contain *R. yavapaiensis* indicates a need to refine our understanding of the distributions and genetic structure (including the possibility of hybridization) of these species in Mexico.

Table 2.1. Exemplar samples of ND2 haplotypes for *Rana onca* (H1-2), *R. yavapaiensis* (H3-23), and tentatively identified *R. magnaocularis* (M1-6). For phylogeographic analysis, each sample was also sequenced for Cyt*b*. Exemplar samples are listed by sample number, site, county, state, and country. Further information on locations is available in Table 2.2. Outgroup samples of *R. forreri* and *R.* 'species 8' are identified by sample number and type locality. Sequences are available from GenBank under accession numbers GU184190-GU184251.

Haplotype Number	Sample Number	Type Locality
H1	LVT3541	Bighorn Sheep Spring, Clark Co., NV, USA
H2	LVT3440	Blue Point Spring, Clark Co., NV, USA
H3	LVT7091	Surprise Canyon, Mohave, Co., AZ, USA
H4	LVT7095	Surprise Canyon, Mohave, Co., AZ, USA
H5	LVT4560	Trout Creek, Mohave, Co., AZ, USA
H6	LVT9531	Río Cocospera, Rancho el Aribabi, SO, MX
H7	LVT4562	Trout Creek, Mohave, Co., AZ, USA
H8	LVT4579	Trout Creek, Mohave, Co., AZ, USA
H9	LVT8814	Santa Maria River, Yavapai Co., AZ, USA
H10	LVT4567	Cottonwood Creek, Yavapai Co., AZ, USA
H11	LVT8092	Coon Creek, Gila Co., AZ, USA
H12	LVT8037	Pinto Creek, Gila Co., AZ, USA
H13	LVT7395	Aravaipa Creek, Graham Co., AZ, USA
H14	LVT8181	Markham Creek, Graham Co., AZ, USA
H15	LVT7190	Muleshoe Hotsprings, Cochise Co., AZ, USA
H16	LVT7983	Cienega Creek, Santa Cruz Co., AZ, USA
H17	LVT9548	Alamo Canyon, Santa Cruz Co., AZ, USA
H18	LVT9534	Río Cocospera, Rancho el Aribabi, SO, MX
H19	LVT9532	Río Cocospera, Rancho el Aribabi, SO, MX
H20	NK3927	Canon Bonito, Rancho Nuevo, SO, MX
H21	NK3929	Canon Bonito, Rancho Nuevo, SO, MX
H22	LVT9990	Canon el Pulpito, SO, MX
H23	LVT9015	Río Tutuaca, Rancho el Nogal, CH, MX
M1	LVT9501	Río Yaqui, SO, MX
M2	LVT9970	Río Sahuaripa, SO, MX
M3	LVT9521	Río Sonora, SO, MX
M4	LVT10354	Arroyo San Ignacio, SO, MX
M5	LVT9503	Río Yaqui, SO, MX
M6	LVT10353	Arroyo San Ignacio, SO, MX
R. forreri	KU194581	37.9 km S. of Escuinapa, SI, MX
R. 'species 8'	KU195346	Río Atoyac at Mexico Hwy. 190, PU, MX

Table 2.2. Sample sites for *Rana onca* and *R. yavapaiensis* by county, state, country, site labels (referenced in figures), geographic coordinates (datum NAD27), and haplotypes observed. Also shown are sites in Sonora where samples have been tentatively identified as *R. magnaocularis*.

Species, Site, County, State, Country	Label	Lat.	Long.	Haplotype (*n*)
Rana onca				
Bighorn Sheep Spring, Clark Co., NV, USA	BH	35.939	-114.733	H1(10)
Blue Point Spring, Clark Co., NV, USA	BP	36.389	-114.432	H2(10)
Boy Scout Canyon, Clark Co., NV, USA	BS	35.984	-114.745	H1(10)
Littlefield, Mohave Co., AZ, USA	LF	36.908	-113.896	H1(10)
Rogers Spring, Clark Co, NV, USA	RS	36.378	-114.443	H2(4)
Salt Cedar Canyon, Clark Co., NV, USA	SC	35.965	-114.743	H1(7)
Rana yavapaiensis				
Alamo Canyon, Santa Cruz Co., AZ, USA	AC	31.365	-111.135	H17(8)
Aliso Spring, Santa Cruz Co., AZ, USA	AS	31.581	-111.099	H6(10)
Aravaipa Creek, Graham Co., AZ, USA	AR	32.878	-110.392	H6(1), H13(9)
Canon Bonito, Rancho Nuevo, SO, MX	RN	31.232	-108.920	H6(1), H20(3), H21(1)
Canon el Pulpito, SO, MX	CP	30.777	-109.005	H20(4), H22(6)
Cienega Creek, Santa Cruz Co., AZ, USA	CN	32.011	-110.623	H16(3), H17(4)
Coon Creek, Gila Co., AZ, USA	CR	33.686	-110.843	H6(9), H11(1)
Cottonwood Creek, Yavapai Co., AZ, USA	CC	33.903	-112.324	H6(8), H10(2)
Hassayampa R., Maricopa Co., AZ, USA	HA	33.931	-112.692	H6(10)
Kayler Spring, Gila Co., AZ, USA	KS	33.945	-111.302	H6(8)
Markham Creek, Graham Co., AZ, USA	MC	33.091	-109.823	H14(10)
Mineral Creek, Pinal Co., AZ, USA	MN	33.251	-110.983	H6(1), H11(5)
Muleshoe Hotspr., Cochise Co., AZ, USA	MH	32.338	-110.250	H6(10), H15(2)
Pinto Creek, Gila Co., AZ, USA	PC	33.457	-111.005	H6(1), H12(9)
Río Bavispe, near Huachinera, SO, Mexico	RB	30.205	-108.957	H6(10)
Río Cocospera, Rancho el Aribabi, SO, MX	RC	30.858	-110.663	H6(2), H18(6), H19(2)
Río Tutuaca, Rancho el Nogal, CH, MX	RE	28.560	-108.356	H6(1), H23(5)

Santa Maria River, Yavapai Co., AZ, USA	SM	34.368	-113.184	H6(10), H9(1)
Sheep Wash, Greenlee Co., AZ, USA	SW	33.303	-109.404	H6(9)
Surprise Canyon, Mohave Co., AZ, USA	SU	35.908	-113.620	H3(15), H4(8)
Tonibabi, SO, MX	TB	29.833	-109.562	H6(10)
Trout Creek, Mohave Co., AZ, USA	TC	35.000	-113.447	H5(4), H7(2), H8(2)
Turkey Creek, Greenlee Co., AZ, USA	TU	33.288	-109.261	H6(7)
Willow Creek, Mohave Co., AZ, USA	WC	35.144	-113.530	H5(3), H6(2)
Rana magnaocularis				
Arroyo San Ignacio, SO, MX	SI	28.699	-109.085	M1(2), M4(2), M5(1), M6(1)
Río Sahuaripa, SO, MX	SR	29.186	-109.277	M1(5), M2(3), M4(1), M5(1)
Río Sonora, SO, MX	SN	29.331	-110.537	M3(10)
Río Yaqui, SO, MX	RY	28.591	-109.560	M1(7), M5(3)

Table 2.3. Sources for observation records of *Rana onca* and *R. yavapaiensis* used in species distribution modeling.

Species	Data Type	Source
R. onca	Literature	Bradford *et al*., 2004
	Museum Records	California Academy of Sciences, San Francisco
		Carnegie Museum of Natural History, Pittsburgh
		Los Angeles County Museum of Natural History, Los Angeles
		Marjorie Barrick Museum of Natural History, University of Nevada, Las Vegas
		Monte L. Bean Life Science Museum, Brigham Young University, Provo
		Museum of Vertebrate Zoology, University of California, Berkeley
R. yavapaiensis	Literature	Jennings, 1995
	Museum Records	Museum of Vertebrate Zoology, University of California, Berkeley
		Museum of Natural History, University of Arizona, Tucson
	Database	Ranid Frog Database – Arizona Game and Fish Department, Phoenix
California records	Literature	Jennings & Hayes, 1994
	Museum Records	Louisiana Museum of Natural History, Baton Rouge
		Smithsonian National Museum of Natural History, Washington, D.C.

Table 2.4. Molecular diversity indices for ND2 sequences of *Rana onca*, the main clade of *R. yavapaiensis*, the Surprise Canyon population of *R. yavapaiensis,* and all *R. yavapaiensis* samples combined. Shown are sample sizes (*n*), numbers of haplotypes (*nh*), haplotype diversity with standard error (*h* ± SE), and nucleotide diversity with standard error (*π* ± SE).

Taxon	*n*	*nh*	*h* ± SE	*π* ± SE (/100)
R. onca	51	2	0.4063±0.0575	0.0393±0.0409
Main *R. yavapaiensis*	202	19	0.6905±0.0357	0.1164±0.0826
Surprise Canyon	23	2	0.4743±0.0668	0.0458±0.0461
All *R. yavapaiensis*	225	21	0.7454±0.0302	0.2418±0.1448

Figure 2.1. **(a)** Sampled sites for genetic analysis with location abbreviations from Table 2.2. Circle shading reference taxa as follows: *Rana onca* (black); Surprise Canyon population of *R. yavapaiensis* (dark gray); *R. yavapaiensis* (light gray); and tentatively identified *R. magnaocularis* from locations originally sampled for *R. yavapaiensis* (white). Circle size is proportional to sample size (largest = 23, smallest = 4). **(b)** Depiction of the phylogenetic relationship of *R. onca* and *R. yavapaiensis* haplotypes based on 50% majority-rule consensus tree (ln L = -5283.75) from Bayesian inference runs. All major nodes are supported by 100% Bayesian inference posterior probabilities and Maximum parsimony bootstrap values (shown).

Figure 2.2. **(a)** Median-joining haplotype network of *Rana onca* and *R. yavapaiensis* with haplotypes coded by number. Crossbars along connection lines indicate a mutational change; the white square represents either an unsampled or an extinct common ancestor haplotype. Haplotypes are identified by shading according to the three major clades depicted in Fig. 2.1b. Circle size reflects the number of sampled individuals sharing a haplotype (largest = 110, smallest = 1). **(b)** The geographic distribution of ND2 haplotypes of *R. onca* and *R. yavapaiensis*. Haplotypes are referenced by code as depicted in the network, and pie size reflects the number of individuals per haplotype at each site.

Figure 2.3. Mismatch distribution analysis of ND2 sequence data from the main *Rana yavapaiensis* clade (excluding the Surprise Canyon samples) under the sudden expansion model.

Figure 2.4. Species distribution models for *Rana onca* under current climate conditions (a) and two glacial models, CCSM (b) and MIROC (c), and *R. yavapaiensis* under current climate (d), CCSM (e) and MIROC (f). White dots indicate sample locations. Higher (dark gray) and lower (lighter gray) logistic probability values for predicted suitable habitats are depicted.
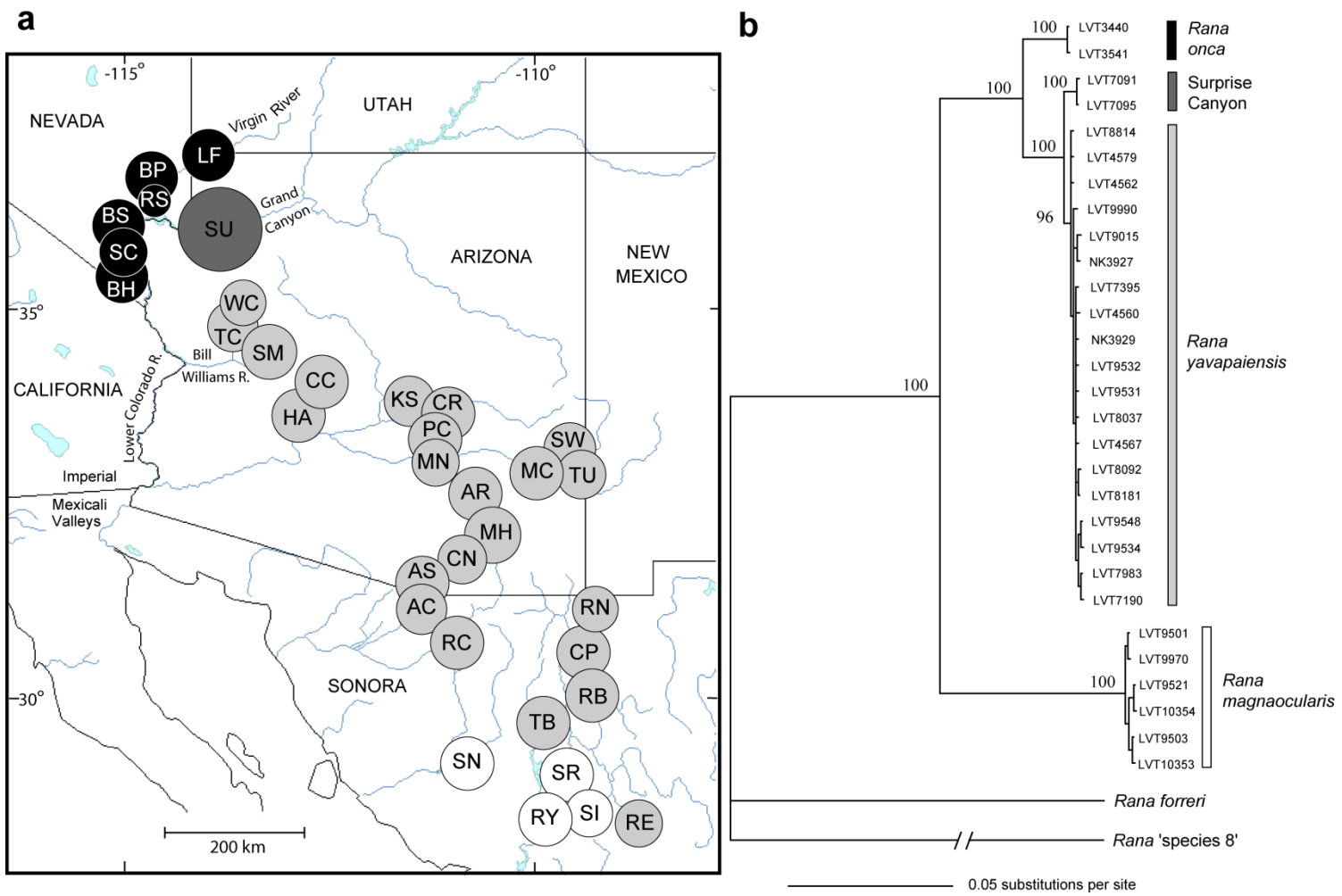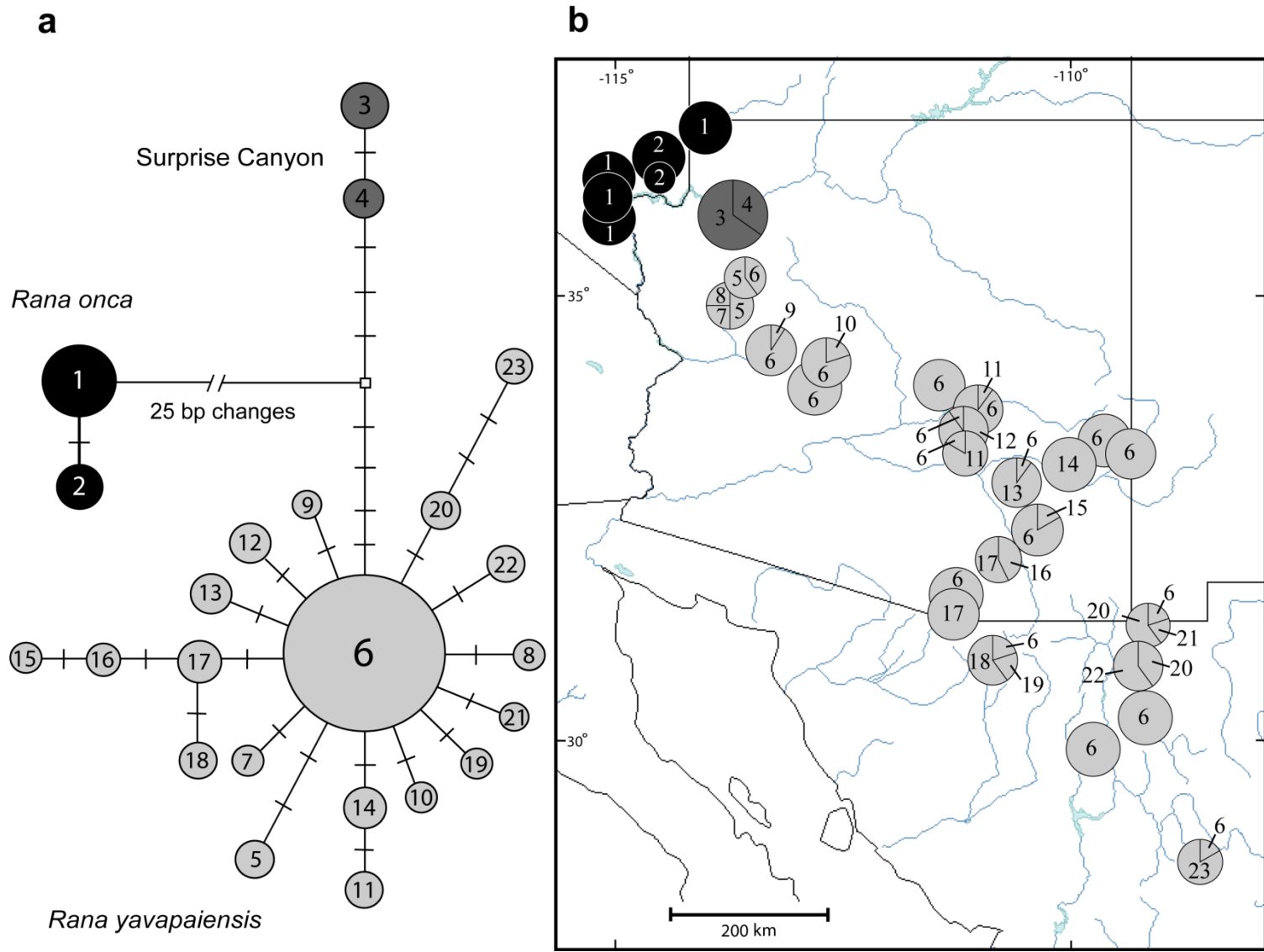
Figure 2.1.

Figure 2.2.

Figure 2.3.

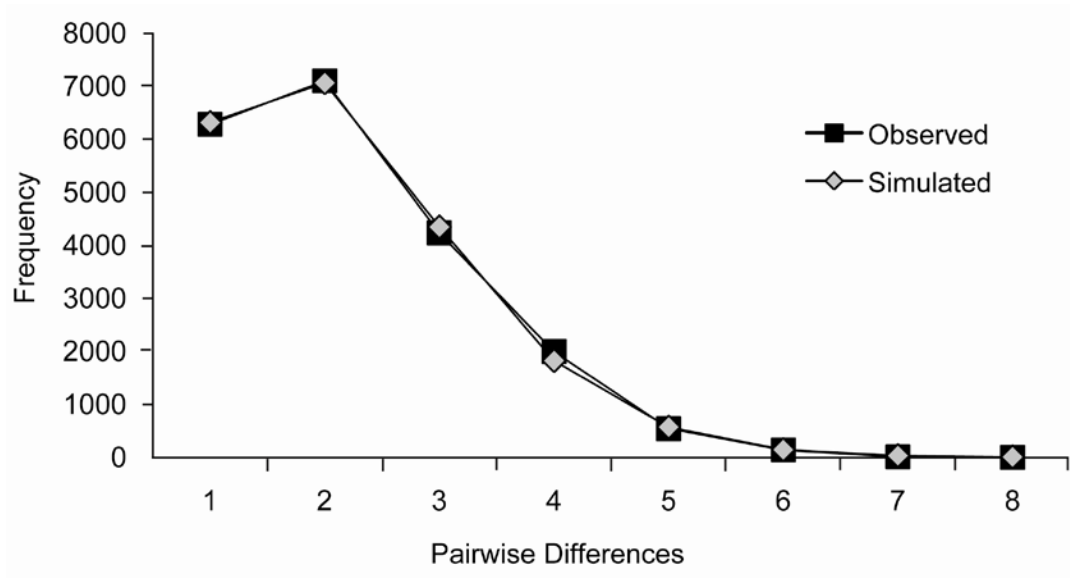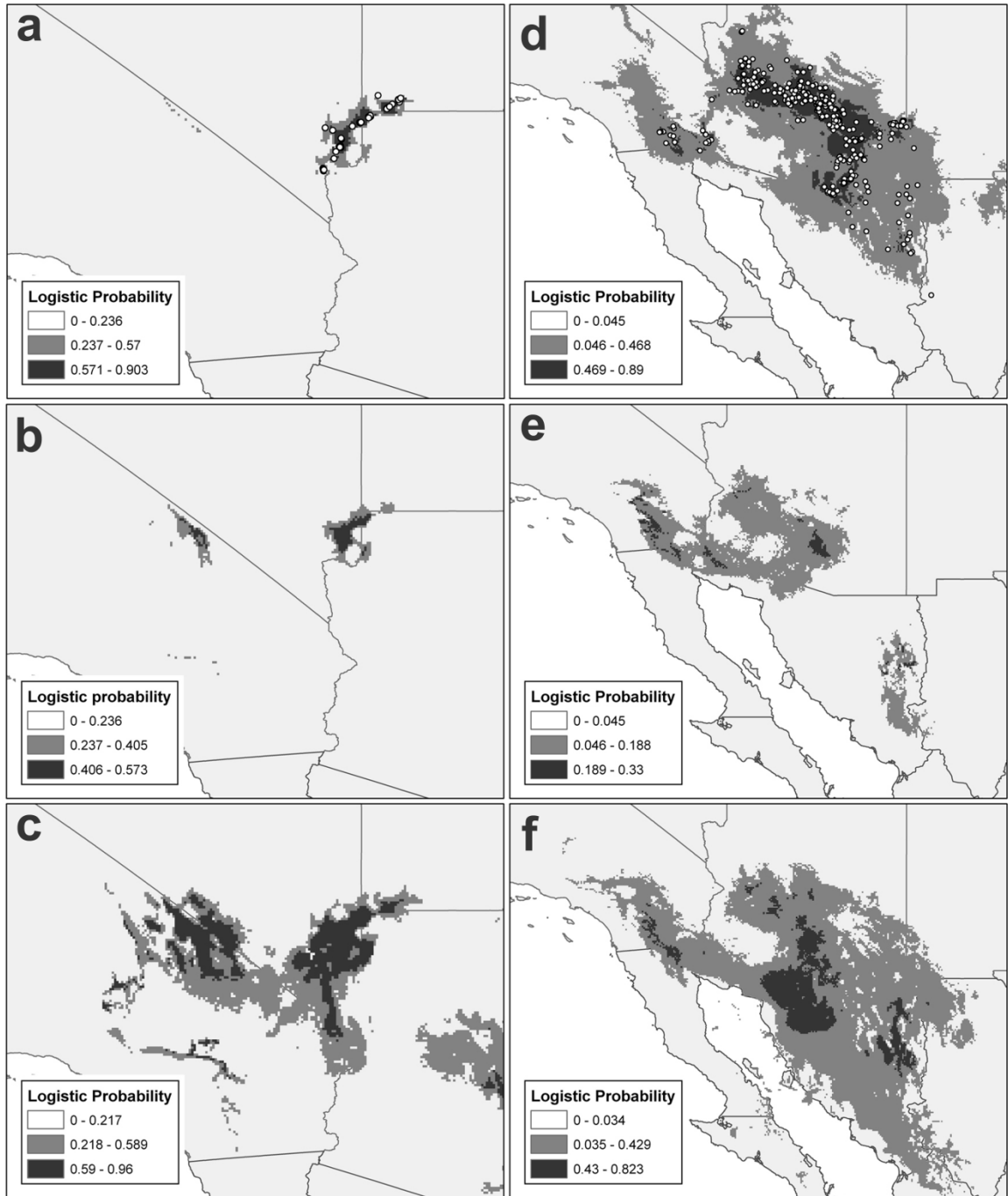**Figure 2.4.**

APPENDIX

Assessment of hierarchical ND2 genetic variation for *Rana yavapaiensis*

Materials and Methods

We assessed genetic variation of ND2 among river basins by conducting an analysis of molecular variance (AMOVA) in ARLEQUIN (10,000 permutations; pairwise difference distances). Within the USA, we grouped sample sites along the Bill Williams, Gila, Upper Gila, Salt, and Santa Cruz rivers by basins using 8 digit Hydrologic Unit Codes (HUCs; U.S. Geological Survey). We grouped sites across HUCs along the Middle Gila and San Pedro rivers that shared contiguous stretches of perennial water. Because no system comparable to 8 digit HUCs exists for Mexico, we grouped sites by major river basins and proximity based on 1:200,000 maps (Table A1.).

Results

River basins explained a significant, although low amount of the total genetic variation (12.8%). Most genetic variation (51.8%) occurred among sites within river basins, likely because of the relatively high levels of fixation within these sites (fixation indices $\Phi_{SC} = 0.594$, $\Phi_{ST} = 0.646$, and $\Phi_{CT} = 0.128$, all $P \leq 0.03$).

Discussion

Our assessments of haplotype distribution and diversity suggest that current environmental conditions may limit regional dispersal of *R. yavapaiensis* among river basins despite a signal of older expansion. While little genetic structure was attributable

to river basins (consistent with and interpretation of high gene flow), this pattern was influenced by the persistence of the most common haplotype in high frequencies across the entire range. Most unique haplotypes are restricted to single or nearby sites and not shared among river basins (Fig. 2.2b) suggesting the possibility that the period of expansion was followed by more recent restricted levels of migration and gene flow among regional populations.

Table A1. Number of *Rana yavapaiensis* samples (*n*) grouped by river basins for AMOVA. Site labels reference Fig. 2.1a and Table 2.2.

Group	Basin	Sites by Label	*n*
1	Bill Williams River	WC, TC, SM	24
2	Lower Gila River	HA, CC	20
3	Middle Gila & San Pedro rivers	MN, AR, MH	28
4	Upper Gila River	MC, SW, TU	26
5	Salt River	KS, CR, PC	28
6	Santa Cruz River	CN, AS, AC	25
7	Río Concepcion	RC	10
8	Río Bavispe	CP, RN, RB	25
9	Río Moctezuma	TB	10
10	Río Tutuaca	RE	6

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Thesis Title:
Phylogeography of two closely related anurans, the relict leopard frog (*Rana onca*) and lowland leopard frog (*Rana yavapaiensis*)

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