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# PHOSPHOGLUCONATE DEHYDROGENASE POLYMORPHISM AND SALINITY IN THE WHITE SANDS PUPFISH

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*Abstract.*—The phosphogluconate dehydrogenase (*Pgdh*) locus is the only polymorphic allozyme locus observed among 37 loci examined in all four populations of a New Mexico state Endangered species, the White Sands pupfish (*Cyprinodon tularosa*). We report evidence suggesting that this polymorphism may be associated with salinity. Salinity levels vary widely within and between habitats occupied by White Sands pupfish. The frequency of the $Pgdh^{100}$ allozyme was correlated with salinity but not with temperature. Frequency of $Pgdh^{100}$ differed between low (3.76 parts per thousand (ppt)) and high (9.23 ppt) salinity sites at Malpais Spring despite no obvious barriers to fish movement. Frequencies of $Pgdh^{100}$ in two introduced populations differed from that of the presumptive founding stock and correlated with salinity in the current habitats.

*Key words.*—*Cyprinodon tularosa, Pgdh*, phosphogluconate dehydrogenase, salinity, selection, translocation, White Sands pupfish.

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Following the desiccation of the Pleistocene lakes in the southwestern United States, numerous aquatic organisms were isolated in remnant water bodies (Miller 1948, 1981). These remnant habitats vary considerably in their physical characteristics, especially temperature and salinity. Such divergent environmental conditions coupled with genetic drift are presumably responsible for the rapid diversification of the Cyprinodontid fishes of the southwestern United States (Miller 1948, 1981). Pupfish exhibit considerable variation in morphology, behavior, and physiology (Miller 1948; Turner 1974; Hirshfield et al. 1980); however, little variation has been reported at allozyme loci (Turner 1974; Echelle 1991; Echelle and Echelle 1993; Stockwell et al. 1998).

Low levels of allozyme variability are probably due to periodic genetic bottlenecks (Echelle 1991). Such bottlenecks should lead to the random loss of genetic variability and the pattern should be consistent across all neutral loci (Nei et al. 1975). Allozyme polymorphisms in otherwise genetically depauperate species allow for the possibility of natural selection. Indeed, the role of selection in maintaining allozyme polymorphisms has drawn considerable attention (Lewontin 1974, 1991; Avise 1994; Watt 1994; Mitton 1997).

Experimental and field studies have provided compelling evidence that selection may act on specific allozyme loci. The phosphoglucose isomerase polymorphism of *Colias* has been shown to be related to temperature (Watt 1977, 1983; Watt et al. 1983); allozymes differ in their kinetic properties and temperature optima. In *Mytilus edulis,* aminopeptidase allozymes have been related to osmotic regulation in varying salinity regimes (Koehn et al. 1980; Hilbish et al. 1982; Hilbish and Koehn 1985). The extensive work of Powers and coworkers with the lactate dehydrogenase-B locus (*Ldh-B*) in *Fundulus heteroclitus* has shown a convincing relationship between allozyme genotype and fish performance under different thermal conditions (for review see Powers et al. 1991).

Relationships between environmental variables and allozymes are often initially based on correlations observed in field populations. In *F. heteroclitus* early work established a correlation between environmental temperature and *Ldh-B* allele frequency in wild populations (Powers 1972; Mitton and Koehn 1975; Powers and Place 1978). Laboratory studies demonstrated that *Ldh-B* is expressed in tissues with high aerobic activity, such as red muscle, and that allelic variants differ in temperature-specific catalytic efficiencies (Powers et al. 1991). Differences in catalytic efficiencies among allozyme genotypes may manifest at the organismal level as differences in hatching times (DiMichele and Powers 1982a), embryonic metabolic rate (Paynter et al. 1991), or temperature-specific swimming performance (DiMichele and Powers 1982b). DiMichele et al. (1986) have verified these results in field selection experiments.

In the previously mentioned studies, allozyme polymorphisms have been associated with environmental heterogeneity. The habitats of the White Sands pupfish (*Cyprinodon tularosa*) vary considerably in salinity levels. Like other pupfish species (Turner 1974; Echelle 1991; Echelle and Echelle 1993), the White Sands pupfish has relatively low allozyme variability (Echelle et al. 1987; Stockwell et al. 1998). Here, we report the distribution of *Pgdh* allozymes in White Sands pupfish and suggest that this polymorphism is associated with environmental salinity. Further, we infer from recently translocated populations that response to habitat salinity may be rapid.

## MATERIALS AND METHODS

The White Sands pupfish is classified as endangered by the State of New Mexico and occupies four localities located

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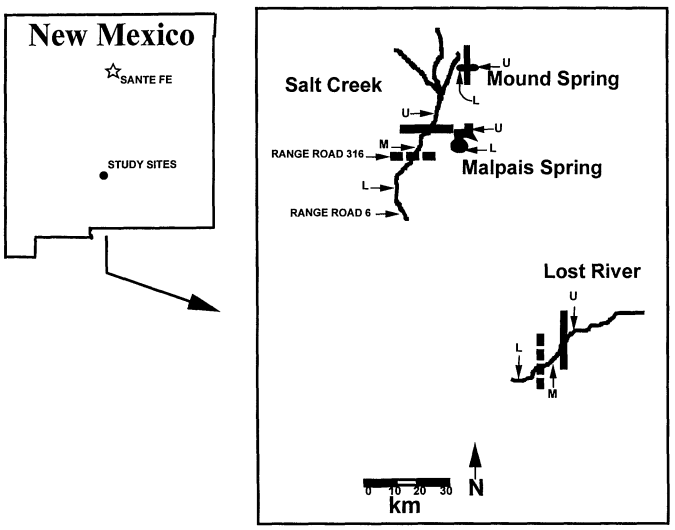


FIG. 1. The distribution of the White Sands pupfish. Barriers to fish movement are both permanent (solid lines) and temporary (dashed lines). Sample sites were located in the lower (L), middle (M), and upper (U) sections of these habitats.

in the Tularosa Basin of New Mexico: Malpais Spring, Salt Creek, Mound Spring, and Lost River (Miller and Echelle 1975; Echelle et al. 1987; Fig. 1). Of 37 loci examined, only *Pgdh* (E. C. 1.1.1.44) was polymorphic in all four populations (Echelle et al. 1987; Echelle and Echelle 1992; Stockwell et al. 1998). Allozyme frequencies for this locus differed significantly among populations of White Sands pupfish (Stockwell et al. 1998).

Salinity and temperature for White Sands pupfish habitats have been recorded on an approximately quarterly basis by New Mexico Department of Game and Fish (NMDGF) beginning in December 1994 (Table 1). Temperature and salinity varied considerably within and among habitats. Temperature variation was mostly seasonal and comparable for the four locations, whereas differences in habitat salinity were substantial. Lost River and Salt Creek exhibited consistently high salinity and Mound Spring consistently low salinity. Malpais Spring exhibited an increasing gradient in salinity from the springhead to the lower ephemeral pools.

All habitats except Malpais Spring were fragmented by natural or man-made barriers (Fig. 1). Salt Creek was fragmented into three sections by a waterfall near the upper end and a culvert at Range Road 316 (Fig. 1). However, during and subsequent to high flows, a side channel may have provided access for fish movement above and below the culvert at Range Road 316 (J. Pittenger, NMDGF, pers. comm.). Lost River had three permanent reaches that were only connected following prolonged precipitation (Fig. 1). A culvert prevented upstream movement of fish to the upper reaches of Lost River. Malpais Spring was not fragmented, but some pools in the lower end of this spring system were ephemeral. Fish occurred throughout Malpais Spring from the upper to lower sampling points. Mound Spring was fragmented by a steep gradient that prevented movement of fish from the lower to the upper pool.

Historical accounts and a recent genetic study have shown that the Mound Spring and Lost River populations were established by translocations from Salt Creek in the 1970s (Stockwell et al. 1998; Pittenger and Springer 1999). It is likely that the founding stocks for introductions to Lost River and Mound Spring were collected at Range Road 316 (middle reach) and/or at Range Road 6 (lower reach). The number of founders for the Mound Spring population is undocumented, but approximately 30 fish were the presumptive founding stock of the Lost River population (Pittenger and Springer 1999).

During 1995 and 1996, fish were collected with minnow

TABLE 1. Minimum, average, and maximum temperatures, salinities, and *Pgdh* allele frequencies in White Sands pupfish for 10 locations.

| Location | Temperature (°C)* | | | Salinity (ppt)* | | | Allele frequency (*Pgdh*) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min. | Avg. | Max. | Min. | Avg. | Max. | $Pgdh^{100}$ | $Pgdh^{91}$ | $Pgdh^{110}$ | *n* |
| Lost River[a] | | | | | | | | | | |
| Lower | 11.00 | 17.58 | 28.00 | 13.50 | 17.25 | 23.00 | 0.840 | 0.050 | 0.110 | 50 |
| Middle | 9.50 | 18.58 | 27.50 | 31.00 | 36.38 | 40.00 | 0.910 | 0.020 | 0.070 | 50 |
| Upper | 11.90 | 17.83 | 22.00 | 23.00 | 27.75 | 40.00 | 0.900 | 0.010 | 0.090 | 50 |
| Malpais Spring[b] | | | | | | | | | | |
| Lower | 3.20 | 16.86 | 29.00 | 5.00 | 9.23 | 21.50 | 0.653 | 0.010 | 0.337 | 49 |
| Upper | 13.20 | 15.71 | 17.60 | 3.50 | 3.76 | 4.00 | 0.449 | 0.000 | 0.551 | 49 |
| Mound Spring[c] | | | | | | | | | | |
| Lower | 8.70 | 18.94 | 27.50 | 2.00 | 3.11 | 4.00 | 0.500 | 0.375 | 0.125 | 48 |
| Upper | 9.40 | 18.15 | 26.30 | 1.50 | 2.94 | 4.00 | 0.560 | 0.380 | 0.060 | 50 |
| Salt Creek | | | | | | | | | | |
| Lower[d] | 3.00 | 14.65 | 27.10 | 10.50 | 16.08 | 21.00 | 0.630 | 0.170 | 0.200 | 50 |
| Middle[e] | 11.50 | 14.79 | 24.00 | 13.00 | 16.47 | 22.80 | 0.680 | 0.120 | 0.200 | 50 |
| Upper[f] | 7.40 | 15.68 | 33.40 | 13.50 | 15.64 | 16.60 | 0.990 | 0.010 | 0.000 | 50 |

* Data were provided by New Mexico Department of Game and Fish. Readings were taken as follows:
[a] 1995: March, June, and October; 1996: January.
[b] 1995: March, June, and October; 1996: January, April, June, and November; 1997: February and May.
[c] 1995: March, June, and October; 1996: January, April, June, and November; 1997: May.
[d] 1995: May; 1996: January, April, August, and November; 1997: February.
[e] 1994: December; 1995: May, June, and October; 1996: January, April, and November; 1997: February.
[f] 1995: April, June, and October; 1996: January, April, August, and November; 1997: February.

traps at 10 localities: lower, middle, and upper fragments of Lost River; lower, middle, and upper fragments of Salt Creek; Malpais Spring 10 m downstream of the springhead (upper) and at the lower end of the marsh complex (lower); and upper and lower pools at Mound Spring (Fig. 1).

Fifty fish per population were prepared for allozyme electrophoresis following methods described by Stockwell et al. (1998). *Pgdh* was scored from liver tissue on horizontal starch gels with the continuous tris citrate (pH 8.0) buffer of Selander et al. (1971). The most common allele was designated 100 and other alleles were designated using their mobility relative to the common allele.

We used BIOSYS-I (Swofford and Selander 1981) to calculate allele frequencies and *F*-statistics. Departures from Hardy-Weinberg expectations were tested via chi-square analyses for all cases where expected numbers were at least three. For allele frequency comparisons, we lumped the rarer alleles into one class.

To evaluate the relationships between environmental variables (salinity and temperature) and *Pgdh* polymorphism we conducted four analyses. First, we regressed the frequency of the common allele $Pgdh^{100}$ against average, minimum, and maximum values for temperature and salinity using all 10 sample sites. Second, we compared allele frequencies of sampling sites within each population. Third, we exploited the historical relationships among populations. Allele frequencies at Mound Spring and Lost River were compared with that of the putative founding stock at Salt Creek by chi-square analysis. We used a mean of allele frequencies of the middle and lower reaches of Salt Creek, the most accessible and most likely source localities of the founding stock, to estimate the allele frequency for the presumptive founding stock. For the introduced populations at Mound Spring and Lost River, we used a mean of allele frequencies of the habitat fragments. Finally, we ran a second series of regressions of the frequency of $Pgdh^{100}$ against minimum, average, and maximum values for temperature and salinity at the population level. For this analysis, we used all data from the Mound Spring and Lost River populations, data from the lower and middle samples for the Salt Creek population, and the upper sample at Malpais Spring. The same patterns were obtained when the lower sample at Malpais Spring was used.

## Results

No significant departures of genotype frequencies from Hardy-Weinberg expectations were observed for *Pgdh* at any of the 10 sample sites. Allele frequency did not differ among the subpopulations at Mound Spring ($\chi^2 = 0.708$, $P > 0.05$) or Lost River ($\chi^2 = 2.78$, $P > 0.05$; Table 1, Fig. 2).

Significant differences in allele frequencies were observed within Malpais Spring ($\chi^2 = 8.24$, $P < 0.005$) and Salt Creek ($\chi^2 = 42.52$, $P < 0.001$; Table 1, Fig. 2). The frequency of $Pgdh^{100}$ differed between upper (0.449) and lower (0.653) reaches of Malpais Spring (Table 1, Fig. 2). The frequency of $Pgdh^{100}$ was 0.630, 0.680, and 0.990 for the lower, middle, and upper reaches of Salt Creek, respectively. Allele frequencies did not differ between the middle and lower reaches of Salt Creek ($\chi^2 = 0.55$, $P > 0.05$), but the upper reach of Salt Creek had significantly different allele frequencies compared to the middle ($\chi^2 = 34.88$, $P < 0.001$) and lower ($\chi^2 = 42.11$, $P < 0.001$) reaches of Salt Creek. The absence of $Pgdh^{110}$ and rarity of $Pgdh^{91}$ in the subpopulation sampled above the waterfall is probably due to either a founding event or a population bottleneck (Table 1, Fig. 2). The $Pgdh^{110}$ allele was present at all sampling sites in Mound Spring and Lost River, but absent in the upper reach of Salt Creek (Table 1). This supports our assumption that the founding stocks for the Lost River and Mound Spring populations originated in either the lower or middle reaches of Salt Creek.

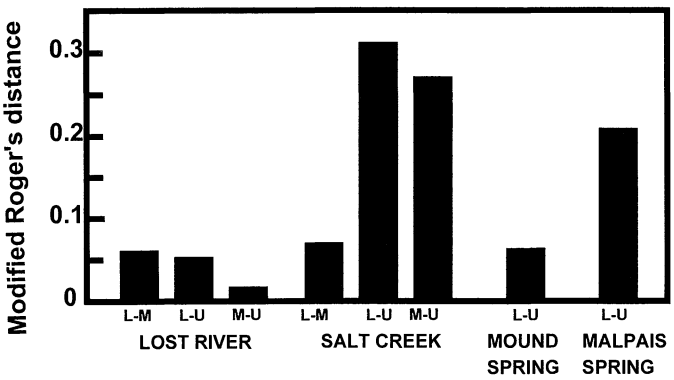


FIG. 2. Pairwise genetic distances among subpopulations within each population. Sample sites were located in the lower (L), middle (M), and upper (U) sections of these habitats.

The frequency of $Pgdh^{100}$ was correlated with minimum, average, and maximum salinity ($r^2 = 0.640$, $P = 0.005$; $r^2 = 0.627$, $P = 0.006$; and $r^2 = 0.566$, $P = 0.012$, respectively). In contrast, $Pgdh^{100}$ frequency was not correlated with minimum, average or, maximum water temperature ($r^2 = 0.000$, $P = 0.960$; $r^2 = 0.005$, $P = 0.839$; and $r^2 = 0.237$, $P = 0.154$, respectively).

Allele frequencies differed significantly between the inferred founding population at Salt Creek ($Pgdh^{100} = 0.66$) and the introduced populations at Mound Spring ($Pgdh^{100} = 0.53$, $\chi^2 = 6.35$, $P < 0.02$) and Lost River ($Pgdh^{100} = 0.88$, $\chi^2 = 37.98$, $P < 0.001$; Fig. 3). The direction of allele frequency changes was consistent with the relationship derived from the regression of $Pgdh^{100}$ and salinity. At Mound Spring where salinity was relatively low, $Pgdh^{100}$ frequency declined, whereas at Lost River where salinity was high, $Pgdh^{100}$ frequency increased.

At the population level, the $Pgdh^{100}$ allozyme was correlated with average salinity ($r^2 = 0.948$, $P = 0.026$) and maximum salinity ($r^2 = 0.962$, $P = 0.019$). A weaker correlation, which was not significant, was observed between $Pgdh^{100}$ and minimum salinity ($r^2 = 0.827$, $P = 0.091$). By contrast, no relationship was observed between $Pgdh^{100}$ and minimum, average, or maximum temperature ($r^2 = 0.109$, $P = 0.670$, $r^2 = 0.063$, $P = 0.749$, $r^2 = 0.458$, $P = 0.323$).

## Discussion

Heterogeneity in environmental salinity represents a potentially important physiological stress for White Sands pupfish and may play a role in maintaining polymorphism at *Pgdh*. Although problems associated with trying to infer natural selection in wild populations have been widely discussed

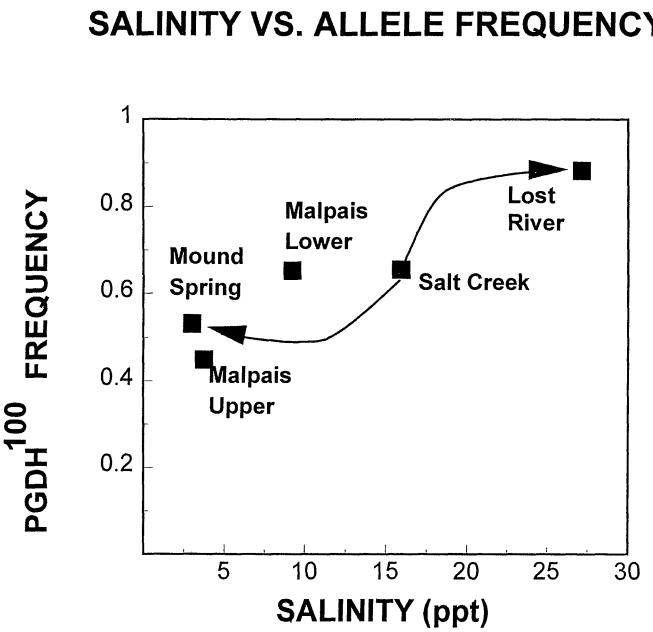


FIG. 3. Frequency of $Pgdh^{100}$ versus mean salinity is shown for Lost River (mean of all three sections), Salt Creek (mean of lower and middle sections), Mound Spring (both pools), and the lower and upper reaches of Malpais Spring. Arrows denote that the Mound Spring and Lost River populations were derived from the Salt Creek population.

(see Endler 1986), an extensive body of literature has accumulated that argues that allozyme polymorphisms may be maintained by habitat heterogeneity (Hedrick 1986; Powers et al. 1991; Vrijenhoek et al. 1992; Mitton 1997). Three pieces of correlative evidence suggest that allele frequencies at *Pgdh* in White Sands pupfish may be related to salinity. First, we observed a correlation between $Pgdh^{100}$ frequency and salinity. This relationship was also evident when we restricted our analyses to the population level. Second, we observed a significant shift in allele frequencies across a salinity gradient at Malpais Spring. This was particularly striking because fish are continuously distributed between the lower and upper sections of Malpais Spring. Third, allele frequencies in populations recently established in high and low salinity environments diverged from the putative founder stock and from each other (Fig. 3). The Lost River and Mound Spring populations were apparently derived from the Salt Creek population (Stockwell et al. 1998) via introductions. The Lost River population was presumably founded in 1970 and the Mound Spring population was founded between 1973 and 1977 (Pittenger and Springer 1999). The frequency of $Pgdh^{100}$ in these two introduced populations was significantly different from the founding stock. Others have argued that shifts in allele frequencies in populations subjected to novel environmental conditions provide evidence for the operation of selection on allozyme polymorphisms (Mitton and Koehn 1975; Smith et al. 1983). Alternatively, random genetic drift could also explain such patterns. Indeed, a small number of founders was used to establish the Lost River population (Pittenger and Springer 1999).

We recognize the lack of independence of some of our arguments for *Pgdh* and salinity in the White Sands pupfish, but this may be unavoidable for a species that occurs in only four locations. The temporal shift in allele frequencies at Lost River and Mound Spring (Fig. 3), and the high genetic distance between the low and high salinity sites at Malpais Spring collectively suggest that selection associated with habitat salinity may operate on *Pgdh* or a tightly linked locus.

*Pgdh* is an important control point in the pentose phosphate shunt (Bewley and Lucchesi 1975; Hughes and Lucchesi 1977; Cavener and Clegg 1981) and evidence from a variety of taxa has been presented to suggest that polymorphism at *Pgdh* may be maintained by selection. Polymorphism for the *Pgdh* locus has been reported to be correlated with latitude in *Drosophila* spp. (Oakeshott et al. 1983; Begun and Aquadro 1994), dark respiration rates in *Lolium perenne* (Rainey et al. 1987), and salinity in *Fundulus heteroclitus* (Powers et al. 1986). Demonstration of a causal relationship between *Pgdh* function and salinity in the White Sands pupfish will require assessment of organismal and cellular function of the alternative genotypes at this locus (Koehn 1978).

Conservation of rare and endangered fish species often requires translocation as a tactic for recovery (Minckley 1995; Stockwell et al. 1996). Indeed, current conservation plans call for the "replication" of strains of White Sands pupfish (Stockwell et al. 1998; Pittenger and Springer 1999). The goal of translocations is to provide a population that serves as a "genetic replicate" as a hedge against extinction. However, our data suggest that translocations may lead to rapid evolutionary responses of the targeted species (Stockwell and Weeks 1999). Data presented for the "replicated" Salt Creek stock at Mound Spring and Lost River suggest that changes in genetic characteristics may arise rapidly following translocation to new environmental conditions. For the *Pgdh* locus these populations display considerable divergence from their Salt Creek founders in fewer than 60 generations.

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