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Overview Article

Conservation genetics in aquatic species: General approaches and case studies in fishes and springsnails of arid lands

Carla Hurt and Philip Hedrick*

School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

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Abstract. Many aquatic species have become endangered because of the elimination and fragmentation of their habitat, human alteration of the environment, and introduced species. New applications and insights from genetic studies of endangered species are being used to formulate management plans to prevent extinction of endangered taxa. Here we discuss three applications of genetic techniques to the conservation of endangered aquatic species in the desert Southwest, USA. First, we discuss estimation of long-term effective population size in three endangered big-river fishes, bonytail chub (*Gila elegans*), humpback chub (*G. cypha*) and razorback suckers (*Xyrauchen texanus*), of the lower Colorado River. For all three species, the current census number is much smaller than the estimated effective population size in which these species evolved. Second, we discuss the determination of species, ESUs, and MUs in the endangered Sonoran topminnow. Molecular genetic data show that the Gila

and Yaqui topminnows are different species. Experimental examination of pre-mating and post-mating reproductive isolation demonstrates that some reproductive isolation has already developed between these two species. Finally, springsnails (*Pyrgulopsis*) of the lower Colorado River basin exist only in remote and isolated springs. Examination of molecular variation generally supports the morphological designation of 16 different species, although a few species have several ESUs. There does not appear to be a correlation of genetic and geographic distance between species, suggesting that they are quite old. These genetic studies provide insight into the conservation of these rare aquatic species. Although mitigating ecological factors may be most significant in preventing extinction, genetic studies can provide the evolutionary context for endangered species and identify what units are most in need of conservation.

Key words. Bonytail chub; effective population size; humpback chub; mtDNA; neutrality; reproductive isolation; razorback sucker; springsnails; topminnows.

Introduction

In the latter half of the 20th century, it was widely recognized that the rate of species extinction was increasing and that many other species were in imminent danger. The major factors related to these extinctions and de-

clines were overharvesting from hunting, fishing, trapping and other killing, loss, degradation and fragmentation of habitat, and introduction of non-native species such as pathogens, parasites, predators, and competitors (Diamond, 1989). A landmark book, edited by Minckley and Deacon (1991), detailed the effects of many of these factors on the native fishes of the arid western USA in the same period. Because of limited geographic distribution for many aquatic species in arid lands, they are particularly susceptible to habitat degradation and fragmenta-

* Corresponding author phone: 480-965-0799;
fax: 480-965-2519; e-mail: philip.hedrick@asu.edu
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tion, introduced non-native species, and other factors that cause population decline and possible extinction.

The problem and the potential causes of extinction were widely recognized for terrestrial and aquatic organisms, but only in the 1970s and 1980s was a new approach, generally called conservation biology, introduced and developed to better understand the processes influencing extinction. Much of the credit for the development of conservation biology, utilizing both ecological and genetic principles, can be attributed to Michael Soulé and the contributors to his edited volumes, (Soulé and Wilcox, 1980; Soulé, 1986; Soulé, 1987). Genetics is an important focus of conservation biology because it helps understand the evolutionary context of species and enables the development of improved management strategies. Although other factors are of primary concern for avoiding extinction of many endangered species, for long-term persistence and understanding the evolutionary context of the species, conservation biologists feel that direct predictions about the history and status of endangered species were possible by measuring genetic variation and interpreting these data in a population genetics context. The framework of population genetics theory furnished an elegant approach to interpret the measured amounts of genetic variation and predict the future effects of evolutionary factors and management strategies.

Many recommendations from population genetics were general; avoiding inbreeding and maintaining genetic variation with caveats (Hedrick and Miller, 1992). In some cases, recommendations were consistent with management ideas from other risk avoidance approaches, such as avoiding prolonged low population numbers or splitting the population into subpopulations. The application of new molecular techniques has made examination of genetics feasible for many more endangered species, and the level of genetic analysis has become much more sophisticated. Improvements in molecular techniques have enabled more definitive analysis of increasingly detailed questions about the genetics of populations and species.

These advances have been applied to genetic variation thought to be neutral, i.e., genetic markers that do not have either detrimental or adaptive effects, and consequently are markers of evolutionary phenomena. Also of significance in conservation are variants that are not neutral, but discussion of these selectively important variants is beyond the scope of this review (see Hedrick 2001 for an introduction). Here we discuss neutral genetic variation that can, e.g., provide us with an evolutionary history of a species and identify important demographic events. Whether a particular variant is neutral, detrimental or adaptive depends upon the environment, population size, genetic background, the relationship between genetic variation and the phenotype, and so on. For example, a

particular allele that is adaptive, such as providing resistance to an infectious disease in one environment, could be detrimental when the pathogen is absent because of a cost associated with that allele. Or, genetic variants neutral in one situation may be adaptive in another.

Neutral variation

The extent and pattern of molecular variation within populations are generally consistent with the neutral theory; a balance predicted by a reduction in variation from genetic drift, an increase in variation from mutation, and selective equivalence or neutrality of different variants (Kimura, 1983; Nei, 1987). Based on the predictions of this elegant theory, molecular data can provide information about the relationships of species and populations, estimates of parameters such as population size and gene flow, and insights often unavailable from other types of data. When the population is small, genetic drift can have a much greater effect on allele frequencies than selection. More specifically, neutrality of genetic variants are assumed when the selection coefficient s (either the selective disadvantage of a detrimental allele or the selective advantage of an adaptive allele) is $< 1/(2N_e)$ where N_e is the effective population size (Kimura, 1983). Because endangered species often have low effective population sizes, genetic variants are more likely to be effectively neutral in endangered than in common species. For example, if $s < 0.01$ ($< 1\%$ difference in fitness between genotypes) and the effective population size is 50, then genetic drift plays a more important role than selection in determining the fate of the variant, while in a population of size 500 with $s = 0.01$, selection should be more important than genetic drift.

Most recent conservation genetics research has focused on the use of neutral molecular markers. Molecular genetic markers hold great promise for estimating fundamental parameters or characteristics important in conservation, such as past effective population size, the occurrence of bottlenecks in population size, population origin of individuals, individual inbreeding level and sex-specific gene flow or founder contribution. Neutral genetic markers have been primarily used in conservation to identify species, evolutionarily significant units (ESUs) and management units (MUs) (e.g., Moritz, 1999). Highly variable genetic markers, such as microsatellite loci, have allowed the quantification of patterns not apparent when using genetic markers with less variation. The use of extensive sequence data or large numbers of single-nucleotide polymorphisms (SNPs) can provide high genetic resolution. Molecular markers can also be used to infer the historical and geographical relationships between groups (Avice, 2000). The power to infer such relationships from molecular data is substantial and data

from ancient specimens can now provide additional insight into relationships of contemporary groups (Barnes et al., 2002). Unfortunately, old samples of most fishes and other aquatic organisms have been stored in formalin, which breaks down DNA, making molecular analysis quite difficult.

Below we give examples of how neutral genetic variation can be used in conservation studies in aquatic species. First, we show how sequence variation in mitochondria DNA (mtDNA) can be used to estimate the evolutionary (long-term) effective population size in three of the big-river fishes, bonytail chub, humpback chub and razorback sucker, of the lower Colorado River. This information is particularly significant now because these fishes are endangered and have greatly declined in numbers in recent decades. Second, we show how genetic variation can be used to determine species, ESUs, and MUs in the Sonoran topminnow. In addition, we discuss recent experimental work to understand pre-mating and post-mating reproductive isolation between Gila and Yaqui topminnows. Finally, we show how molecular variation in the springsnail *Pyrgulopsis* in the lower Colorado River basin can be used to identify species and ESUs. In addition, we discuss the relationship of genetic and geographic distance between springsnail populations and species to understand the process of evolutionary divergence in this group.

Long-term effective population size in big-river fishes from the lower Colorado River

Four fishes of the Colorado River, bonytail chub, humpback chub, razorback sucker and Colorado pikeminnow (Colorado squawfish), are endangered and have been in deep decline in recent decades (Minckley et al., 2003). Two factors that greatly contributed to this decline were the damming of the river, which changed water flow and temperature, and the introduction of non-native fish. The last wild Colorado pikeminnow in the lower Colorado River was caught in 1975, but they were once so abundant that early settlers used them as fertilizer. Bonytail chub are critically imperiled, persisting only in Lake Mohave, Arizona-Nevada, and perhaps Lake Havasu, Arizona-California, as less than 100 wild fish, augmented by hatchery reintroductions (Fig. 1). Humpback chub are represented by one viable population in the Little Colorado River-Grand Canyon complex. This population hovered near 10,000 adults into the early 1990s, but recently is thought to have declined substantially to around 2,000 adults. Although annual spawning of razorback suckers occurs, the population consists mostly of large, very old adults and there is no evidence of natural recruitment success. A large population apparently formed when Lake Mohave filled in the early 1950s but annual

estimates of adult fish have consistently declined to around 9,000 in 1999 (Minckley et al., 2003) and are now thought to be around 4,000 old adults. Indications are that historically there were large populations of all four fishes in the lower Colorado River as late as the mid-20th century (Minckley et al., 2003).

Conserving genetic variation has been a major focus of recovery efforts for many endangered species. Retaining variation for adaptation to environmental change is of great concern, particularly because many imperiled taxa inhabit in recently altered habitats and are exposed to new biological threats, such as non-native predators, competitors, and pathogens. In general, the amount of genetic variation within a population available for future adaptation results from a balance between mutation, introducing new variation, and genetic drift that reduces it. Efforts to measure the effective population size in wild populations have often concentrated on the effective size in a given generation or over a few generations (Hedrick, 2005).

The amount of genetic variation potentially available for adaptation is determined by the long-term, or what could be called the evolutionary, effective population size. The long-term effective size may not be reflected in estimates of contemporary effective population size or as a percentage of the contemporary census number because the ancestral effective population size, particularly for endangered species, could have been much larger, than contemporary estimates. Below, we explain how Garrigan et al. (2002) used molecular data to estimate the long-term effective population size in the endangered fishes of the lower Colorado River and show that the contemporary effective size or census number is only a small proportion of the estimated long-term effective size. Estimating the evolutionary effective population size is particularly crucial at this point because recommendations for recovery of these species suggest numbers of only a few 1000 are necessary. For further discussion of the effective population size in relationship to the conservation and recovery of these species, see Minckley et al. (2003).

To estimate the long-term effective population size in three of these fishes (no samples of Colorado pikeminnow were available), Garrigan et al. (2002) examined mtDNA sequence data and used the maximum likelihood approach in the program FLUCTUATE (Kuhner et al., 1998). Because the generation time is long for these big-river fishes, and the decline is only over the past few generations, one would expect estimates of long-term effective population size from molecular data would not be overwhelmed by their recent declines. The maximum likelihood method assumes that new sequence variants appear by mutation and are eliminated by genetic drift. For a given mutation rate and N_e , a sample of mtDNA sequences should exhibit an appropriate pattern of pairwise differences. These estimates are for the effective population size of a species throughout a substantial portion of



Figure 1. Sites of the remaining populations of the bonytail chub (Lake Mohave), humpback chub (Little Colorado River), and the razorback sucker (Lake Mohave) in the lower Colorado River.

its evolutionary history and do not necessarily reflect historical or recent effective population sizes.

Examination of mtDNA sequence variation in samples of bonytail chub, humpback chub, and razorback sucker showed substantial variation (Garrigan et al., 2002). For humpback chub, bonytail chub, and razorback sucker, there were 5, 3, and 10 haplotypes found in samples of 18, 16, and 49 individuals, respectively (Table 1). The humpback chub and razorback genealogies (Garrigan et al., 2002) showed similarity in that rare haplotypes were the most divergent and the most common haplotypes were closely related. In addition, humpback chub and razorback sucker showed similar divergence over all sequences, about 1.5 nucleotides between all pairwise comparisons, while the bonytail chub had an average of 2.8 nucleotide differences.

Assuming that the mutation rate is 4×10^{-9} per nucleotide (Hedrick, 2005), then using the program FLUC-

TUATE, we can estimate the long-term effective population size from these data (Table 1). If the population size is assumed to be constant over evolutionary time, estimates of effective population size are 48,800, 44,800, and 223,000 for humpback chub, bonytail chub, and razorback sucker, respectively (this assumes an equal sex ratio because mtDNA is maternally inherited and generation lengths of 10 and 15 years for the two chubs and the sucker, respectively). When population growth is taken into account, the estimates suggest that bonytail chub has been declining and razorback sucker expanding in numbers over evolutionary time (Table 1). Overall, this genetic analysis suggests the three species had large numbers until recently. Although there is less genetic variation for the bonytail chub, and estimates of effective population size are the smallest, the three remaining haplotypes are quite divergent suggesting that present genetic variation still reflects a high degree of the ancestral variation.

Table 1. Estimates of mtDNA variation in three endangered big-river fishes from the Colorado River. Using these data, the maximum likelihood estimates are given of the long-term effective population size, N_e , if the population is assumed constant over evolutionary time and if the population is allowed to grow or contract and the direction of that change (from Garrigan et al., 2002 with changes as given in Hedrick, 2005). For comparison, the current census numbers of the species are also given.

	Species		
	Humpback chub	Bonytail chub	Razorback sucker
Data			
mtDNA gene	ND2	ND2	Cytochrome B
No. nucleotides	790	763	311
Sample size	18	16	49
No. haplotypes	5	3	10
Estimates of N_e			
N_e (constant size)	48,800	44,800	223,000
N_e (variable)	74,400	31,000	313,600
Population trend	stable	declining	expanding
Current census	2,000	100	4,000

Variation in the other two species remains even more intact. Overall, the present census number for all three species is orders of magnitude less than the estimated long-term effective population size. In addition, the current effective population size may be much smaller than the current census number, Frankham (1995) suggests that the ratio of effective population size to census number may be around 0.1.

Many endangered species, such as these fishes, probably evolved when their population size was much larger than present or even historically known. As a result, there may be substantial genetic variation remaining for potential adaptation but this variation could be quickly lost if the present population size is small. Further, if the current effective population size is reduced, either naturally or through inappropriate management, genetic variation will diminish and less new variation generated, increasing the potential for reduced fitness due to fixation of detrimental alleles. Such reduction in fitness when the effective population size declines appears to be a particularly severe problem in species with large ancestral populations and, consequently, high historical genetic loads (Hedrick and Kalinowski, 2000).

Gila and Yaqui topminnows

The Sonoran topminnow is a small live-bearing fish of the family Poeciliidae that occurs in Arizona, USA and Sonora, Mexico. Two endangered Sonoran topminnow taxa, the Gila topminnow and the Yaqui topminnow, occur in southeastern Arizona. The Gila topminnow was once considered among the most abundant fishes in the lower Gila River basin in Arizona. They now persist in at only a few watersheds in southeastern Arizona (Fig. 2), primarily because of loss and fragmentation of adequate

shallow-water habitat and the widespread introduction of the non-native western mosquitofish (*Gambusia affinis*). The Yaqui topminnow was never widespread in the USA because the Rio Yaqui drainage includes only a small part of southeastern Arizona now within the San Bernardino National Wildlife Refuge, and it has faced similar threats to its persistence.

The Sonoran topminnow has undergone several changes in taxonomy since its initial description as two distinct species, *Poeciliopsis occidentalis* (Gila topminnow) and *P. sonoriensis* (Yaqui topminnow) (Girard, 1859). Several authors either synonymized or retained these taxa (for a review, see Quattro et al., 1996). Based on subtle morphological differences, Minckley (1969, 1971) redescribed the two taxa as different subspecies, *P. o. occidentalis* (Gila topminnow) and *P. o. sonoriensis* (Yaqui topminnow). Since being recognized as endangered, Sonoran topminnows have been surveyed for a variety of molecular markers in an effort to understand their population structure within and differentiation between taxa.

Collectively, these molecular genetic studies suggest that *P. occidentalis* and *P. sonoriensis*, though morphologically very similar, have long been isolated. First, Quattro et al. (1996) examined mtDNA variation using six-cutter restriction enzymes and found that the Gila and Yaqui topminnows were fixed for two very divergent haplotypes. Recently, sequences of three mtDNA regions (ND2, Cytochrome B, and the d loop) comprising over 2600 base pairs (bp) were found to be nearly invariant within the two taxa but with non-overlapping sequences that were about 1% divergent between the two taxa (Lee et al., unpublished).

Second, Hedrick et al. (2001) found extensive variation at a MHC (major histocompatibility complex) locus within both Gila (17 alleles) and Yaqui topminnows (12

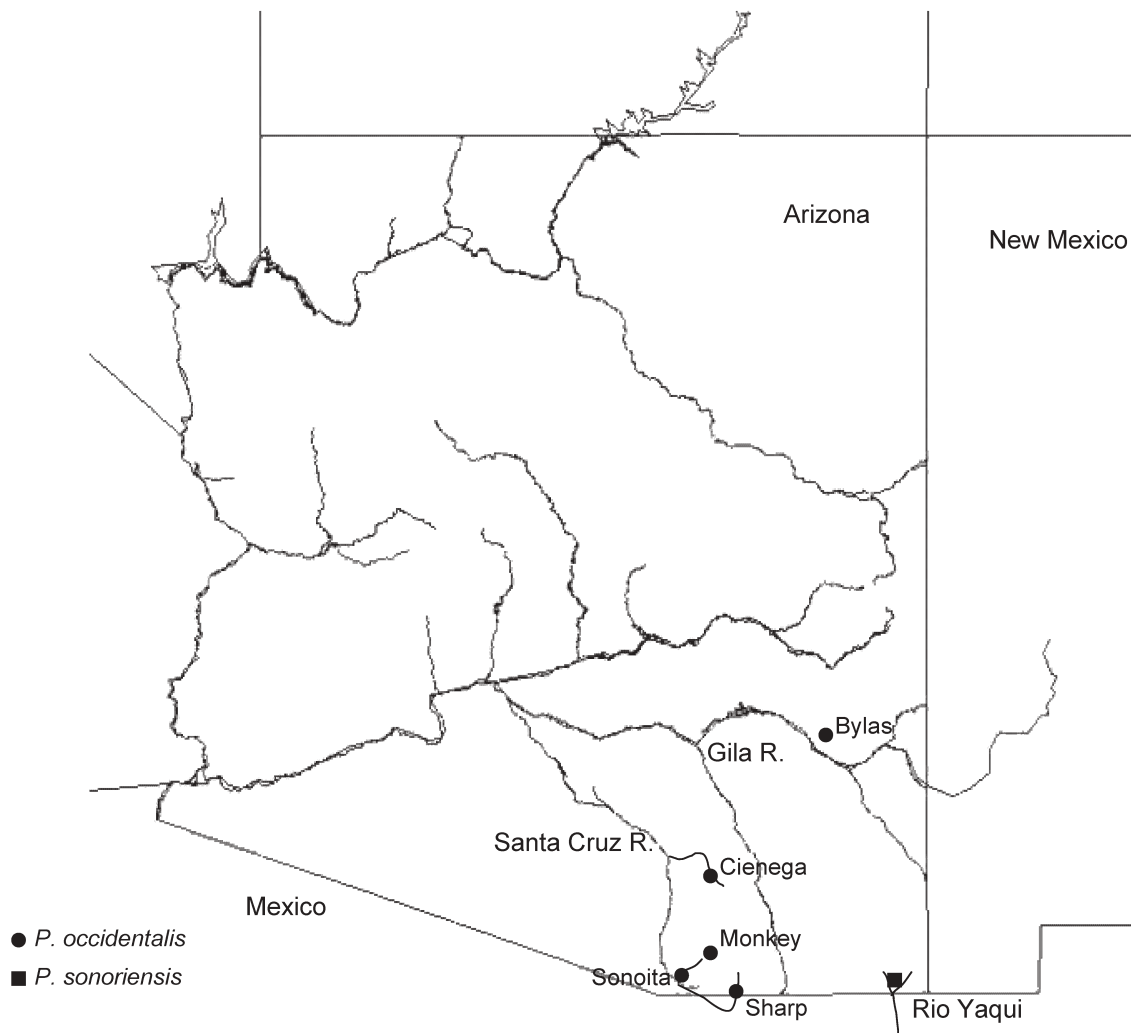


Figure 2. Location of natural populations of the Gila and Yaqui topminnows in the USA.

alleles). These 29 alleles comprised two non-overlapping sets in the two taxa and can be used as diagnostic for the two taxa.

Third, Hedrick et al. (2001) also examined variation at 10 microsatellite loci. For seven microsatellite loci that could be scored in both taxa, the alleles formed nearly non-overlapping (diagnostic) sets of alleles at these loci (Table 2). Only two of 39 microsatellite alleles found in the Gila topminnow were present in any Yaqui topminnow and only two of 22 alleles found in the Yaqui topminnow were present in any Gila topminnow. Overall, only 1.2% of the Yaqui alleles were in Gila samples and only 1.1% of the Gila alleles were in Yaqui samples.

For three microsatellite loci, the size of the alleles in the two species were greatly divergent. The largest difference was for locus OO56 that differed by more than 100 base pairs. However, this locus is a complicated repeat (Parker et al., 1998) so it is not clear how many mutational steps are responsible for the difference in size be-

tween species. The other two loci that have disjunct size distributions, C-15 and LL53, are simple GT dinucleotide repeats. Because the most common mutation at perfect repeat microsatellite loci is thought to differ by only a single repeat, it appears that isolation between these taxa must have been long and complete for so many differences to accumulate. Based on this genetic evidence and other considerations, Minckley (1999) suggested that the two taxa should be considered different species, *P. occidentalis* (Gila topminnow) and *P. sonoriensis* (Yaqui topminnow).

Recently, we have examined in experimental populations both pre-zygotic (Hurt et al., 2004) and post-zygotic (Hurt and Hedrick, 2003) reproductive isolation between these two species. In no-choice mating trials (two within-species and two reciprocal between species pairings), there was evidence that mating patterns of the two species have diverged significantly and for conspecific mate preference (Hurt et al., 2004). In multiple-choice trials

Table 2. The seven microsatellite loci that could be examined in the Gila and Yaqui topminnows with their alleles (sizes in bp) and frequencies. The average gives the frequency of diagnostic Gila and Yaqui alleles in Gila and Yaqui topminnows (from Hedrick et al., 2001).

Locus	Alleles	Species	
		Gila	Yaqui
G-49	149–159	0.917	–
	161	0.083	0.486
	163	–	0.514
C-15	164–192	–	1.0
	202–248	1.0	–
OO56	143–153	1.0	–
	256	–	1.0
LL53	110–116	–	1.0
	136–164	1.0	–
4-44	106	–	0.526
	108	1.0	0.080
	114, 118	–	0.394
Acc	124	–	0.794
	128	1.0	–
	130	–	0.206
G53	96, 102, 104	–	1.0
	100	1.0	–
Average	Gila alleles	0.988	0.011
	Yaqui alleles	0.012	0.989

(males of both species with females of one species or the other), there was stronger evidence for conspecific mate preference. In these experiments, males of each species spent more time performing mating behaviors and attempting more copulations towards conspecific than heterospecific females (Hurt et al., 2004).

To assess post-zygotic reproductive isolation, Hurt and Hedrick (2003) examined the reproductive potential of a series of crosses and backcrosses between the two species. Previous to this study, no crosses between these two species had been successfully carried out. Thirteen crosses of each within and between species type were made and both interspecific crosses were successful. The lowest success (54%) was for Gila male × Yaqui female cross, a cross that also produced a very skewed sex ratio (only 8% females) and a time to produce the first brood that was 45% longer than the average of the other crosses (Table 3).

Table 3. The proportion of successful crosses within and between Gila and Yaqui topminnows, the proportion of female F₁ progeny, and the days until the F₁ females produced their first brood (Hurt and Hedrick, 2003).

Male	Female	Successful crosses	Female progeny	Days to first brood
Gila	Gila	0.85	0.52	62.4
Gila	Yaqui	0.54	0.08	87.1
Yaqui	Gila	0.69	0.59	53.6
Yaqui	Yaqui	0.69	–	63.7

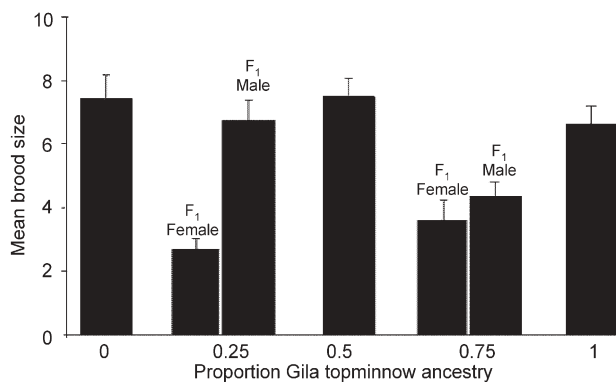


Figure 3. The mean brood size for pure species of Gila (indicated by 1) and Yaqui (0) topminnows, F₁ crosses between them (0.5), and the two types of backcross categories (0.25 and 0.75) where these crosses are separated by the sex of F₁ individuals. Here the two types of backcrosses in which F₁ of a given sex were produced by reciprocal matings were combined (Hurt and Hedrick, 2003).

To determine whether there was a further reproductive barrier that would inhibit crosses between these F₁ progeny and the two species, five replicates of each of the eight types of backcrosses were made. For example, the four crosses that produced 75% Gila topminnow ancestry were Gila males × F₁ females (from either a Gila male × Yaqui female cross or a Yaqui male × Gila male cross) and F₁ males (from either a Gila male × Yaqui female cross or a Yaqui male × Gila male cross) × Gila females. High reproductive success was observed across the eight categories of backcrosses with at least four of five crosses in each category producing offspring. However, brood size varied greatly by individual cross type. In particular, crosses between F₁ females and pure species males had a lower average brood size than the reciprocal cross category (or the pure species or F₁ categories) (Fig. 3).

Overall, there was a generally high degree of reproductive success and all hybrid classes including backcrosses successfully produced offspring. The low fitness of the cross Gila male × Yaqui female and the low brood size of F₁ females crossed to males of either species indicate that initial post-mating isolating mechanisms have developed between Gila and Yaqui topminnows.

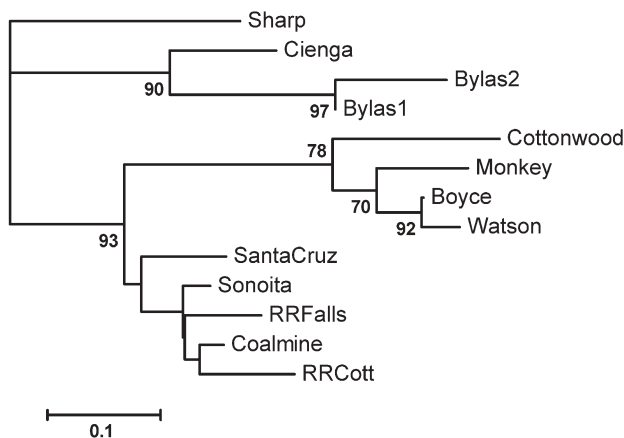


Figure 4. An unrooted neighbor-joining tree for the 13 samples of Gila topminnows based on allele frequencies at the polymorphic microsatellite loci and the MHC locus. The numbers indicate bootstrap values for the nodes in the tree (Hedrick et al., 2001).

Gila and Yaqui topminnows, ESUs and MUs

Hedrick et al. (2001) surveyed the 10 known natural populations of Gila topminnows for variation at microsatellite loci and a MHC (major histocompatibility complex) locus (along with two introduced populations, Boyce Thompson Arboretum and Watson Wash, and one recolonized population, Santa Cruz River) to determine the population structure in this species. The 10 natural populations (Fig. 2) fell into five groups based on the data from these loci (Fig. 4). In addition, other distributional and ecological attributes of these populations support these groupings (see Parker et al., 1999 and Minckley, 1999 for discussions of the ecological, life history, and other attributes of the sites). First, the two populations Bylas Spring 1 and Bylas Spring 2, the only samples from the mainstem Gila River, were very similar to each other, have low genetic variation, and are different from the others. The Bylas populations are isolated by at least 580 km of stream channel from the closest other populations with extensive dry reaches.

Second, the Monkey and Cottonwood Springs samples were nearby in the upper Sonoita Creek watershed and are similar, have intermediate levels of genetic variation, and are different from all other natural populations. Monkey Spring appeared to be the most distinctive sample in the Parker et al. (1999) survey and this site probably has been isolated from Sonoita Creek, into which it flows, by a travertine dam formed perhaps 10,000 years ago. As indicators of long-term isolation of this site by immigration, it was occupied by an extinct species of pupfish and an extinct, morphologically distinct, form of the Gila chub. In addition, we found a substantial life history difference in that development time was 50% longer in Monkey Spring males than males

from other sites. As a result of the totality of these findings, Parker et al. (1999) concluded that Monkey Spring should be considered a separate ESU. With the genetic similarity of the Cottonwood sample, it could be included in this ESU.

Third, the other samples from the Sonoita Creek drainage, Sonoita Creek, Coalmine Canyon, Red Rock at Cott tank, and Red Rock Falls, cluster together with Coalmine Canyon and Sonoita Creek being very similar. Except for Red Rock at Cott tank, which is variable for only one locus, these populations are intermediate in genetic variation. The sample from the recolonized Santa Cruz River population clusters with these nearby Sonoita Creek samples.

The Cienega Creek and Sharp Spring samples form the fourth and fifth groups, respectively. Although they do not appear as different on the microsatellite phylogenetic tree as the other groups, they have nearly non-overlapping sets of alleles for two microsatellite loci and do not share any alleles at the MHC locus. Based on these data and the discussions of other factors in Parker et al. (1999), we recommended that there be two ESUs; one composed of Monkey and Cottonwood Springs, and one composed of the remaining eight natural populations, which itself should be subdivided into four MUs (Bylas Springs, Cienega Creek, Sharp Springs, and Sonoita Creek populations, except Monkey Spring and Cottonwood Spring). Management in this manner should allow both conservation of genetic variation and evolutionary differences and provide an approach to the long-term persistence of the Gila topminnow.

There are only two Yaqui samples from Arizona that have been examined for microsatellite and MHC loci, North Pond and Tule Spring, both from the SBNWR (Hedrick et al., 2001). These two Yaqui topminnow samples were quite similar, sharing alleles or very similar alleles at all loci. Genetic variation in the two samples was also substantial and similar. Therefore, these samples could be considered the same ESU, and the only known one in the USA.

Springsnails in the lower Colorado River basin, species and ESUs

Extinction of non-marine molluscs from human-related causes outnumber those of birds and mammals combined (Ponder et al., 1995). Despite their often small size and inconspicuous nature, molluscs play a key role in maintaining ecosystem health; occupying an important niche in basal food chains as major consumers of aquatic plants and as a critical food source for fish, amphibians, reptiles, and birds. The Hydrobiid genus *Pyrgulopsis* is the most species-rich molluscan genus in North America with an estimate of approximately 130 species. Springsnails pos-

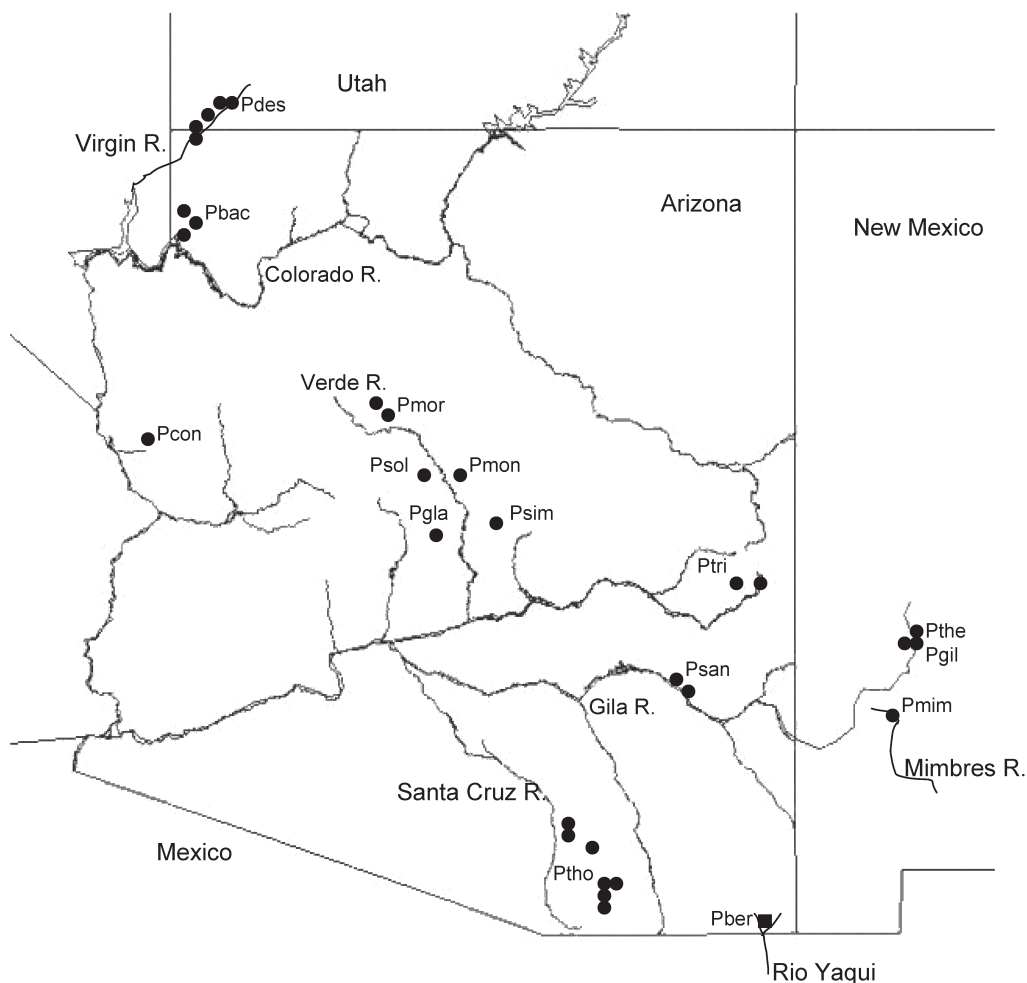


Figure 5. Map showing collection sites for *Pyrgulopsis* specimens where the notations indicate the different species, e.g., Ptho is *Pyrgulopsis thomsoni* (see Hurt, 2004 for other abbreviations).

sess several life history traits, habitat specificity and low dispersal, that make them exceptionally vulnerable to extinction. These traits have contributed to a high degree of endemism in this genus, with many species occurring only within a single spring or seep. Currently, over one third of the recognized species of *Pyrgulopsis* are candidates for listing under the Endangered Species Act (Hershler, 1994). Three described species of *Pyrgulopsis* have gone extinct since their description in the early 1900's and it is likely that other species have been lost before being discovered. Loss and alteration of spring habitat appear to be the major factor causing the decline of springsnails.

Effective management of endangered species requires reliable information on occupied habitat, population structure, and taxonomic relationships. Early studies of springsnail taxonomy relied on shell morphology, but Hershler and Thompson (1986) redefined the genus based on penial morphology. Because there may be cryptic species not readily detected by morphological criteria

(Liu et al., 2003), detailed genetic studies are needed to reliably document the extent and patterns of divergence between morphologically defined springsnail species and to identify populations or groups of populations that qualify for special protection as ESUs.

We examined the geographical distribution of genetic variation in the mitochondrial gene Cytochrome oxidase I (COI) within and between the 40 springsnail populations belonging to the 16 described *Pyrgulopsis* species that occur in the lower Colorado River drainage (Hurt, 2004) (Fig. 5). Overall, we identified 56 unique alleles among the 1056 surveyed individuals.

Phylogenetic analysis supports nearly all morphologically defined species; the exception being the paraphyletic species pair, *P. thomsoni* and *P. conicus*. In general, species groups appear to form a nested hierarchy of clades (Fig. 6). There is a large, well-supported clade that includes all taxa from the Verde, Colorado and Santa Cruz watersheds. *P. thermalis*, a warm spring species from New Mexico, is the most genetically distinct of all

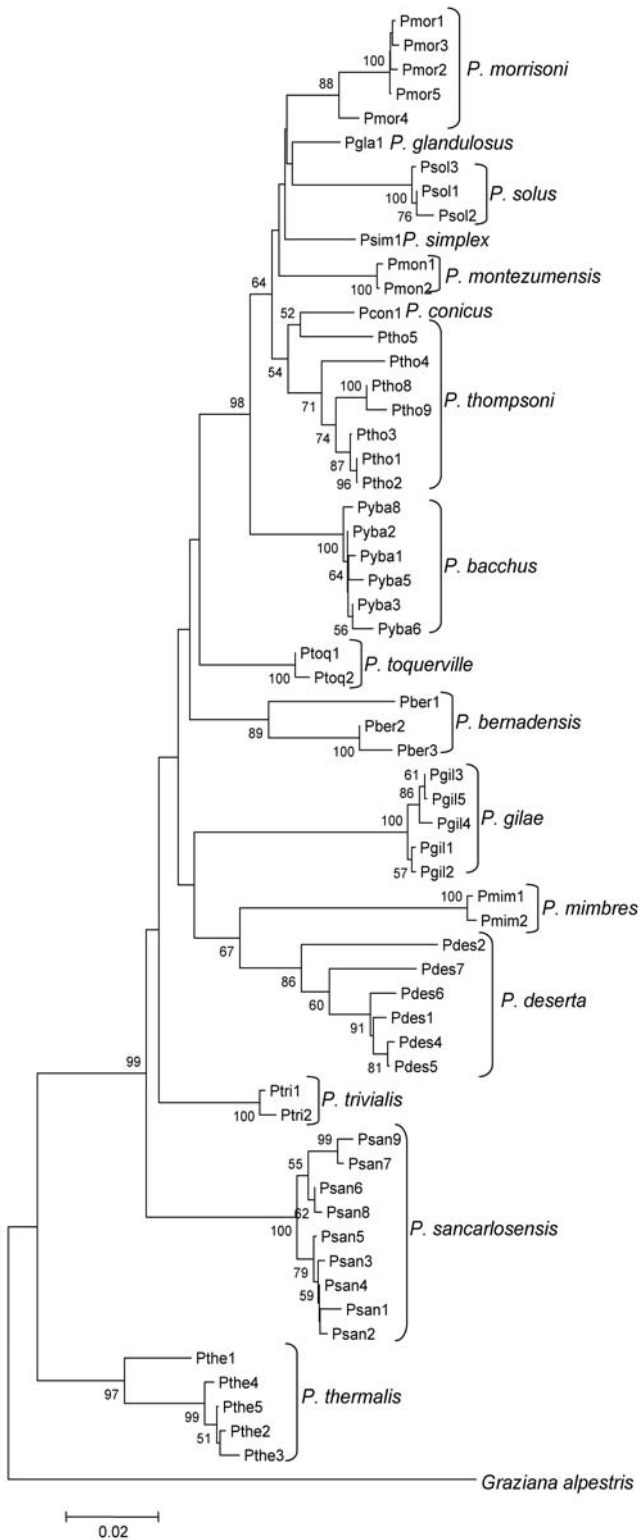


Figure 6. Neighbor-joining tree of mtDNA sequence data. Numbers are bootstrap percentages for well-supported clades (Hurt, 2004).

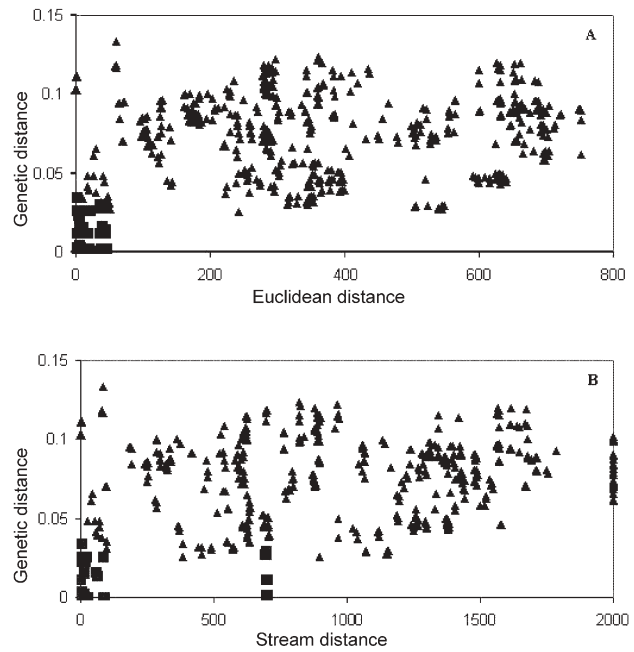


Figure 7. Population pairwise genetic distances plotted against (A) Euclidean distances and (B) stream distances. Squares indicate comparisons between conspecific populations and triangles indicate comparisons between populations belonging to different species (Hurt, 2004).

the examined species with an average net genetic distance of 10.7% from all other species.

The correlation of genetic distance with geographic Euclidean distances was highly significant (Fig. 7). However, the correlation within species and between species separately was not significant between genetic and geographic distances, indicating that the relationship between geographic and genetic distance is the result of small genetic and geographic distances between conspecific populations and larger genetic and geographic distance between species.

High levels of genetic subdivision are found below the species level in four taxa, warranting the establishment of distinct classification units for conservation and management purposes. Based on these genetic differences, population geography and habitat we identified an additional seven potential ESUs (see Hurt, 2004). For instance, we have identified 4 ESUs among the nine examined *P. thompsoni* populations. Two ESUs comprise populations on the east and west slopes of the Huachuca Mountains, and two ESUs are in Cottonwood Spring, a cold-water spring along Sonoita Creek, and Monkey Springs, an isolated constant temperature thermal spring that once supported several unique fish taxa and still supports a topminnow ESU (Minckley, 1999; Parker et al., 1999).

Our molecular phylogeny of Colorado springsnails demonstrates a complex pattern of speciation that cannot

be explained by drainage pattern or geographic Euclidean distances. Molecular analysis shows high levels of genetic divergence between geographically close, conspecific populations, indicating that many have been isolated for extended periods of time. Despite isolation, habitat specificity and recent compromises to habitat quality, many populations have maintained haplotype diversity. This result is a positive sign for the future persistence of springsnail species, many of which are currently listed as threatened or endangered. Management efforts should strive to maintain maximum genetic diversity by preserving as many populations as possible; particular emphasis should be placed on those populations with highly diverged haplotypes and unique environmental traits.

Conclusions

Here we present an overview of applied genetic approaches to the conservation of three different groups of endangered species: the big-river fishes of the lower Colorado River, the Sonoran topminnow of southeastern Arizona, and springsnails in the lower Colorado River basin. All of these species have suffered large declines in the last 50 years, mainly because of human-altered habitat and other human-caused threats. As a result, either their present population sizes are greatly reduced or the remaining populations are extremely isolated. Conservation genetic analysis provided insight into these changes that otherwise could only be conjectured. It also has provided information that can be used to guide recovery and management as well as a baseline for evaluating future changes in these taxa.

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