

Final Technical Report: Microsatellite Variation in Topminnows (I95051)
Arizona Game and Fish Department
Heritage Grant IIPAM No. I95051

Molecular Variation and Evolutionarily Significant Units in the Endangered Gila Topminnow

KAREN M. PARKER, RUBY J. SHEFFER, AND PHILIP W. HEDRICK

Department of Biology, Arizona State University, Tempe, AZ 85287-1501

Running head: *Variation in the Gila topminnow*

Word count: 7,240

Key words: evolutionarily significant units, Gila topminnow, major histocompatibility complex, microsatellite loci

Address correspondence to Philip W. Hedrick, email hedrick@hedricklab.la.asu.edu

Address: as above

Tel: 602-965-0799

Fax: 602-965-2519

Abstract: *Variation in microsatellite loci for the endangered Gila topminnow from the four watersheds in Arizona which they are still naturally extant was examined. Bylas Spring had quite low variation while the other populations all had significant variation. All of the populations had "diagnostic" alleles and the genetic divergence, based on several quantitative measures, between populations was substantial. The amounts and patterns of genetic variation are consistent with known historical and physical differences between sites. Further, the sites differ in a number of important factors in their physical habitat, biota, and the life-history of the topminnows. Based on these considerations, we recommend that the four watersheds all be considered as evolutionarily significant units and managed in a manner appropriate for separate evolutionarily significant units.*

Introduction

Highly variable molecular genetic variants have recently been used to address a number of issues previously difficult to answer in conservation and evolutionary biology (Avice & Hamrick 1996; Smith & Wayne 1996). Determining the unit of conservation is particularly controversial, with some suggesting the species as the appropriate unit (e.g. Caughley & Gunn 1996) while others consider evolutionarily significant units (ESUs) that are on independent evolutionary trajectories, whether they be populations, stocks, subspecies, or species (e.g. Moritz 1994; Waples 1995), should be the units of conservation. Until recently, molecular markers most commonly used were allozymes or mitochondrial DNA (mtDNA) variants. In recent years, highly variable nuclear molecular markers are being widely applied. Although it is tempting to use the latest molecular findings exclusively to determine ESUs, other important historical, ecological, and distributional data need to be included to provide a comprehensive perspective (Waples 1995). Here we attempt to provide such an integrated perspective for the endangered Gila topminnow.

The Gila topminnow (*Poeciliopsis o. occidentalis*), once one of the most abundant fishes in the Gila river drainage, is federally and state-listed as endangered and exists in the United States in only four isolated Arizona watersheds (Figure 1) (e.g. Sheffer et al. 1997a). Major factors influencing their decline were the loss and fragmentation of adequate shallow-water habitat and the establishment of the non-native central mosquito fish, *Gambusia affinis* (Meffe et al. 1983). The present report is one of a series in which we have examined genetic variation and fitness-related traits in the Gila topminnow in an effort to understand some of the genetic factors important for its conservation (see Discussion).

Below we describe genetic variation in polymorphic microsatellite loci identified in the Gila topminnow and indicate what evolutionary factors appear to have resulted in the pattern of variation observed. Variation in microsatellite loci is compared to that found in previous studies for allozymes (Vrijenhoek et al. 1985; Meffe & Vrijenhoek 1988), mtDNA (Quattro et al. 1996), and a potentially adaptive major histocompatibility complex (MHC) locus (Hedrick & Parker 1998). In light of these findings and other data on distribution, habitats, and life history of extant Gila topminnow populations, we discuss appropriate ESUs and potential conservation for the Gila topminnow.

Materials and Methods

Microsatellite loci, which have short, tandem repeats of variable number that are considered different alleles (Ashley & Dow, 1994), have become an important nuclear molecular markers to determine population differentiation. Variation at microsatellite loci is particularly appropriate for this application because they generally evolve rapidly, are not within transcribed genes, and allelic variation at these loci is thought to be selectively neutral.

Using standard techniques (Parker et al. 1998), we identified 10 microsatellite loci in Gila topminnows, five of which are polymorphic in our present samples. Three of the five polymorphic loci (*Pooc-6-10*, *Pooc-C15*, and *Pooc-LL53*) are simple dinucleotide repeats while the other two (*Pooc-G49* and *Pooc-OO56*) are composed of dinucleotide repeats interrupted by other sequences.

Natural populations of Gila topminnows exist in the United States in only four Arizona watersheds, represented here by samples from Bylas Spring, Cienega Creek, Monkey Spring, and Sharp Spring (see Discussion below and in Sheffer et al. 1997a). For the present study, we examined variation at microsatellite loci in 160 individuals, 40 individuals sampled from stocks derived from each population - the same fish previously examined for variation at a MHC locus (Hedrick and Parker 1998). We also compare variation in these individuals for microsatellite loci and the MHC locus.

Results

Variation within populations

Table 1 gives the observed allelic frequencies for the five polymorphic loci in the four samples (allele designations indicate the number of base pairs for that allele). First, notice that each population has several alleles (in boldface) that appear "diagnostic" for the specific population. For example for Bylas Spring, allele *Pooc-G49-161* was absent in other samples and allele *Pooc-C15-240* was fixed in Bylas Spring and in very low frequency in Cienega Creek and Sharp Spring. Overall, Bylas Spring has diagnostic alleles at two loci, Cienega Creek at three, Monkey Springs at four, and Sharp Spring at two.

The loci vary both in sharing alleles over different populations and the amount of variation. The highest sharing is for loci *Pooc-G49* and *Pooc-OO56* which both have an

allele common in all, or nearly all, of the samples. The other three loci are quite divergent, with Monkey Spring fixed for an allele at locus *Pooc-6-10* (and different by five dinucleotide repeats) not found in the others and high divergence between nearly all pairs of populations for *Pooc-C15* and *Pooc-LL53*, although the size range and total number of alleles is higher for *Pooc-C15*.

The amounts of within-population variation, as determined by observed and expected heterozygosity and the observed number of alleles, is in Table 2. In all locus-population combinations, observed heterozygosity was not significantly different than that expected from Hardy-Weinberg proportions. The lowest variation was in the Bylas Spring sample, polymorphic for only one locus. However, At this locus (*Pooc-G49*), however, the Bylas Spring sample had the highest heterozygosity of all four because of a second allele in substantial frequency that differed by a single dinucleotide repeat. The other three samples were each polymorphic for three loci, with Cienega Creek and Sharp Spring having the highest average heterozygosity. Sharp Spring had the most alleles averaged over loci (3.8), primarily because of 12 different alleles at *Pooc-C15*. The locus with the lowest within-population variation was *Pooc-6-15*, fixed for an allele in all populations, while the most variable was *Pooc-C15*, with 6.2 alleles per sample, even though Bylas Spring was fixed for this locus.

We examined the variation over individuals within a population in several different ways to determine if there was interlocus association. First, we calculated the correlation coefficient for individual homozygosity (scored as 0) versus heterozygosity (scored as 1) for each polymorphic pair of loci over all 40 individuals within a sample. The average of these nine correlation coefficients was near zero (0.049) and they ranged from -0.125 to 0.200 (all non-significant). Second, we calculated linkage (gametic) disequilibrium between pairs of polymorphic loci for which we had sufficient statistical power to detect it. For example, for *Pooc-C15* the 40 individuals from Sharp Spring were represented by 20 different genotypes, making linkage disequilibrium estimation

infeasible for any locus pair that included *Pooc-C15*. In fact, estimation was possible only for the three locus pairs in Cienega Creek and there was no evidence of linkage disequilibrium for any of these using the approach of Slatkin & Excoffier (1996). Finally, we calculated the observed variance in individual heterozygosity over all five loci and compared it to that randomly generated from a sample of size 40 (Table 3). In all cases, observed variance was close to that expected and the probability of obtaining a variance greater than that observed by chance using a Monte Carlo approach ranged from 0.20 to 0.42, with a mean of 0.31, consistent with the hypothesis that there were no interlocus associations (e.g. Brown et al. 1980).

Variation between populations

We examined differences between populations using several different measures. First, the probability of a unique allele per locus was calculated, which indicates the frequency of allelic differences in kind for a given population compared to another population (Hedrick 1971). Table 4 gives probabilities of a unique allele for the five polymorphic loci for all sample pairs, the values range from 0.150 for the probability of a unique allele in Bylas Spring when compared to Cienega Creek to 0.597 for Monkey Spring when compared to Bylas Spring. Averages across the bottom vary from 0.350 for Bylas Spring with the least unique alleles compared to other samples, to 0.590 for Monkey Spring which has the highest proportion. The largest asymmetry in the averages across the bottom of the table and those on the right side is for Bylas Spring, indicating that Bylas Spring has fewer unique alleles when compared to other samples (0.350) than the other samples have when compared to Bylas Spring (0.486).

We also calculated genetic distance (Nei 1972) and F_{ST} (Nei 1987) between pairs of samples for the ten microsatellite loci. We did not calculate a size-based genetic-distance measure because of large differences between loci in the variation in size (see

discussion in Boyce et al. 1997; see also Takezaki & Nei 1996). Genetic distance and F_{ST} values varied from 0.098 and 0.223 for Cienega Creek - Sharp Spring comparison to 0.440 and 0.712 for the Bylas Spring - Monkey Spring comparison (Table 5). For all pairs of populations, the F_{ST} values were statistically significant.

Using the genetic distance values from Table 5 for the microsatellite loci, Figure 2 is a phylogenetic tree using the UPGMA approach (Kumar et al. 1993). Monkey Spring is the most divergent, consistent with that seen from calculating the probability of a unique allele in Table 4. Cienega Creek and Sharp Spring cluster together, with Bylas Spring also closely related. Using a neighbor-joining approach to obtain a phylogenetic tree (not shown), Monkey Spring is again quite divergent but Bylas Spring and Cienega Creek first cluster together, with Sharp Spring being closely related.

Comparison of MHC and Microsatellite Variation in the Same Individuals

We examined the association of microsatellite and MHC variation in the same individuals in several ways. First, we calculated the correlation coefficient of individual heterozygosity for each locus pair in each population in which they were variable (nine different combinations) and none was significant. The mean value of these correlations was -0.018 and ranged from -0.15 to 0.16. Second, we calculated the extent of linkage disequilibrium between the microsatellite loci and the MHC locus in the only three combinations for which the calculation could be done. All were in the Cienega Creek, and none showed a statistically significant association. Finally, we calculated the variance of individual heterozygosity including the MHC locus and the five polymorphic microsatellite loci. The probability that the variance was greater than that expected at random was similar to that for microsatellite loci alone, with an average probability of 0.39. Overall then, there appears to be no association between variation among individuals within a population for microsatellite loci and the MHC locus.

Discussion

Gila topminnows have become one of the most thoroughly examined endangered species genetically, with molecular genetic studies of allozymes, mtDNA, a MHC locus, and microsatellite loci and research on traits related to fitness by Quattro & Vrijenhoek (1989), Sheffer et al. (1997a), Sheffer et al. (1997b), and Cardwell et al. (1998). Along with the extensive information from earlier studies on the natural history and ecology of the species and the factors influencing its decline (see Meffe et al. 1983 and references therein), it appears possible to understand relationships between present-day populations and make well-educated recommendations for future conservation efforts.

Comparison of Data from Different Molecular Markers

New, within-population genetic data from the microsatellite loci (expected heterozygosity and observed number of alleles) are summarized in Table 6 along with the data from previous studies (allozymes from Meffe & Vrijenhoek, 1988; mtDNA from Quattro et al. 1996; and MHC from Hedrick & Parker, 1998). These data are generally consistent over types of loci, allowing for sampling error over loci, thus making several generalizations possible. However, there are several observations that merit discussion.

First, Bylas Spring has the lowest overall variation (the only polymorphic locus for this sample was *Pooc-G49*) and had the lowest variation for both microsatellite loci and the MHC locus. The other three populations all have substantial polymorphism, with Sharp Spring somewhat higher for both measures of genetic variation. Vrijenhoek et al. (1985), after their allozyme survey, suggested that Sharp Spring as the most genetically variable of the four sites, and the other three had low variation. Sharp Spring still appears

the most genetically variable of the four sites when examining all types of loci given in Table 6, but both Cienega Creek and Monkey Spring have substantial overall variation.

Second, genetic distance is substantial for all pairs of populations for microsatellite loci, with Bylas Spring-Monkey Spring the largest (Table 5). This comparison did not share alleles at the MHC locus, making the MHC genetic distance infinite. Vrijenhoek et al. (1985) found the different sites were similar for allozyme loci. In fact, Bylas and Monkey Springs shared alleles (and were monomorphic for them) at all 25 allozyme loci examined, making allozyme genetic distance zero between these populations. Sharing of alleles for the allozyme loci is surprising because Bylas and Monkey Springs now are the most physically isolated from each other. The lack of any differences may reflect common ancestry in the distant past and a low rate of evolution for these loci, along with low power to determine the level of differentiation because of generally low variation at allozyme loci.

Third, variation in mtDNA region examined by Quattro et al. (1996) shows far less variation within and between populations than either microsatellite loci or the MHC locus. It is thought generally that mtDNA evolves rapidly but this does not appear the case for a number of fishes (e.g. Moritz et al. 1987). In addition, Quattro et al. (1996) used six-cutter restriction endonucleases, so that their results were of relatively low resolution, probably not recognizing differentiation that occurred in the last several hundred thousand years (Wilson et al. 1985).

Finally, there is evidence that genes in the MHC are under balancing selection (Hedrick 1994), most likely from parasite or pathogen resistance (Hedrick & Kim 1997). If so, the pattern of variation within and between populations may reflect this phenomenon, given reasonably strong balancing selection. The pattern of variation within individuals for the MHC locus, however, is consistent with Hardy-Weinberg expectations for the samples from Cienega Creek and Monkey Spring (Bylas Spring is not variable) and shows a deficiency of heterozygotes for Sharp Spring, the opposite of the expectation

for balancing selection (possibly due to the presence of a null allele, Hedrick & Parker 1998).

Between-population differentiation for the MHC locus is significant and appears to be as large or larger than for the polymorphic microsatellite loci (see discussion of measures of differentiation in Hedrick & Parker 1998). While it is possible that the MHC differentiation may have resulted from divergent selection among sites, the amount of differentiation is not unlike the pattern observed for microsatellite loci *Pooc-C15* and *Pooc-LL53*, suggesting the pattern of MHC differentiation may be primarily influenced by non-selective factors (see below). In fact, the pattern of variation over samples for microsatellite locus *Pooc-LL53* is nearly identical to that observed for the MHC locus, i.e., both are monomorphic in Bylas Spring, share an allele in high frequency between Bylas Spring and Cienega Creek, are nearly fixed for a third allele in Monkey Spring, and Sharp Spring has several different alleles but shares one with Monkey Spring. Because the measures of association within individuals for *Pooc-LL53* and the MHC locus were non-significant, it is likely that these nearly identical patterns of genetic variation for these two loci are similar reflections of population history and are not the result of linkage. When comparing the patterns of microsatellite and MHC variation over samples in bighorn sheep, Boyce et al. (1997) concluded that the greater similarity of MHC variation as compared to the microsatellite variants over samples provided support that MHC had a similar selective history over the different populations. Such a pattern is not obvious in the Gila topminnow data.

Explanation of the Observed Variation Within and Between Populations

There are number of non-selective factors that may play a role in determining variation within and between populations. Table 7 lists primary ones that may have been important in the four populations and gives an estimate of the relative effect for each factor.

Probably the most important factor determining lower variation observed in Bylas Spring is the low effective population size. This is both a function of the small habitat, the amount of water flowing from the spring (actually the middle spring or S-II) averages only a few liters per minute which can support at most a few hundred adults, and the recurring bottlenecks observed since the site was discovered (Marsh & Minckley 1990; Minckley et al. 1991). In fact, during one of these bottleneck periods in 1990, Gila topminnows became extinct at Bylas Spring (our stock was derived from a refugium population). In addition, because its location is elevated from the Gila River, it may be that there were only a few founders to originally colonize the site and that immigration has since been unlikely.

Monkey Spring appears to have been isolated from a tributary of Sonoita Creek through formation of a natural, 10-meter high travertine dam perhaps 10,000 years bp (Minckley et al. 1991). In other words, there may have been little or no immigration into Monkey Spring for more than 20,000 generations, assuming that there is two or more generations per year in the warm, constant-temperature habitat. Although the size of the site is somewhat reduced from its time of discovery by water development, population size is substantial and varies seasonally less than other sites because of its constant temperature and flow, and absence of floods. Long-term isolation with little or no immigration provides an explanation for the high probability of unique microsatellite alleles observed in Monkey Spring.

Both Sharp Spring and Cienega Creek have large amounts of variation shared in part with other sites. Because the population sizes have been large historically and there was substantial connectedness between these and other sites, such patterns of variation are not unexpected. Sharp Spring has several MHC alleles that differ substantially from those at other sites (Hedrick & Parker 1998) and has a large number of alleles at microsatellite locus *Pooc-C15*, suggesting Sharp Spring (and the upper Santa Cruz River that it now represents) may have had a very large population size in the recent past (also

indicated by museum collections) and may have been somewhat isolated from other populations.

Evolutionarily Significant Units in the Gila Topminnow

The molecular genetic studies of microsatellite and MHC variation generally support the importance of all four populations as significant ESUs. Monkey Spring, which has the most unique alleles and is quite genetically divergent, and Sharp Spring, which has the most genetic variation, are the most strongly supported as separate ESUs by these data. In addition, there are a number of other non-genetic attributes that support their significance (Table 8).

First, Bylas Spring is the only remaining population representing the mainstem Gila River stock (see Marsh & Minckley 1990; Minckley et al. 1991), isolated from others by at least 580 km by stream channel, much of which now is dry except during flooding. The other populations are also isolated, with 250 km separating Cienega Creek and Monkey Spring and 101 km separating Monkey Spring and Sharp Spring. There are extensive dry reaches between all sites except during floods. In other words, even though there may have been ancestral exchange between these sites through intermediate populations, it is unlikely that there has been exchange in recent decades and natural exchange is unlikely in the future.

Monkey Spring, besides appearing to be the most distinctive population based on the microsatellite data, also was occupied by a now-extinct species of pupfish, genus *Cyprinodon* (Minckley 1973; Minckley et al. 1991), and a now extinct, morphologically distinct form of *Gila intermedia*, the Gila chub (Rinne 1976; DeMaris 1986). Monkey Spring topminnows appear to have relatively low fecundity in nature (e.g. Constanz 1979) although this difference disappeared under laboratory conditions (Sheffer et al. 1997a). On the other hand, Cardwell et al. (1998) found that in a common laboratory

environment, male development time was approximately 50% longer in Monkey Spring topminnows than for males from any of the other three sites. Because *Gila* topminnows are members of a tropical genus, perhaps delayed development is a relict trait retained (or expressed) because of the warm, constant temperature at Monkey Spring. Perhaps, other populations evolved faster male development because of higher male-male competition for mates in seasonally variable environments.

Finally, neither Cienega Creek nor Monkey Spring has been infested with central mosquito fish while Bylas and Sharp Spring have been impacted by these non-native fish. Presence of mosquito fish not only introduces a competitor and predator but potentially, non-native parasites and pathogens. The suite of selection pressures experienced by the populations with or without mosquito fish may be substantial and itself of evolutionary significance.

Moritz (1994) suggested that genetic criteria for evolutionarily significant units is that they show phylogeographic differentiation for mtDNA variants and significant divergence of allele frequencies at nuclear loci. The four populations examined here show significant divergence both at the microsatellite loci and the MHC locus. Although no variation was found for mtDNA by Quattro et al. (1996) and there was little phylogeographic differentiation at the MHC locus, there is some indication of the size of the microsatellite alleles sorting out geographically. For example, at *Pooc-LL53* the three largest alleles are found in the Sharp Spring sample and for *Pooc-C15* in Monkey Spring there is cluster of seven alleles with sizes from 204 to 224 while Sharp Spring has three small alleles and nine alleles with sizes between 226 and 240.

Let us put these conclusions in perspective. After a laboratory study of fitness correlates by Vrijenhoek et al. (1985) indicated Sharp Spring fish had higher fitness than Monkey Spring fish, they recommended "the Sharp Spring stock currently offers the best choice for stocking the Gila River system." As a result, the U. S. Fish and Wildlife Service stopped using Monkey Spring fish for reintroductions and has since used only

Sharp Spring stock. Genetic results presented here, those of Sheffer et al. (1997a), Hedrick & Parker (1998), and Cardwell et al (1998) are, on the other hand, consistent with the recommendations by Simons et al. (1989) that "at least one representative lineage is preserved from each of the four geographic areas in Arizona." These genetic results support the suggestion in the draft recovery plan (USFWS 1993) that reintroductions be "from the hydrographically nearest natural population."

On a cautionary note, the new, highly variable markers used here are considered indicative of recent, non-selective factors. It is therefore possible that significant differences between populations may be observed that are not correlated with variation in adaptively important traits (Hedrick 1996; Hedrick & Savolainen 1996). However, the size of the genetic differences observed in these topminnow populations for both microsatellite loci and the MHC locus suggest that their isolation has been long enough term to reasonably suggest that the populations are on independent evolutionary trajectories, i.e., that they are significantly different evolutionarily significant units.

Acknowledgments

We appreciate the knowledge, inspiration, and comments on this manuscript by W. L. Minckley and the statistical assistance of Steven Kalinowski. This research was supported in part through funding from the Arizona Game and Fish Department Heritage Fund.

Disclaimer

The findings, opinions, and recommendations in this report are those of the investigators who have received partial or full funding from the Arizona Game and Fish Department Heritage Fund. The findings, opinions, and recommendations do not necessarily reflect those of the Arizona Game and Fish Commission or the Department, or necessarily represent official Department policy or management practice. For further information, please contact the Arizona Game and Fish Department.

Literature Cited

- Ashley, M. V., and B. D. Dow. 1994. The use of microsatellite analysis in population biology: background, methods, and potential applications. Pages 185-201 in B. Sherwater, B. Streit, G. P. Wagner, and R. DeSalle (eds.). *Molecular ecology and evolution: approaches and applications*. Birkhauser, Basel, Switzerland.
- Awise, J. C., and J. L. Hamrick (editors). 1996. *Conservation genetics: case histories from nature*. Chapman and Hall, New York.
- Boyce, W. M., P. W. Hedrick, N. E. Muggli-Cockett, S. Kalinowski, M. C. Penedo, and R. R. Ramey. 1997. Genetic variation of major histocompatibility complex and microsatellite loci: a comparison in bighorn sheep. *Genetics* **145**:421-433.
- Brown, A. H. D., M. F. Feldman, and E. Nevo. 1980. Multilocus structure of natural populations of *Hordeum spontaneum*. *Genetics* **96**:523-536.
- Cardwell, T. N., R. J. Sheffer, and P. W. Hedrick. 1998. Differences in male development time among populations of the endangered Gila topminnow. *Journal of Heredity* (submitted).
- Caughley, G., and A. Gunn. 1996. *Conservation biology in theory and practice*. Blackwell Science, Cambridge, Massachusetts.
- Conzanz, G. D. 1979. Life history patterns of a livebearing fish in contrasting environments. *Oecologia* **40**:189-201.
- DeMarais, B. D. 1986. Morphological variation in *Gila* (Pisces: Cyprinidae) and geologic history: Lower Colorado river basin. M.S. Thesis. Arizona State University, Tempe, Arizona.
- Hedrick, P. W. 1971. A new approach to measuring genetic similarity. *Evolution* **25**:276-280.
- Hedrick, P. W. 1994. Evolutionary genetics of the major histocompatibility complex. *American Naturalist* **143**:945-964 .

- Hedrick, P. W. 1996. Conservation genetics and molecular techniques: a perspective. Pages 459-477 in T. B. Smith and R. K. Wayne, editors. Molecular genetic approaches in conservation. Oxford University Press, New York.
- Hedrick, P. W., and T. Kim. 1997. Genetics of complex polymorphisms: parasites and maintenance of MHC variation. in R. S. Singh and C. K. Krimbas, editors. Evolutionary genetics from molecules to morphology. Cambridge University Press, New York.
- Hedrick, P. W., and K. M. Parker. 1998. MHC variation in the endangered Gila topminnow. *Evolution* (in press).
- Hedrick, P. W., and O. Savolainen. 1996. Molecular and adaptive variation: a perspective for endangered plants. Pages 92-102. in J. Machinski, D. H. Hammond, and L. Holter, Editors. Southwestern rare and endangered plants: proceedings of the second conference. General technical report RM-GTR-283. U. S. Department of Agriculture, Forest Service, Fort Collins, Colorado.
- Kumar, S., K. Tamura, and M. Nei. 1993. MEGA: molecular evolutionary genetics analysis, version 1.01. Pennsylvania State University, University Park, Pennsylvania.
- Marsh, P. C., and W. L. Minckley. 1990. Management of endangered Sonoran topminnow at Bylas Springs, Arizona: description, critique, and recommendations. *Great Basin Naturalist* **50**:265-272.
- Meffe, G. K., D. A. Hendrickson, W. L. Minckley, and J. N. Rinne. 1983. Factors resulting in decline of the endangered Sonoran topminnow, *Poeciliopsis occidentalis* (Atheriniformes: Poeciliidae), in the United States. *Biological Conservation* **25**:135-159.
- Meffe, G. K., and R. C. Vrijenhoek. 1988. Conservation genetics in the management of desert fishes. *Conservation Biology* **2**:157-169.

- Minckley, W. L. 1973. Fishes of Arizona. Arizona Game and Fish Department. Phoenix Arizona.
- Minckley, W. L., G. K. Meffe, and D. L. Soltz. 1991. Conservation and management of short-lived fishes: the cyprinodontoids. Pages 247-282. in W. L. Minckley and J. E. Deacon, editors. Battle against extinction: native fish management in the American west. University of Arizona Press, Tucson, AZ.
- Moritz, C. 1994. Defining "evolutionary significant units" for conservation. Trends in Ecology and Evolution **9**:373-375.
- Moritz, C., T. E. Dowling, and W. M. Brown. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annual Reviews of Ecology and Systematics **18**:269-292.
- Nei, M. 1972. Genetic distance between populations. American Naturalist **106**:283-292 .
- Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.
- Parker, K. M., K. Hughes, T. J. Kim, and P. W. Hedrick. 1998. Isolation and characterization of microsatellite loci from the Gila topminnow (*Poeciliopsis occidentalis*) and examination in guppies (*Poecilia reticulata*). Molecular Ecology (in press).
- Quattro, J. M., P. L. Leberg, M. E. Douglas, and R. C. Vrijenhoek. 1996. Molecular evidence for a unique evolutionary lineage of endangered Sonoran desert fish (genus *Poeciliopsis*). Conservation Biology **10**:128-135.
- Quattro, J. M., and R. C. Vrijenhoek. 1989. Fitness differences among remnant populations of the endangered Sonoran topminnow. Science **245**:976-978.
- Rinne, J. N. 1976. Cyprinid fishes of the genus *Gila* from the lower Colorado River basin. Wassman Journal of Biology **34**: 65-107.
- Sheffer, R. J., P. W. Hedrick, W. L. Minckley, and A. L. Velasco. 1997a. Fitness in the endangered Gila topminnow. Conservation Biology **11**:162-171.

- Sheffer, R. J., P. W. Hedrick and C. Shirley. 1997b. No bilateral asymmetry in wild-caught, endangered Gila topminnows. *Heredity* (in press).
- Simons, L. H., D. A. Hendrickson, and D. Papoulias. 1989. Recovery of the Gila topminnow: a success story? *Conservation Biology* **3**:11-15.
- Slatkin, M., and L. Excoffier. 1996. Testing for linkage disequilibrium in genotypic data using the Expectation-Maximization algorithm. *Heredity* **76**:377-383.
- Smith, T. B., and R. K. Wayne (editors). *Molecular genetic approaches in conservation*. Oxford University Press, New York.
- Takezaki, N, and M. Nei. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* **144**:389-399.
- U. S. Fish and Wildlife Service. 1993. Draft Gila topminnow recovery plan. U. S. Fish and Wildlife Service. Albuquerque, New Mexico.
- Vrijenhoek, R. C., M. E. Douglas, and G. K. Meffe. 1985. Conservation genetics of endangered fish populations in Arizona. *Science* **229**:400-402 .
- Waples, R. S. 1995. Evolutionary significant units and the conservation of biological diversity under the Endangered Species Act. *American Fisheries Society Symposium* **17**:8-27.
- Wilson, A. C., R. L. Cann, S. Carr, M. George, U. B. Gyllensten, et al. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society* **26**:375-400.

Table 1. Allelic frequencies for the five polymorphic microsatellite loci in the four populations. Frequencies in boldface are for "diagnostic" alleles, those only in a single population (except those in low frequency) or those in substantially higher in a given sample than all others.

<i>Locus</i>	<i>Allele</i>	<i>Bylas Spring</i>	<i>Cienega Creek</i>	<i>Monkey Spring</i>	<i>Sharp Spring</i>	<i>Average</i>
<i>Pooc-G49</i>	149	---	---	---	0.038	0.009
	159	0.250	1.000	1.000	0.962	0.803
	161	0.750	---	---	---	0.188
<i>Pooc-6-10</i>	287	---	---	1.000	---	0.250
	297	1.000	1.000	---	1.000	0.750
<i>Pooc-C15</i>	202	---	---	---	0.050	0.012
	204	---	---	0.025	0.200	0.056
	208	---	---	0.088	0.012	0.025
	210	---	---	0.012	---	0.003
	214	---	---	0.612	---	0.153
	216	---	---	0.225	---	0.056
	218	---	0.012	---	---	0.003
	222	---	---	0.012	---	0.003
	224	---	---	0.012	---	0.003
	226	---	---	---	0.012	0.003
	228	---	---	---	0.100	0.025
	230	---	---	---	0.012	0.003
	232	---	0.362	0.012	0.025	0.100
	234	---	---	---	0.100	0.025
	236	---	---	---	0.338	0.084
	238	---	0.612	---	0.112	0.181
240	1.000	0.012	---	0.025	0.259	
246	---	---	---	0.012	0.003	
<i>Pooc-OO56</i>	143	---	0.200	---	---	0.050
	145	1.000	0.800	0.762	1.000	0.890
	149	---	---	0.238	---	0.060
<i>Pooc-LL53</i>	142	---	---	0.988	---	0.247
	144	---	0.488	---	---	0.122
	146	1.000	0.512	---	---	0.378
	150	---	---	0.012	0.425	0.109
	154	---	---	---	0.550	0.138
	164	---	---	---	0.025	0.006

Table 2. The observed heterozygosity (H_O), expected heterozygosity (H_E), and observed number of alleles (n) for the five polymorphic microsatellite loci in the four populations.

<i>Locus</i>	<i>Measure</i>	<i>Bylas Spring</i>	<i>Cienega Creek</i>	<i>Monkey Spring</i>	<i>Sharp Spring</i>	<i>Average</i>
<i>Pooc-G49</i>	H_O	0.350	0.000	0.000	0.075	0.106
	H_E	0.375	0.000	0.000	0.075	0.112
	n	2	1	1	2	1.5
<i>Pooc-6-10</i>	H_O	0.000	0.000	0.000	0.000	0.000
	H_E	0.000	0.000	0.000	0.000	0.000
	n	1	1	1	1	1.0
<i>Pooc-C15</i>	H_O	0.000	0.600	0.600	0.800	0.500
	H_E	0.000	0.493	0.625	0.812	0.476
	n	1	4	8	12	6.2
<i>Pooc-OO56</i>	H_O	0.000	0.300	0.325	0.000	0.171
	H_E	0.000	0.320	0.362	0.000	0.171
	n	1	2	2	1	1.5
<i>Pooc-LL53</i>	H_O	0.000	0.625	0.025	0.500	0.288
	H_E	0.000	0.500	0.025	0.516	0.260
	n	1	2	2	3	2.0
Average	H_O	0.070	0.305	0.190	0.275	0.210
	H_E	0.075	0.263	0.202	0.281	0.205
	n	1.2	2.0	2.8	3.8	2.45

Table 3. The number of heterozygous loci observed per individual for the five microsatellite loci and the mean over loci (\bar{H}) and the mean observed and expected variance of heterozygosity per individual, $O(s_H^2)$ and $E(s_H^2)$, respectively, and the probability (P) that the observed variance is greater than that expected at random.

<i>Population</i>	<i>Heterozygosity</i>					\bar{H}	$O(s_H^2)$	$E(s_H^2)$	P
	0	1	2	3	4				
Bylas Spring	26	14	-	-	-	0.35	0.23	0.23	0.41
Cienega Creek	4	17	13	6	-	1.52	0.77	0.70	0.21
Monkey Spring	12	18	10	-	-	0.95	0.56	0.50	0.20
Sharp Spring	3	21	14	2	-	1.38	0.50	0.49	0.42

Table 4. Probability of a unique allele for a given population at the top of the table when compared to the population at the side, averaged over the five polymorphic microsatellite loci.

	<i>Bylas Spring</i>	<i>Cienega Creek</i>	<i>Monkey Spring</i>	<i>Sharp Spring</i>	<i>Average</i>
Bylas Spring	-	0.335	0.600	0.522	0.486
Cienega Creek	0.150	-	0.597	0.370	0.372
Monkey Spring	0.550	0.567	-	0.470	0.529
Sharp Spring	0.350	0.242	0.572	-	0.388
Average	0.350	0.381	0.590	0.454	

Table 5. The genetic distance (D) and F_{ST} between the population pairs for the four types of loci where the number of loci is given in parentheses.

<i>Comparison</i>	<i>Microsatellites (10)</i>		<i>Allozymes (25)</i>		<i>mtDNA (1)</i>		<i>MHC (1)</i>	
	D	F_{ST}	D	F_{ST}	D	F_{ST}	D	F_{ST}
Bylas-Cienega	0.188	0.479	0.080	1.000	0.0	0.0	0.35	0.33
Bylas-Monkey	0.440	0.712	0.000	0.000	0.0	0.0	∞	0.87
Bylas-Sharp	0.174	0.506	0.016	0.300	0.0	0.0	∞	0.46
Cienega-Monkey	0.294	0.494	0.080	1.000	0.0	0.0	∞	0.51
Cienega-Sharp	0.098	0.223	0.030	0.431	0.0	0.0	∞	0.24
Monkey-Sharp	0.271	0.466	0.020	0.300	0.0	0.0	2.35	0.37
Overall F_{ST}	-	0.591	-	0.750	-	0.0	-	0.55

Table 6. The expected heterozygosity (H_E) and observed number of alleles (n) for the different types of loci and the four populations where the number of loci is given in parentheses.

<i>Loci (number)</i>	<i>Bylas Spring</i>		<i>Cienega Creek</i>		<i>Monkey Spring</i>		<i>Sharp Spring</i>	
	H_E	n	H_E	n	H_E	n	H_E	n
Microsatellite (10)	0.038	1.10	0.132	1.50	0.101	1.90	0.140	2.40
Allozyme (25)	0.000	1.00	0.000	1.00	0.000	1.00	0.037	1.08
mtDNA (1)	0.000	1.00	0.000	1.00	0.000	1.00	0.000	1.00
MHC (1)	0.000	1.00	0.500	2.00	0.141	3.00	0.729	5.00

Table 7. Characteristics that are potentially relevant to understanding the amount of genetic variation within and the differentiation between the populations.

<i>Characteristic</i>	<i>Bylas Spring</i>	<i>Cienega Creek</i>	<i>Monkey Spring</i>	<i>Sharp Spring</i>
Population size (number of adults)	Small (<100)	Large (>>10,000)	Intermediate (<10,000)	Large (>>10,000)
Age of population (years before present)	Recent (<100)	Old (>>10,000)	Old (~10,000)	Old (>>10,000)
Relative historic immigration from other populations	Low	High	Low	High
Number of founders	Very low	High	Low	High

Table 8. Characteristics of the four populations divided into those of the physical habitat, the biota, and the life history of *Gila topminnows*.

<i>Characteristic</i>		<i>Bylas Spring</i>	<i>Cienega Creek</i>	<i>Monkey Spring</i>	<i>Sharp Spring</i>
<i>Physical</i>	Location	Mainstem Gila	Tributary to Santa Cruz	Above natural dam, tributary to Santa Cruz	Headwaters of Santa Cruz
	Temperature*	Variable	Variable	Constant, warm spring	Variable
	Habitat size	Very small	Large	Small	Large
	Flooding frequency	Very low	High	Low	Intermediate
<i>Biota</i>	Endemic vertebrates	None	None	Pupfish, distinct chub	None
	Mosquito fish	Present	Absent	Absent	Present
	Food supply	Seasonally variable	Seasonally variable	Constant	Seasonally variable
<i>Life history</i>	Male development**	Fast	Fast	Slow	Fast
	Fecundity***	High	High	Low	High
	Female reproduction	Seasonal	Seasonal	Year-round	Seasonal

* All these habitats are thermally ameliorated, either by low-volume spring inflow or groundwater exchange.

**Under laboratory conditions (Cardwell et al. 1998).

***In the field.

Figure Legends

Figure 1. Map showing the location of the four samples used.

Figure 2. The phylogenetic tree for the four populations using the UPGMA approach based on the allelic frequencies at the five polymorphic microsatellite loci.

Appendix Table 1.

<i>Locus</i>	<i>Allele</i>	<i>Bylas Spring</i>	<i>Cienega Creek</i>	<i>Monkey Spring</i>	<i>Sharp Spring</i>
<i>Pgd</i>	<i>a</i>	1.00	-	1.00	0.45
	<i>f</i>	-	1.00	-	0.55
<i>Est-4</i>	<i>a</i>	1.00	-	1.00	0.70
	<i>b</i>	-	1.00	-	0.30
<i>MHC - DRB</i>	<i>Pooc-1</i>	1.000	0.500	-	-
	<i>Pooc-2</i>	-	-	-	0.325
	<i>Pooc-3</i>	-	-	-	0.175
	<i>Pooc-4</i>	-	-	0.025	-
	<i>Pooc-5</i>	-	0.500	-	-
	<i>Pooc-6</i>	-	-	0.925	0.050
	<i>Pooc-7</i>	-	-	0.050	-
	<i>Pooc-8</i>	-	-	-	0.350
	<i>Pooc-9</i>	-	-	-	0.100