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# ALLOZYMIC VARIATION IN DESERT PUPFISH FROM NATURAL AND ARTIFICIAL HABITATS: GENETIC CONSERVATION IN FLUCTUATING POPULATIONS

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**Abstract**

*We studied patterns of allozymic variation among wild and captive populations of endangered desert pupfish* Cyprinodon m. macularius. *Much of the existing variation can be attributed to different founder sources, founder and bottleneck effects, or a combination of all three. Local isolation, either natural or artificial, promotes divergence of both wild and captive populations. Divergence was countered over the natural long-term condition of drought by dispersal and population mixing during brief wetter periods. Such gene flow among populations is now precluded by anthropogenic control of hydrologic pattern. Recovery of this species to self-sufficiency will likely require re-establishment of a natural hydrology, which, for the foreseeable future, is unlikely in the lower Colorado River basin. If this charismatic desert species is to survive, resource managers must combine knowledge of ecological and genetic patterns with political realities. Until natural habitats are re-established, a captive management program that more closely mimics patterns of alternating gene flow and isolation should be implemented.* © *1998 Elsevier Science Ltd. All rights reserved.*

*Keywords*: conservation genetics, desert pupfish, *Cyprinodon macularius*, Colorado River, Salton Sea, translocations, founder effects, México.

## INTRODUCTION

Preservation of biological diversity at the most basal level entails maintenance of genetic variation (Frankel and Soulé, 1981; Schoenewald-Cox *et al.*, 1983). Differing patterns in magnitude and distribution of genetic variation among taxa demand different conservation measures (Templeton *et al.*, 1991). As a result, biochemical surveys of genetic variation are becoming routine parts of the recovery process (Hedrick and Miller, 1992).

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One drawback of such surveys is the uncertainty of quantifying the effects of factors contributing to observed patterns (Rockwell and Barrowclough, 1987). For designing a conservation plan, genetic variation must be interpreted in light of ecological and demographic information. Unfortunately, ecological and demographic data are often unavailable and difficult to obtain. Studies of translocated populations with known histories may therefore prove particularly useful in interpreting patterns of genetic variation observed in the field (Stockwell *et al.*, 1996).

Recovery efforts for imperiled desert fishes in North America frequently involve translocations (Minckley, 1995). Translocations of imperiled fishes may reduce short-term extinction probabilities by increasing the total number of individuals and populations, but such efforts are arguably ineffective in maintaining the genetic integrity of imperiled taxa (Hendrickson and Brooks, 1991). Studies of genetic variation in translocated desert fishes have helped to identify important population patterns and processes and provide a basis for directing future recovery efforts (see Echelle, 1991 and references therein; DeMarais and Minckley, 1993).

This paper reports allozymic variation in wild and captive populations of desert pupfish (Cyprinodontidae, *Cyprinodon macularius* Baird & Girard), an endangered fish native to the lower Colorado River (Fig. 1). Our purpose was to address two questions: Do patterns of genetic variation differ among wild and captive populations? If so, what factors are responsible? Patterns in captive populations were examined in light of number of founders, time since founding, patterns of gene flow, and relative habitat sizes. Results of this analysis were used to evaluate current recovery efforts for desert pupfish in the context of probable historical patterns of gene flow and isolation.

## MATERIALS AND METHODS

### Study organism

Desert pupfish formerly occurred throughout much of the vast lower Colorado River basin of northwestern

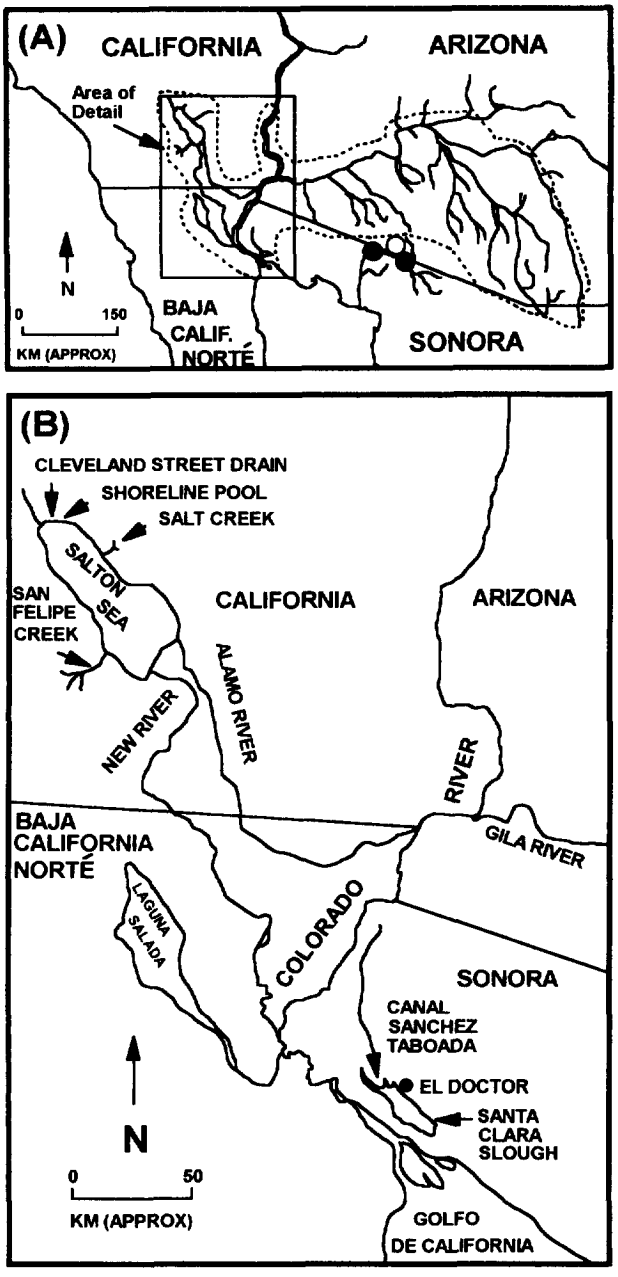


**Fig. 1.** (A) Outline of probable historic range of desert pupfish (dotted line); ●, extant localities of the Rio Sonoyta pupfish (*C. macularius* ssp.); ○, single natural locality for Quitobaquito pupfish (*C. m. eremus*). Modified from Minckley (1973), Miller and Fuiman (1987), and Marsh and Sada (1993). (B) Map of lower Colorado River and Delta with names of same localities mentioned in text.

México (Baja California, Sonora) and southwestern USA (Arizona, California) (Fig. 1). Two subspecies are now recognized, *C. m. eremus* Miller & Fuiman, from Quitobaquito Spring, Organ Pipe Cactus National Monument, Arizona, and *C. m. macularius* Baird & Girard in most of the remainder of its geographic range. A third form in the Río Sonoyta, Sonora, is as yet named (Miller and Fuiman, 1987). We deal with only *C. m. macularius*.

This taxon is part of a declining native fish fauna in the American Southwest (Minckley and Deacon, 1991). Habitat destruction and impacts of exotic species have extirpated and fragmented desert pupfish populations throughout its range (Schoenherr, 1988; Minckley *et al.*, 1991), and it was listed as endangered by the US Fish and Wildlife Service (USFWS, 1986). A recovery plan (Marsh and Sada, 1993) included provisions for maintaining genetic variation, some of which are incorporated here.

**Sampling localities and collections**

Pupfish were obtained from nine captive populations: Dexter National Fish Hatchery and Technology Center, Chavez County, New Mexico; private pond (W. L. Minckley residence: WLM), and Desert Botanical Garden, Maricopa County, Arizona; Boyce Thompson Arboretum, Pinal County, Arizona; Howard Well, Graham County, Arizona; Flowing Wells Junior High School, Pima County, Arizona; Simone/McCallum Pond and Living Desert Reserve, Riverside County, California; Palm Canyon Pond, San Diego County, California. Wild fish were obtained from four California sites around the Salton Sink (Imperial and Riverside counties): Salt Creek, Cleveland Street Drain, unnamed shoreline pool *c.* 1.0 km east of Cleveland Street Drain, and San Felipé Creek (Fig. 1). Fish were frozen at capture and stored at −80°C or transported and maintained alive until processed. Sample sizes in our collections and those provided by other collectors varied considerably, but were large enough to allow detection of rare ($p = 0{\cdot}05$) alleles (Sjögren and Wyöni, 1994).

Historical data on captive populations (Fig. 2) are from unpublished notes of Brian Bagley (formerly with Arizona Game & Fish Department), Betsy Bolster (California Department of Fish & Game), Gerald Burton (USFWS), and Paul C. Marsh and W. L. Minckley (Arizona State University). Captive fish in Arizona and New Mexico were of two separate lineages from Sonora: Canal Sanchez Taboada (Dexter, Desert Botanical Garden), and 'Terrace Springs' on the Colorado River Delta (WLM, Howard Well, Flowing Wells). Boyce Thompson fish were a mixture of both Mexican sources. California captive populations each were from a different wild stock: Living Desert from the Salton Sea, Palm Canyon from San Felipé Creek, and Simone/McCallum from Salt Creek.

**Electrophoretic techniques**

Living fish were euthanized with chlorobutanol before dissection. Water-soluble protein extracts were obtained from eye, muscle and liver. Tissues were placed in 1.5-ml microcentrifuge tubes, homogenized in equal volumes of distilled water, and centrifuged for 5 min at *c.* 18,000 G (Murphy *et al.*, 1990). Supernatants were loaded on filter-paper wicks, inserted into 12% starch gels, electrophoresed for *c.* 12 h at 25–50 mA then sliced and stained for enzyme activity. Proteins, tissue

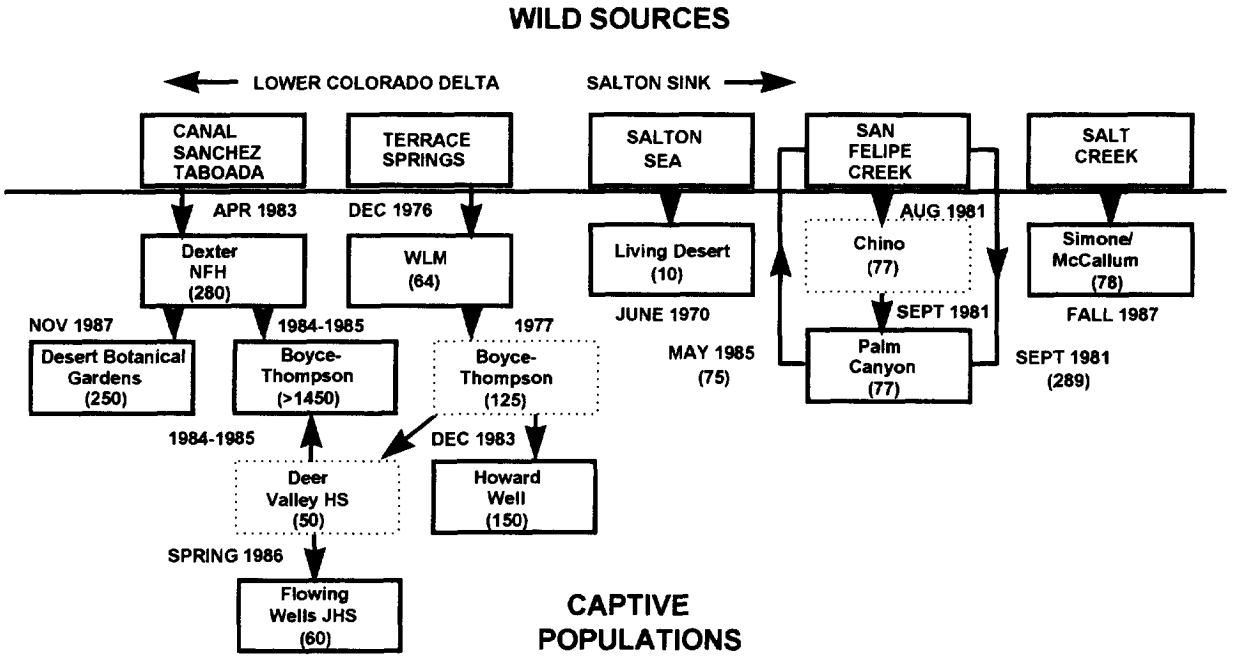


**Fig. 2.** History of captive desert pupfish populations founded from collections on the lower Colorado River delta and Salton Sink. The solid horizontal line separates wild from captive populations, and dotted lines indicate extirpated populations. Numbers in parentheses are number of founders for each population. Arrows indicate direction of fish transfers.

**Table 1. Presumptive loci, proteins, tissue sources, and buffer systems**

Enzyme nomenclature follows recommendations of the International Union of biochemistry (1984). Locus designations follow Buth (1983).

| Protein (EC number) | Locus | Tissue scored | Buffer system[a] |
|---|---|---|---|
| Aconitase hydratase (4.2.1.3) | sAh-A | liver | 2 |
| Glucose-6-phosphate isomerase (5.3.1.9) | Gpi-A | muscle-eye | 1 |
| | Gpi-B | muscle-eye | 1 |
| L-Lactate dehydrogenase (1.1.1.27) | Ldh-A | muscle-eye | 1 |

[a]Buffer systems as follows: (1) electrode buffer: 0·04 M citric acid monohydrate, adjust to pH 6·1 by adding 10–15 ml/litre of *n*-3-aminopropylmorpholine; gel buffer: 1 in 19 dilution of electrode buffer. (2) electrode buffer: 0·1 M tris hydroxymethylaminomethane (= 'tris'), 0·3 M citric acid, pH 7·5; gel buffer: 1 volume of electrode solution + 6 volumes $H_2O$.

distributions and buffers are given in Table 1. Proteins surveyed were selected from those known to be polymorphic ($p < 0{\cdot}99$) in *C. macularius* (Four of six loci recorded by Turner (1983), Turner (1984) and other pupfishes (Echelle *et al.*, 1987). Other criteria included cost and ease of resolution.

**Data analyses**

Data analyses used the BIOSYS-1 computer program of Swofford and Selander (1981). Loci were deemed polymorphic if frequency of the most common allele was $< 0{\cdot}99$. Average heterozygosity per locus per individual was calculated following Nei (1978). The chi-square goodness-of-fit test with Levene's (1949) correction was used to test for deviations from Hardy–Weinberg expectations. For analysis of genetic differentiation, populations were divided into lineages, defined above as a group of populations descended from a common source (Fig. 2). Differentiation within lineages was examined with an exact Fisher test for each locus (Raymond and Rousset, 1995*a*) with the GENEPOP package (Raymond and Rousset, 1995*b*). Mantel tests (Douglas and Endler, 1982; Manly, 1991) were used to compute associations between changes in genetic variation (heterozygosity) and four variables: number of founders; relative habitat size; time since founding; and number of known founding events.

Populations derived from sources of differing heterozygosity may differ genetically for that reason alone. To control for this potentially confounding factor, we used the standardized difference between source and descendent population heterozygosity, $H_{\mathrm{DIFF}}$, defined as follows:

$$H_{\mathrm{DIFF}} = \mathrm{ABS}(H_{x-1} - H_x)/H_{x-1} \qquad (1)$$

where $H_{x-1}$ is the source population heterozygosity and $H_x$ is descendent population heterozygosity. Mantel tests for association with number of founders, relative habitat size and time since founding used $H_{\mathrm{DIFF}}$ as the response variable.

The difference between original source and descendent population heterozygosity ($H_{\mathrm{SOURCE}}$) was used as

the response variable to determine the effect of number of founding events. $H_{SOURCE}$ was defined as follows:

$$H_{SOURCE} = ABS(H_{x-0} - H_x)/H_0 \quad (2)$$

where $H_0$ is the original source heterozygosity. Differences in heterozygosity ($H_{SOURCE}$, $H_{DIFF}$) were determined for six populations with genetic information on founder sources (Fig. 2).

Gaps in the history of pupfish populations were many (Fig. 2), and potentially problematic. For example, the source of Flowing Wells had been extirpated by the time of this study, so it was compared with its nearest population, Howard Wells. One population, Boyce Thompson, was founded from a mixture of two source populations (Fig. 2), so heterozygosity values from each source were averaged for comparisons. The same was true for the source of Living Desert pupfish. In cases such as the desert pupfish, where critical information is incomplete or possibly biased, the most pragmatic approach is to analyze the data first and then carefully examine the results for any discrepancies due to such inadequacies (Elliot, 1992).

## RESULTS AND DISCUSSION

Overall, only four polymorphic loci were resolved by procedures outlined in Echelle *et al.* (1987) and Turner (1983, 1984), but this is consistent with generally low levels of allozymic variation in desert pupfish. Statistically significant deviation from Hardy–Weinberg expectations occurred in only two of 39 chi-square tests, an excess of heterozygotes for sAh-A at Boyce-Thompson and a deficiency of heterozygotes for Gpi-A at Simone/McCallum (Table 1).

Genetic variation among desert pupfish populations (Tables 2 and 3) was marked. Populations originating on the delta were fixed for the same allele at the Gpi-B locus that dominated in Salton Sink populations (>90% frequency in six of seven populations and fixed in Living desert). Among delta populations, Dexter and Desert Botanical Garden fish lacked variation at the Gpi-A and Ldh-A loci. Two populations (Howard Well, Flowing Wells) showed marked differences in allele frequency at the sAh-A locus. Variation was less among Salton Sink samples, but some differences in frequencies were nonetheless apparent (e.g. sAh-A and Ldh-A at Living Desert). Differentiation within lineages (=captive populations with common ancestry, Fig. 2) was significant in three of four cases (Table 4).

In general, evidence from genetic studies of animal populations indicates that translocations may reduce allelic diversity and heterozygosity (Stockwell *et al.*, 1996), presumably due to drift, bottleneck effects, or founder effects. Levels of heterozygosity within source and descendent populations of desert pupfish did not consistently fit this pattern (Table 3), but with only four variable loci and six populations, our ability to detect such patterns could be limited (Leberg, 1992).

Significant patterns were found, however, with regard to changes in heterozygosity among populations. Absolute levels of heterozygosity at individual loci may transiently increase or decrease as a result of drift, population bottlenecks, or founder events (Wright, 1931). Accordingly, when only a limited number of variable loci are considered, the effects of such events should be more reliably manifested through changes in heterozygosity. We found changes in overall heterozygosity among the captive populations of desert pupfish were strongly associated with founder population size (Mantel test, $p = 0{\cdot}016$), but not time since founding, relative habitat size, or number of founding events. This result is consistent with theoretical expectations for species that can quickly expand in population size following a founding event or population bottleneck (Nei *et al.*, 1975). This also may explain why time since founding has little influence on retention of genetic variability in captive populations.

Lack of association between genetic variability and relative habitat size may be explained by the highly variable nature of pupfish habitat. Habitat instability, especially fluctuations in water levels, can lead to dramatic changes in population size. This is certainly the case with Howard Well and Boyce Thompson pupfish (B. Bagley, pers. comm. 1990). Such variations may also obscure the impact of multiple founding events as well.

Levels of genetic variation in the original wild sources of captive pupfish populations are unknown and form an important and potentially confounding gap in the historical data used in this study. Overall, source and captive stock variation are not significantly related (Mantel test $p > 0{\cdot}05$), but we have no data on genetic variation of source populations at the times when collections were made for founding captives. If significant genetic changes in source populations occurred after captives were founded, present genetic patterns may not be valid for comparisons.

Observed allele frequencies in Dexter and WLM captive populations point strongly to the influence of source populations on genetic variation. The Dexter stock was founded with 280 fish in 1983 and subsequently maintained as a few thousand individuals. The WLM stock originated with only 64 fish in 1976 and persisted in the low hundreds. Founder effects should thus be more pronounced in the WLM stock and less in the Dexter stock had the sources been the same, exactly opposite the lack of variation in Dexter relative to the originally and chronically smaller and older WLM stock (Table 2). Sources for the two populations were both from the same part of the Colorado Delta (Fig. 1), yet the pattern of variation indicates differentiation despite geographic proximity.

Wild founders for Dexter were from a pool formed by heavy equipment near the terminus of Canal Sanchez Taboada at Ciénega de Santa Clara (G. Burton, pers.

**Table 2. Allelic variation at four variable loci in 13 populations of *Cyprinodon macularius***

| Population | Locus | | | |
|---|---|---|---|---|
| | sAh-A | Gpi-A | Gpi-B | Ldh-A |
| Salt Creek | (30) | (30) | (30) | (30) |
| | a: 0·217 | 0·933 | 0·917 | 0·017 |
| | b: 0·783 | 0·067 | 0·083 | 0·983 |
| Cleveland Street drain | (29) | (29) | (29) | (29) |
| | a: 0·155 | 0·914 | 0·966 | 0·086 |
| | b: 0·845 | 0·069 | 0·034 | 0·914 |
| | c: | 0·017 | | |
| Shoreline pool | (29) | (30) | (30) | (30) |
| | a: 0·172 | 0·900 | 0·933 | 0·167 |
| | b: 0·828 | 0·050 | 0·067 | 0·833 |
| | c: 0·050 | | | |
| San Felipe Creek | (29) | (29) | (29) | (29) |
| | a: 0·172 | 0·966 | 0·931 | 0·052 |
| | b: 0·828 | 0·034 | 0·069 | 0·948 |
| Simone/McCallum | (50) | (50) | (50) | (50) |
| | a: 0·150 | 0·700 | 0·990 | 0·120 |
| | b: 0·850 | 0·210 | 0·010 | 0·880 |
| | c: 0·090 | | | |
| Palm Canyon | (49) | (50) | (50) | (50) |
| | a: 0·194 | 0·880 | 0·990 | 0·060 |
| | b: 0·806 | 0·060 | 0·010 | 0·940 |
| | c: 0·060 | | | |
| Living Desert | (35) | (35) | (35) | (35) |
| | a: 0·700 | 1·000 | 1·000 | 0·257 |
| | b: 0·300 | | | 0·743 |
| WLM | (39) | (39) | (39) | (39) |
| | a: 0·410 | 0·885 | 1·000 | 0·154 |
| | b: 0·590 | 0·115 | | 0·846 |
| Howard Well | (55) | (68) | (68) | (38) |
| | a: 0·055 | 0·647 | 1·000 | 0·158 |
| | b: 0·945 | 0·353 | 0·842 | |
| Flowing Wells JHS | (30) | (30) | (30) | (30) |
| | a: | 0·741 | 1·000 | 0·083 |
| | b: 1·000 | 0·259 | | 0·917 |
| Dexter NFH | (33) | (94) | (96) | (95) |
| | a: 0·333 | 1·000 | 1·000 | 1·000 |
| | b: 0·667 | | | |
| Desert Botanical Gardens | (51) | (51) | (51) | (51) |
| | a: 0·402 | 1·000 | 1·000 | 1·000 |
| | b: 0·598 | | | |
| Boyce-Thompson | (53) | (83) | (83) | (88) |
| | a: 0·462 | 0·831 | 1·000 | 0·114 |
| | b: 0·538 | 0·169 | | 0·886 |

Numbers in parentheses indicate sample sizes.

**Table 3. Measures of genetic variation calculated from four polymorphic loci in 13 populations of *Cyprinodon macularius* (with standard errors)**

| Population | Mean number of alleles per locus | Number of loci polymorphic | Heterozygosity |
|---|---|---|---|
| Salt Creek | 1·6 (0·2) | 4 | 0·081 (0·050) |
| Cleveland street drain | 1·7 (0·3) | 4 | 0·103 (0.045) |
| Shoreline pool | 1·7 (0·3) | 4 | 0·125 (0·054) |
| San Felipe Creek | 1·6 (0·2) | 4 | 0·064 (0·029) |
| Simone/McCallum | 1·7 (0·3) | 4 | 0·111 (0·051) |
| Palm Canyon | 1·7 (0·3) | 4 | 0·101 (0·052) |
| Living Desert | 1·3 (0·2) | 2 | 0·143 (0·093) |
| Howard Well | 1·4 (0·2) | 3 | 0·120 (0·069) |
| Flowing Wells JHS | 1·3 (0·2) | 2 | 0·078 (0·055) |
| WLM | 1·4 (0·2) | 3 | 0·136 (0·071) |
| Dexter NFH | 1·1 (0·1) | 1 | 0·069 (0·069) |
| Desert Botanical Gardens | 1·1 (0·1) | 1 | 0·064 (0·064) |
| Boyce-Thompson | 1·4 (0·2) | 3 | 0·104 (0·051) |

**Table 4. Probability values for Fisher exact tests of population differentiation at variable loci in four desert pupfish lineages**

Lineages are defined as a group of populations descended from a common locality (Fig. 2).

| Lineage | locus | p |
|---|---|---|
| Salt Creek[a] | | |
| | sAh-A | 0·29 |
| | Gpi-A | 0·001 |
| | Gpi-B | 0·03 |
| | Ldh-A | 0·04 |
| San Felipe[b] | | |
| | sAh-A | 0·83 |
| | Gpi-A | 0·14 |
| | Gpi-B | 0·06 |
| | Ldh-A | 1·00 |
| Canal Sanchez-Taboada[c] | | |
| | sAh-A | 0·41 |
| Terrace Springs[d] | | |
| | sAh-A | <0·001 |
| | Gpi-A | <0·001 |
| | Ldh-A | 0·40 |

[a]Salt Creek lineage includes Salt Creek and Simone/McCallum refugium.
[b]San Felipe lineage includes San Felipe Creek and Palm Canyon refugium.
[c]Canal Sanchez-Taboada lineage includes Dexter NFH and Desert Botanical Gardens refugia.
[d]Terrace Springs lineage includes WLM, Howard Well and Flowing Wells JHS refugia.

comm.). The latter is a variably sized (up to *c.* 2·0 km$^2$) marsh on the southeastern edge of the delta, now partially maintained by artificial inflow from the canal. Low genetic variation in Dexter is explained if the wild source stock either originated from a few individuals, suffered a severe bottleneck(s) after establishment, or both. Founders of the WLM stock were from a series of five separate springheads (collectively flowing an estimated < 15 litres/min) rising a few tens of meters apart along the base of an alluvial terrace, almost level with and flowing toward the adjacent Cienéga de Santa Clara (Fig. 1). They thus included individuals from five separate populations, none of which likely exceeded *c.*200 fish, and greater variability might be anticipated. Natural habitats like these springs may, however, appear isolated at a point in time, when in fact they are periodically interconnected. Nearby vegetation was inundation tolerant (desert plants were absent) indicating periodic flooding. A rise in water level of < 1·0 m later inundated four and perhaps all five springs, allowing gene flow.

Genetic patterns among delta captives indirectly suggest some degree of genetic fragmentation of wild populations on the Colorado Delta. this is consistent with the historic and present-day desiccated condition (owing now to upstream dams and agricultural diversions) of the habitat at the time the wild collections were made (Hendrickson and Varela Romero, 1989), but this cannot be confirmed without direct study of these populations.

While circumstantial evidence suggests genetic fragmentation of wild pupfish populations on the delta, wild populations in the Salton Sink are remarkably homogeneous over a much larger geographic scale. Lack of genetic variation among these populations may be due to a recent dramatic increase in pupfish abundance and gene flow. Prior to 1904, pupfish populations in the sink were restricted to isolated springs and seeps around the periphery of the basin (Hubbs and Miller, 1948). In 1904–1907, flooding on the Colorado River filled the Salton Sink, to form the present Salton Sea (Sykes, 1937). Following this, pupfish formed remarkably large shoreline populations (Coleman, 1929; Barlow, 1958, 1961). The species remained abundant until the mid-1960s, followed by dramatic declines in response to introduction and establishment of non-native fishes (Schoenherr, 1988). More recently, declines in non-native fishes, presumably in response to ever-increasing salinity accumulated through irrigation return flow and evaporation (B. Bolster, A. Schoenherr, *pers. comm.* 1992), have been paralleled by a rebound of pupfish populations. Thus, over the past century, the population of desert pupfish in the Salton Sink has dramatically expanded and contracted in response to both natural and artificial limiting factors (desiccation and exotic species, respectively).

A similar 'boom and bust' cycle seems to have occurred on the hydrologically connected Colorado Delta, where pupfish populations must have endured natural and now increasing periods of desiccation following upstream agricultural diversions beginning in the 1900s (Sykes, 1914) and impoundment of the river's flows, beginning with Hoover (Boulder) Dam in 1935 (Graf, 1985). Rewatering of the delta by extensive flooding in 1982–1983 resulted in increased distribution and abundance of pupfish (Hendrickson and Varela, 1989) which persists in part today (Abarca *et al.*, 1992). The genetic impacts of this rewatering are unknown. Recent surveys on the Colorado Delta failed to locate pupfish in some areas where they were formerly collected (Hendrickson and Varela Romero, 1989; Abarca *et al.*, 1992), but found locally abundant populations in at least one locality within Ciénega de Santa Clara (Zengal and Glenn, 1996).

## CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Results of this study indicate significant genetic differences among captive and wild desert pupfish populations. Furthermore, it seems likely that founder effects may have played a major role in determining the patterns of genetic variation observed in captive populations considered in this study. Previous study of the genetics of captive populations of desert pupfish (Turner, 1984), and other *Cyprinodon* (Edds and Echelle, 1989) found fewer genetic differences between

captive and wild populations. Both studies found evidence for loss of rare alleles, but little or no difference in heterozygosity between captive and wild populations. While Turner found fewer genetic differences between captive and wild desert pupfish populations, he cautioned that limited genetic sampling (among other potential explanations) could account for lack of differences. Results of this work confirm his cautionary message. Similar problems with 'negative data' have been observed in other studies of allozyme variability in fishes (e.g. Utter *et al.*, 1992).

We conclude that genetic differences among captive populations have resulted, at least in part, from changes in variability stemming from limited numbers of founders, and from continued isolation of captive populations. Circumstantial evidence suggests differences among wild populations on the lower Colorado Delta also may exist due to isolation and fragmentation of habitats that were formerly interconnected, at least periodically, by flooding. Similar effects of human influences have been implied for other pupfishes. For example, Echelle *et al.* (1987) found evidence that changes in habitat structure and hybridization with non-native pupfish may have increased genetic divergence among sub-populations of native pupfishes in the Chihuahuan desert.

In large and continuous habitats, genetic differentiation among populations may be reduced. We found evidence for this in recently expanding populations of desert pupfish in the Salton Sink. A parallel example is provided by the Red River pupfish *Cyprinodon rubrofluviatilis*, a species that is currently widespread and abundant in large river systems of Oklahoma and Texas (Ashbaugh *et al.*, 1994). Populations of Red River pupfish in the same drainage had uniformly high levels of genetic variability within populations and little structuring among populations (i.e. over 96% of the total genic diversity was explained by within-sample variability).

Current recovery efforts for desert pupfish focus on maintaining isolated local populations, and can only result in a systematic decline in genetic diversity of captive populations individually and as a whole (see Hedrick and Gilpin, 1996). Furthermore, we found limited evidence to suggest the small number of populations of desert pupfish in captivity may not represent all of the genetic diversity remaining in wild populations (sensu Waples, 1991; Lesica and Allendorf, 1995). Searches for, and study of wild populations should therefore continue for a time. The possibility of local adaptation (e.g. Hirshfield *et al.*, 1980) should also be considered before groups of wild pupfish populations are managed as relatively homogeneous units. Once a determination is made that no new variability nor wild populations are forthcoming, efforts should begin to manage pupfish in a manner more consistent with their natural history.

Historically, pupfish populations on the Colorado Delta occupied perennial habitats, such as permanent springs and streams that were periodically connected by flooding (Sykes 1914, 1937; Kniffen 1932; Hendrickson and Varela-Romero, 1989; Miller *et al.*, 1991). Such a system can persist in hot deserts only if reliable habitats and flooding are sufficient to allow local persistence and periodic gene flow, respectively. The original geographic range of desert pupfish exceeded 40,000 $km^2$ over an altitudinal range of −50 to 1500 m (Minckley, 1973). Upstream lay the seventh largest watershed in the coterminous USA, part of which yielded at least some surface water each year (Minckley, 1991). Faulting and other phenomena associated with active tectonism created fracture zones where springs were concentrated (Minckley *et al.*, 1986). Large snowpack in unusually wet years assured periodic floods. In this situation the pupfish enjoyed a high probability of survival.

The prognosis for continued survival of desert pupfish is poor. With disappearance from the entire Gila River Basin (Fig. 1), an unknown but substantial proportion of intraspecific diversity was lost. Perhaps most importantly, hydrological control over the rivers precludes gene flow among populations. Desert pupfish will not likely be recovered to self sufficiency until natural hydrologic conditions, including major flooding, are restored. Considering demands on the Colorado River water by more than 20 million people (Graf, 1985), such seems unlikely in the foreseeable future. Therefore, management of captive populations in a way that mimics a more natural state should be implemented.

The severe selective arenas occupied by desert pupfish are not difficult to replicate. The fish needs little more than water to survive as long as non-native species are excluded. Fortunately, most non-native species, considered critical problems in the conservation of most native southwestern species (Minckley and Deacon, 1991), cannot survive the severe conditions pupfish accommodate readily. The ecological and genetic stresses pupfish have experienced for millennia include, among others, isolation, sudden expansions and reductions in habitat size or quality, or mixing and interbreeding of populations. Of these, none is difficult nor expensive to provide. In fact, some comparable 'manipulations' inadvertently occur through human error during refuge operations (Minckley *et al.*, 1991; Minckley, 1995). The desert pupfish has become somewhat of a standard bearer for imperiled fishes of western North America, with charismatic status in the public eye as a representative for desert aquatic ecosystems. Its perpetuation should be of high priority.

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