Evolution, 37(4), 1983, pp. 690-700

GENIC VARIATION AND DIFFERENTIATION OF REMNANT NATURAL POPULATIONS OF THE DESERT PUPFISH, *CYPRINODON MACULARIUS*

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Received April 15, 1982. Revised August 26, 1982

The fish fauna of the southwestern American deserts has attracted wide attention because it is a fauna of relict populations isolated by harsh terrestrial environments. Most attention has focused on "pupfishes" of the genus *Cyprinodon* (family Cyprinodontidae) among which isolation, presumably coupled with both natural selection and stochastic forces, has seemingly led to the development of a large number of morphologically distinctive populations. Many of these are recognized as different species (reviewed in Miller, 1981). Pupfishes of the Death Valley region and geologically related waters (the Colorado River and its affluents and associated drainages) offer the most distinctive examples of rapid (frequently post-Pleistocene) divergence (Miller, 1948, 1950).

The only biochemical genetic study of the pupfishes of the Death Valley region and the Colorado basin is that of Turner (1974). That study compared described species; intraspecific or interpopulation variation was not addressed. Thus, despite environmental, life history, and/or breeding structure differences which might influence genetic variation among conspecific pupfish isolates, few genetic data are available.

This paper presents the results of an intensive allozyme survey of remnant populations of the desert pupfish, *C. macularius*. The data reveal that intraspecific differentiation is at the low level of the interspecific differentiation noted earlier, and suggest that the role of geographic isolation per se in fostering the differentiation of relict fish populations may have been overestimated.

Distribution and History of the Desert Pupfish

Modern *Cyprinodon macularius* is probably a direct descendant of the pupfish that lived along the shores of the Bouse Embayment, an extensive Mio-Pliocene estuary extending from the Gulf of California (Miller, 1981; Smith, 1981). Historically, the species enjoyed a broad distribution, ranging throughout the lower Colorado River and its affluents. Its historical distribution can be divided into four areas (Fig. 1; see also Miller, 1979; Black, 1980). 1. Swamps, springs, small streams ("sloughs") and similar habitats in and near the lower Colorado itself. Along the river proper the species may have ranged as far north as Blythe, California, and south to the Colorado delta, where it was abundant until the 1950's. 2. A large number of isolated springs (and their distributary streams) in the Colorado desert west and east of the Colorado River in California, northeastern Baja California, and Sonora. This area included springs in or bordering depressions such as the Laguna Salada and the Salton Sink. The species has been recorded from springs as far north as Indio, California. These spring populations were probably relicts of those in Lakes Le Conte and Pattie, Wisconsinan waters intimately associated with the lower Colorado (Miller, 1981), but some may have been older. 3. Springs and sloughs tributary to the Gila River and its affluents at least as far upriver as Tucson, Arizona. A population

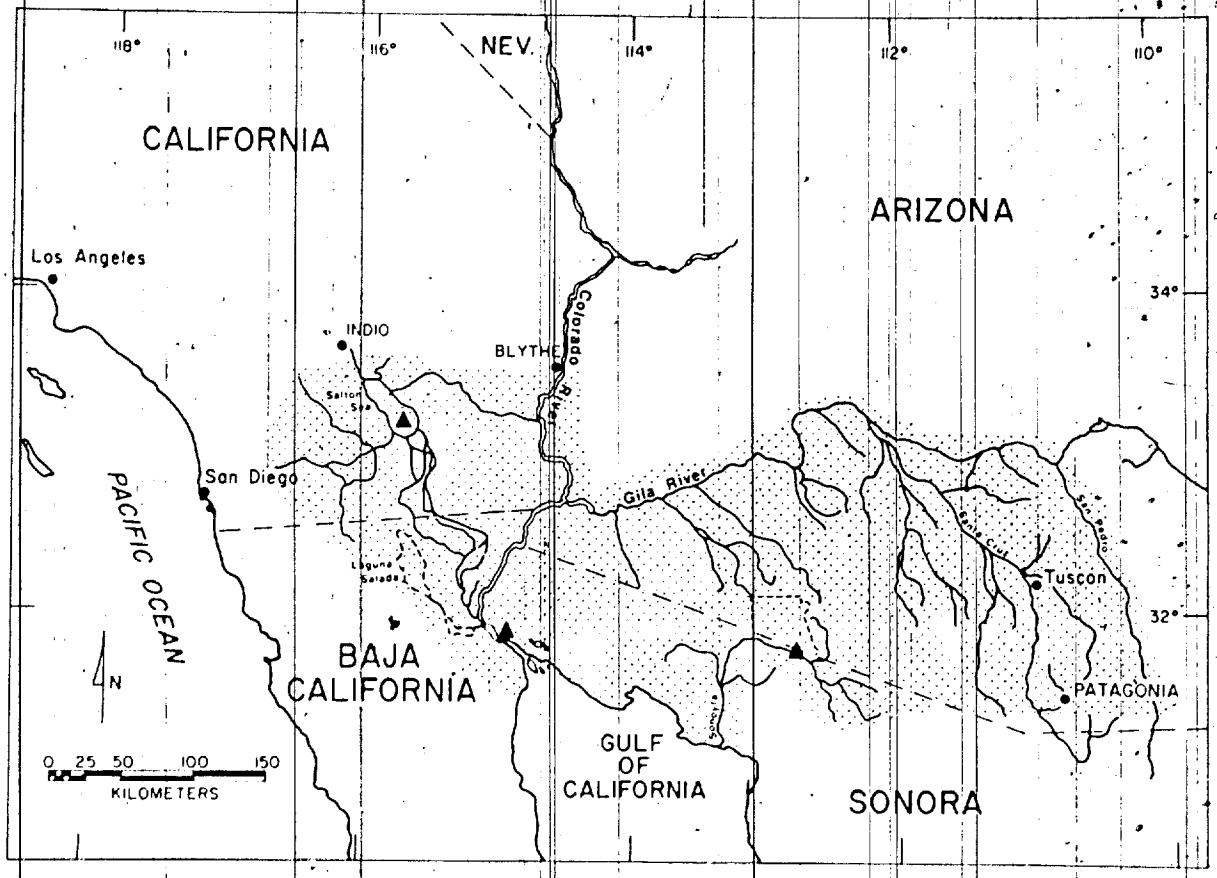

FIG. 1. Approximate historical distribution of *Cyprinodon macularius*. Map depicts extent of range only; a continuous distribution is not implied. Triangles designate sites of remnant natural populations.

further upriver at Monkey Springs near Patagonia, Santa Cruz County, Arizona was morphologically differentiated from other Gila populations (W. L. Minckley, pers. comm.). 4. Similar habitats in the Río Sonoyta basin of northwestern Sonora, Mexico and extreme southern Arizona. The Río Sonoyta is a deltaic affluent of the Colorado whose mouth has been shifted southward by lava flows from the nearby Sierra Pinacate Volcanic Field (Ives, 1936, 1964). This shift has been dated as "Pleistocene" (e.g., Hubbs and Miller, 1948), but the geochronology is complex (Donnelly, 1974). Significant diversion of the Sonoyta probably did not start until roughly 50,000–100,000 years ago (Donnelly's Group III eruptions) and may not have been complete until 13,000–17,000 years ago (M. F. Donnelly, pers. comm.).

Cyprinodon macularius has been extirpated from most of its historical range by human activity. Remnant natural (or quasinatural) populations are as follows:

1. Santa Clara slough, Sonora, Mexico near the delta of the Colorado River (R. R. Miller and W. L. Minckley, pers. comm.).
2. Quitobaquito Springs, Organ Pipe Cactus National Monument, Arizona. The springs are on low volcanic ridges that form the northern boundary of the Sonoyta valley. The outlets of the springs flowed into the adjacent Río Sonoyta in historical times, but were diverted for agriculture by the Papago Indians (Ives, 1936). The status of populations elsewhere in the Sonoyta basin is not presently known in detail, but at least some have apparently been eliminated by agricultural activity. The Quitobaquito pupfish are morphologically differentiated from other Sonoyta populations (R. R. Miller, pers. comm.).
3. Several shoreline pool habitats and one natural spring area around the Salton Sea, Imperial and Riverside counties,

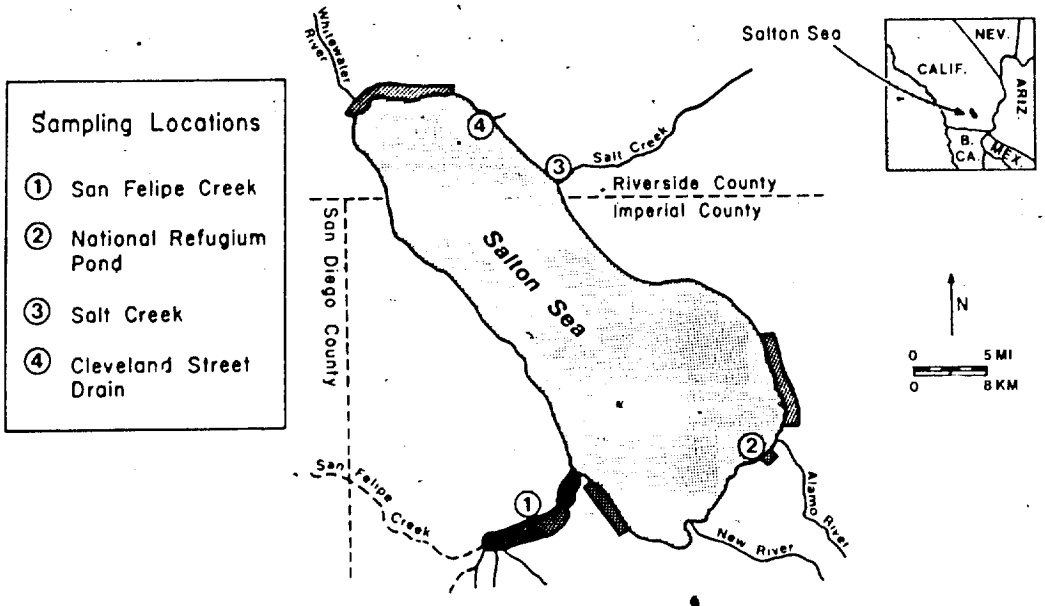

FIG. 2. Distribution of Salton Sea samples, locality designations as in Materials and Methods.

California. These populations have a special history: the Salton Sink was dry in historical times, though a natural lake, Holocene Lake Cahuilla, existed in the Sink in the relatively recent past, and Wisconsinan Lake LeConte also occupied the same area. From 1905 to 1907 the Colorado River broke through irrigation channels and filled the Salton Sink to form a lake, the Salton "Sea." The new lake flooded or connected springs containing pupfish in the sink area and these, possibly along with stock from the Colorado itself, became the progenitors of its *C. macularius* population. Pupfish were common in the Salton Sea within about 15 years of its flooding, and possibly even sooner, (Thompson, 1920) and were abundant shortly thereafter (Coleman, 1929). High population densities persisted through the early 1960's (Cowles, 1934; Barlow, 1958, 1961; Walker et al., 1961). For example, Barlow (1961) estimated that there were about 10,000 juvenile *C. macularius* in a single shoreline pool. The lake populations contracted markedly in the late 1960's (R. K. Liu and Turner, observ. 1967, 1968; Crear and Haydock, 1970), apparently in response to the introduction of exotic species, and with the exception of the four surviving populations, pupfish are no longer a significant component of the Salton Sea fish fauna. The present shoreline populations are thus relicts of a marked population "crash" that followed a rapid increase in the early part of the century. The progenitor populations of the lake were presumably a mixture of spring populations that were themselves relicts of one or more previous lacustrine connections of the sink area with the Colorado River. Given the geology of the area, some may have had a history of several rounds of isolation and expansion into new lake environments.

MATERIALS AND METHODS

Specimens were collected from six locations as follows: Salton Sea area: 1. San Felipe Creek (13 males, 13 females). 2. U.S. National Wildlife Service Refrigium Pond (19 males, 26 females); this is probably the largest remnant Salton Sea population. 3. Salt Creek (27 males, 19 females). 4. Cleveland Street drain, Colorado delta area: "Sta. Clara slough" (13 males, 13 females). Rio Sonoyta basin: Quitobaquito Springs, Organ Pipe Cactus National Monument (9 males, 17 females). The four locations from the Salton Sea are mapped in Figure 2. The San Felipe creek ecosys-

tem is the only known natural Colorado desert spring pupfish habitat still extant. It is now a tributary to the Salton Sea, at least in periods of heavy rainfall; the other Salton Sea samples are remnants of the once abundant lake population. The "Santa Clara slough" sample is not a direct sample of the Colorado delta population, but is taken from a pupfish refugium population established at the Boyce Thompson Arboretum of the University of Arizona, in Superior (Gila County), Arizona. The approximately 40 progenitors of this population were collected in September, 1976 by W. L. Minckley and R. R. Miller at the Santa Clara slough. Thus, the gene frequencies measured in this sample may be different from those of the original population. The sample locality is placed in quotes in recognition of this caveat.

Fish were air freighted live to the laboratory within 24 hours of collection. Eye, liver and muscle homogenates were prepared and stored at −80 C. Fish size (standard length) averaged 34.4 mm (range 23–57 mm). Allozyme surveys were by techniques outlined in Table 1; these are of wide applicability to cyprinodontoid fishes. The protein products of 38–39 structural gene loci were included in the routine surveys.

Results and Discussion

Proportions of polymorphic loci and average heterozygosities of the six population samples are compared in Table 2. Ten loci (those listed in Table 3 plus *Phi1* and *Pgm*) were polymorphic at the ".99 level" (criterion: variant allele frequency: $q \geq .01$ in at least one population) and eight at the .95 level. A very rare variant of the locus *Ldh2* ($q \leq .004$) was present in two Salton Sea populations; no other very rare alleles were detected. The proportions of polymorphic loci and average heterozygosities of these populations are rather similar, though the National Refugium (sample no. 2) seems to be slightly more variable and heterozygous than the others. That population also had the highest background of "rare" alleles ($.05 > q \geq .01$). The values reported for P (average proportion of polymorphic loci) in all the pupfish samples do not differ markedly from averages compiled from 51 fish species by Nevo (1978); $P = .152$. The pupfish heterozygosity values ($H = .042$) seem slightly lower than Nevo's average of .0513. However, in view of the enormous variances of Nevo's averages (their coefficients of variation are respectively 65% and 66%), a more meaningful comparison of the pupfish data is with related or ecologically similar fish species. The euryhaline killifish, *Aphanius dispar*, is a rough ecological and morphological analog of *Cyprinodon* in the Middle East. Kornfield and Nevo (1976) report the following values for samples from the Dead Sea, Red Sea, and Mediterranean: $P = .152$, $H = .049$, average number of alleles per locus = 1.25. The equivalent data for the pupfish samples from the present study are $P = .152$ to .185, $H = .042$, average number of alleles = 1.18. There is little difference between the two data sets, especially in view of differences in the number of loci surveyed (19 in *Aphanius*; 38–39 in *Cyprinodon*).

Interpopulation Comparisons

Salton Sea Populations.—Four of eight polymorphic loci are significantly heterogeneous in gene frequency among the Salton Sea samples, two at very high levels. At two heterogeneous loci, *Aco* and *Mdh1*, the San Felipe sample is apparently monomorphic while the lake populations contain a variant allele. At the *Mdh1* locus, the significant heterogenity is due to the inclusion of the San Felipe creek data (χ^2 samples 2–4 = 2.03, $d.f. = 2$, $P = .42$) but the heterogeneity at the *Aco* locus persists when the San Felipe creek data are eliminated (χ^2 samples 2–4 = 13.01, $d.f. = 2$, $P < .005$). The presence of secondary alleles in the lake samples that are absent in the San Felipe creek population probably results from polymorphism within or differentiation among original founder stocks rather than mutations since the Sink was flooded, for both secondary alleles are present in the "Santa Clara slough" and

TABLE 1. *Presumptive loci, encoded proteins, their tissue distributions and analytical systems used for allozyme surveys in cyprinodontoid fishes.*

Locus	Protein	Tissue distribution[1]			Analytical systems[2]
		Eye	Liver	Muscle	
Aco	Aconitase[3]		X*	X	1
Ada	Adenosine deaminase		X	X*	4
Adh	Alcohol dehydrogenase[3]		X		1 or 4
Agp	Alphaglycerophosphate dehydrogenase (=Glycerol-3-phosphate dehydrogenase)		X	X	1
Cpk1	Creatine (phospho) kinase-1[3]	X			4
Cpk2	Creatine (phospho) kinase-2			X	4
Dip1	Dipeptidase-1[4]	X	X*	X	3
Dip2	Dipeptidase-2[4]	X	X*	X	3
Fum	Fumarase (Fumarate hydratase)[3]			X	4
Gdh	Glucose dehydrogenase (=Hexose-6-phosphate dehydrogenase)[3]		X		4
Gld1	Glyceraldehyde-3-phosphate dehydrogenase-1	X*	X	X	4
Gld2	Glyceraldehyde-3-phosphate dehydrogenase-2	X			4
Got1	Glutamate-oxaloacetate transaminase-1[5] (=Aspartate aminotransferase)	X	X	X*	4
Got2	Glutamate oxaloacetate transaminase-2[5]		X		4
Got3	Glutamate oxaloacetate transaminase-3[5]	X	X	X*	4
Idh1	Isocitrate dehydrogenase-1			X	1
Idh2	Isocitrate dehydrogenase-2		X		1
Ldh1	Lactate dehydrogenase-1	X			4
Ldh2	Lactate dehydrogenase-2	X*	X	X*	1, 2 or 4
Ldh3	Lactate dehydrogenase-3	X*	X	X*	1, 2 or 4
Mdh1	Malate dehydrogenase-1			X	1 or 2
Mdh2	Malate dehydrogenase-2	X	X	X*	1 or 2
Mdh3	Malate dehydrogenase-3	X	X	X*	1 or 2
Mec	Major Eye Carboxylesterase[3]	X	—	—	4
Mlc	Major Liver Carboxylesterase[3]	—	X	X	4
Mor1	Malate oxidoreductase (NADP)-1 (Malic enzyme)			X	1 or 2
Mor2	Malate oxidoreductase-2			X	1 or 2
Mpi	Mannose-phosphate isomerase		X*	X	3 or 4
Par1	Parvalbumin-1			X	5
Par2	Parvalbumin-2			X	5
Par3	Parvalbumin-3			X	5
Pdp	Prolyldipeptidase (=prolidase)[4]	X	X	X	3
Pdg	6-Phosphogluconate dehydrogenase	X*	X	X	4
Pgm	Phosphoglucomutase[3]		X*	X	1 or 2
Phi1	Phosphohexose isomerase-1 (=Glucosephosphate isomerase)		X	X*	1 or 2
Phi2	Phosphohexose isomerase-2		X	X	1 or 2
Sod	Superoxide dismutase[3]	X	X*	X	4
Trp	Tripeptidase[4]		X*	X	3
Xdh	Xanthine dehydrogenase			X	4

[1] In cases of broad tissue specificity, asterisk indicates tissue sources for routine surveys.

[2] Analytical system as follows (horizontal starch gel electrophoresis unless otherwise noted): 1. Electrode buffer: 0.745 M aminopropylmorpholine, 0.04 M citric acid, pH 6.1; gel buffer: 0.0035 M aminopropylmorpholine, 0.002 M citric acid, pH 6.1. 2. Electrode buffer: 0.1 M Trishydroxymethylaminomethane (="Tris"), 0.1 M maleic acid, 0.01 M disodium EDTA, 0.01 M $MgCl_2$, pH 7.4; gel buffer: 1 in 10 dilution of electrode buffer. 3. Electrode buffer: 0.06 M citric acid. 0.0006 M LiOH. 0.003 M boric acid, pH 8.1; gel buffer: 0.0297 M Tris. 0.0594 M citric acid. 0.0006 M LiOH 0.003 M boric acid, pH 8.45. 4. Stock solution: 0.9 M Tris, 0.5 M boric acid, 0.1 M disodium EDTA, pH 8.6; electrode buffer 1 vol stock solution + 6.9 vols H_2O; gel buffer: 1 vol stock solution + 24 vol H_2O. 5. Polyacrylamide gel electrophoresis in Ornstein-Davis discontinuous buffer system: 16% (total monomer) gels, 2.5% bisacrylamide crosslinker.

[3] Other proteins with identical or overlapping enzymatic activities are frequently detected, but are not included in routine surveys. In the case of creatine phosphokinases, these include adenylate and pyruvate kinases.

[4] Four oligopeptidases are surveyed. Dipeptidases 1 and 2 are routinely detected with leucylalanine or leucylglycine. They have broad substrate specificities. In some species dipeptidase 1 has an apparent preference for leucyltyrosine; in others, dipeptidase 2 preferentially hydrolyzes glycylleucine. Prolyl dipeptidase is specifically detected with leucylproline. Tripeptidase is detected with leucylglycylglycine, but hydrolyzes several other leucyltripeptides.

[5] There are two *Got* loci that encode cytosolic enzymes that are present in cyprinodontoid fishes. *Got2* is active only in liver, sometimes to the exclusion of *Got1*. *Got1* is active in all other tissues, and in the liver of some species. When both cytosolic *Got* loci are active in liver, their products sometimes form a heterodimer. In a few species, the products of the *Got2* and *Got3* ("mitochondrial") loci comigrate near the origin, leading to confusion if one or both are polymorphic.

TABLE 2. *Genic variation and heterozygosities in samples of six populations of* Cyprinodon macularius.

	Salton Sea[1]				"Santa Clara slough"[2]	Quitobaquito
	1	2	3	4		
$P_{0.99}$[3]	.10	.26	.23	.23	.16	.13
$P_{0.95}$	.10	.18	.18	.16	.16	.13
$\bar{H}$	.037	.053	.047	.042	.038	.037

[1] See Fig. 2 for locality designations.
[2] Computed with a total of 38 loci, all others with 39 loci.
[3] Abbreviations: $P_{0.99}$ = proportion of polymorphic loci; $P \leq .99$. $P_{0.95}$ = proportion of polymorphic loci; $P \leq .95$. $\bar{H}$ = average heterozygosity per individual per locus.

Quitobaquito samples, and these were never connected to the Salton Sea. Of the four loci that are heterogeneous among the Salton Sea samples, three, *Aco, Ada* and *Xdh,* are significantly heterogeneous in samples from the lake itself.

"Santa Clara Slough" Populations.—Comparison of this sample with those from the Salton Sea reveals statistically significant heterogenity at four loci, *Aco, Ada, Ldh3,* and *Phi2*. The first two loci are heterogeneous among the Salton Sea samples alone. At the *Aco* locus, quite heterogeneous among the Salton Sea samples, inclusion of the "Santa Clara slough" data *decreases* the coefficient of variation from 7% to 5.6%; i.e., the Salton Sea samples are somewhat more differentiated from one another than they are, on the average, from that from the Santa Clara slough. At the *Ada* locus, the relative proportions of the *Ada-80* and *Ada-100* alleles are reversed in the Santa Clara slough sample; its inclusion in the analysis raises the coefficient of variation from 2.3% to 12%, and reduces the probability level to <.005. At the *Ldh3* and *Phi2* loci the Salton Sea samples are not significantly heterogeneous, but at both loci the frequencies of secondary alleles are significantly higher in the "Santa Clara slough" population.

Quitobaquito Sample.—Comparison of the Quitobaquito population data to those from the Salton Sea area reveals seven loci with statistically heterogeneous gene frequencies. At four of these loci, *Aco, Ada, Mdh1* and *Xdh,* the Salton Sea samples are themselves heterogeneous. At the *Aco* locus, the comparison with the Quitobaquito sample has the same result as that from the Santa Clara slough; the Salton Sea samples are so heterogeneous that inclusion of the Quitobaquito sample actually reduces the overall coefficient of variation. The picture is somewhat similar at the *Xdh* locus: the Salton Sea samples are quite heterogeneous (frequency of the *Xdh-100* allele varies from .27 to .64) and addition of the Quitobaquito sample only increases the coefficient of variation from 3.2% to 4.0%. At the *Adh* and *Mdh1* loci, however, addition of the Quitobaquito sample radically alters the heterogeneity. A minor allele (*Mdh1-87*, $q = .06$) at the *Mdh1* locus in the Salton Sea samples is the major allele in the Quitobaquito sample ($q = .9$). At the *Ada* locus, the allele *Ada-100,* the most frequent in the Salton Sea samples ($p = .63$), is a relatively minor one ($p = .1$) in the Quitobaquito sample, and the third allele, *Ada-64*, unique to the Quitobaquito sample, is in fact predominant ($p = .48$). Thus, though the Salton Sea samples are heterogeneous at these loci, the Quitobaquito sample markedly increases both the coefficient of variation and the level of statistical significance. The Quitobaquito sample is also divergent at three polymorphic loci, *Ldh3, Mpi,* and *Phi2,* that are not heterogeneous within the Salton Sea samples. At the *Ldh3* and *Phi2* loci, the Quitobaquito sample is monomorphic, while the Salton Sea populations contain variant alleles ($q = .1$ and .07, respectively); at the *Mpi* locus the comparison is reversed; the frequency of a minor allele is higher in the Quitobaquito sample.

"Santa Clara Slough" versus Quitobaquito.—The two samples are statistically distinct at four polymorphic loci. At each of these one or both are also distinct from the Salton Sea samples, and at two, the latter are themselves heterogeneous.

The most important polymorphic loci and their geographic differentiation are summarized in Figure 3. Genetic similarity and distance measures (Nei, 1975) are presented in Table 4. The general picture

TABLE 3. *Genic differentiation among samples of six populations of* Cyprinodon macularius.

Locus and allele		Salton Sea[1] 1	2	3	4	$\bar{p}_1$[2]	CV[3]	χ^2_1[4]	d.f.	P	"Sta. Clara slough"	$\bar{p}_2$	CV
Aco	*71*	1.0	.84	.68	.87	.89	7%	27.3	3	<.005*	.96	.90	5.6%
	100	—	.16	.32	.13						.04		
	(N)	(27)	(45)	(45)	(53)						(26)		
Ada	*64*	—	—	—	—	.63	2.3%	11.3	3	.01*	—	.52	12%
	80	.54	.35	.26	.36						.85		
	100	.46	.65	.74	.64						.15		
	(N)	(26)	(44)	(43)	(51)						(26)		
Idh2	*89*	1.0	.97	1.0	.94	.99	1.6%	4.77	3	.25	1.0	.99	1.4%
	100	—	.03	—	.06						—		
	(N)	(27)	(45)	(45)	(53)						(26)		
Ldh3	*57*	.87	.87	.91	.94	.90	0.4%	3.75	3	.36	.79	.88	8.0%
	100	.13	.13	.09	.06						.21		
	(N)	(27)	(45)	(45)	(53)						(26)		
Mdh1	*87*	—	.12	.14	.08	.94	3.2%	8.83	3	.033*	.08	.93	2.5%
	100	1.0	.88	.86	.92						.92		
	(N)	(26)	(45)	(46)	(51)						(26)		
Mpi	*95*	—	.06	.02	—	.99	1.5%	5.27	3	.20	.08	.99	1.3%
	100	1.0	.94	.98	1.0						.92		
	(N)	(27)	(45)	(45)	(51)						(26)		
Phi2	*85*	.92	.91	.92	.95	.93	0.1%	—	—	n.s.	.57	.87	4.5%
	100	.08	.09	.08	.05						.43		
	(N)	(27)	(45)	(45)	(49)						(26)		
Xdh	*84*	.64	.64	.27	.39	.57	3.2%	17.6	3	<.005*	n.d.[6]	—	—
	100	.46	.46	.73	.61								
	(N)	(25)	(45)	(45)	(51)								

that emerges from these comparisons is essentially a geographically nested or hierarchical one. The Salton Sea populations are distinctive at some loci; the Santa Clara slough sample is, on the average, more distinctive from them than they are from one another, and the Quitobaquito sample is additionally distinctive. This general picture is illustrated by the cluster analysis presented in Figure 4.

Geographic isolation, because it eliminates the homogenizing effects of gene flow, has long been regarded by many biologists as an evolutionary event of great consequence. This view is vividly expressed by M. J. D. White (1978 p. 107): "There can be no doubt about the reality of allopatric speciation; if populations are geographically isolated for a long enough period of time, they *will* [White's italics] evolve into different species." Western pupfish populations, most especially those in desert springs, are certainly geographically isolated. This isolation has been repeatedly invoked as a major (and sometimes the only) factor causing the divergence of these populations (Hubbs and Miller, 1948; Miller, 1948, 1950, 1981; Liu, 1969; Turner, 1974; Soltz and Hirshfield, 1981). The samples of *C. macularius* compared here represent three isolated groups of populations: Colorado desert (Salton Sea), the Colorado River (Santa Clara slough) and the Sonoyta River (Quitobaquito). Two of them, San Felipe Creek and Quitobaquito Springs, are from archetypical isolated desert spring habitats. The average genetic similarity (I_N) among the three population groups is .970 (range .961–.986). This value is in good agreement with the values reported in the literature for *within-drainage* comparisons among freshwater fish populations, where there is presumably little or no isolation. For example, Avise and Smith (1974) report similarity values (I_R) of .938–.990 ($\bar{x}$ =

TABLE 3. *Continued.*

χ^2_2	*d.f.*	*P*	Quitobaquito	$\bar{p}_3$	*CV*	χ^2_3	*d.f.*	*P*	χ^2_4	*d.f.*	*P*	P_4	*CV*
37.5	4	<.005*	.87 .13 (26)	.88	4.7%	28.8	4	<.005*	1.95	1	.26	.90	4.6%
53.8	4	<.005*	.48 .42 .10 (26)	.51	15%	165	8	<.005*	27.9	2	<.005*	.46	17%
—	—	n.s.[5]	1.0 — (26)	.99	1.4%	—	—	n.s.	—	—	n.s.	.99	1.3%
5.74	1	.018*	1.0 — (26)	.93	2.5%	5.55	1	.017*	10.2	1	<.005*	.92	2.9%
9.21	4	.058	.90 .10 (26)	.81	21%	186	4	<.005*	71.1	1	<.005*	.83	21%
0.69	1	.46	.10 .90 (26)	.98	2.1%	6.26	1	.012*	3.36	1	.071	.99	2.0%
50.6	1	<.005*	1.0 — (52)	.95	1.7%	4.11	1	.044*	27.9	1	<.005*	.91	5.8%
—	—	—	.17 .83 (26)	.62	4.9%	29.9	4	<.005*	—	—	—	—	—

[1] See map, Fig 2, for locality designations.
[2] $\bar{p}_1$ = mean freq. Salton Sea samples. $\bar{p}_2$ = mean freq. Salton Sea and Sta. Clara slough samples. $\bar{p}_3$ = mean freq. Salton Sea and Quitobaquito samples. P_4 = overall mean freq.
[3] *CV* = Coefficient of variation (standard deviation as % of mean value, calculated with the arc sine $\sqrt{p}$ transform).
[4] χ^2_2 = heterogeneity χ^2, Salton Sea samples. χ^2_2 = heterogeneity χ^2, Salton Sea and Sta. Clara slough samples. χ^2_3 = heterogeneity χ^2, Salton Sea and Quitobaquito samples. χ^2_4 = heterogeneity χ^2, Sta. Clara slough and Quitobaquito samples.
[5] n.s. = not significant by inspection.
[6] n.d. = no data.
[7] Statistically significant heterogeneity.

.967) for comparisons of the bluegill (*Lepomis macrochirus*), and Brett (1981) reports similarities (I_N) of .950–.997 ($\bar{x}$ = .984) and .980–.983 ($\bar{x}$ = .988) respectively for within-drainage population comparisons of *Poecilia mexicana* and *P. sphenops*. The range of similarity values for *C. macularius* is also similar to those which characterize broadly distributed fish species such as brook trout (.973–.995, Stoneking et al., 1981), cutthroat trout (.963–.990, Loudenslager and Gall, 1980) and milkfish (.986–1.00, Winans, 1980).

There is therefore no evidence that geographic isolation has had a profound effect upon the divergence of *C. macularius* populations. This is even more apparent if the slight differentiation of the *Cyprinodon* populations is compared to the divergence which has occurred among populations and species of the fossorial mammal genus *Geomys* in a similar time period (Penney and Zimmerman, 1976). Genetic similarities among *Geomys* isolates range from .514 to .735, far lower than those of *Cyprinodon*. Similarly, the similarity values for the pupfish samples are well above most values reported by Yanev and Wake (1981) from comparisons of isolated relict populations of a desert salamander, *Batrochoseps campi* (I_N = .887). Nominally interspecific comparison of Death Valley pupfish yield values similar to the interpopulation values reported here for *C. macularius* (Turner, 1974). In fact, there is apparently more differentiation among

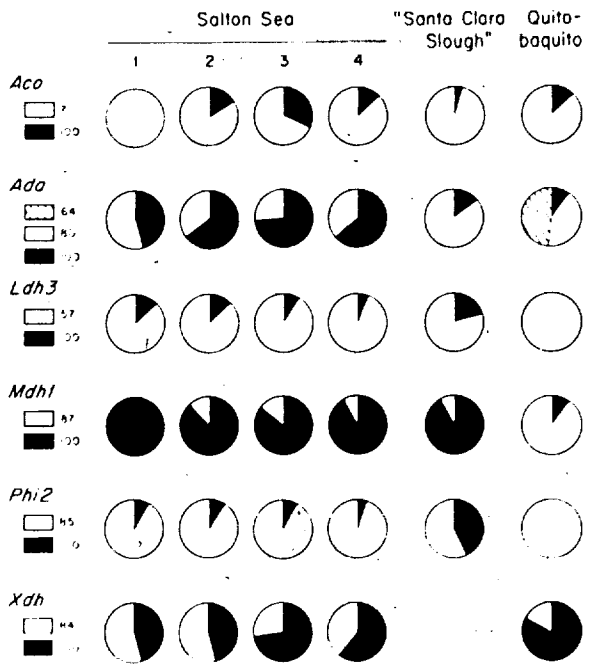

FIG. 3. Population frequency pie diagrams of the most variable loci in samples of *Cyprinodon macularius*.

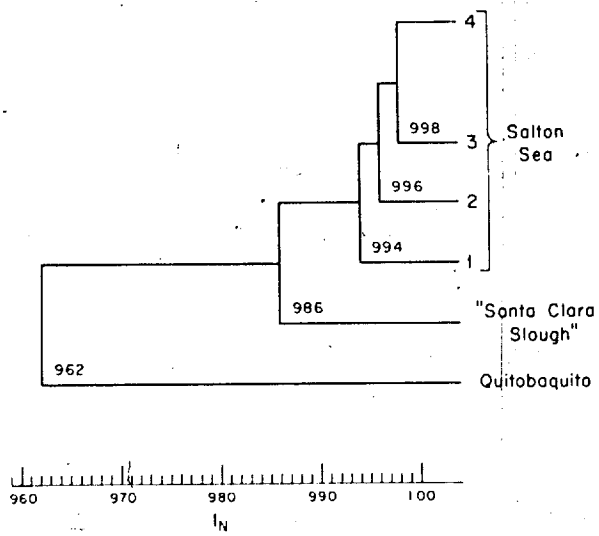

FIG. 4. Cluster analysis of genic similarity values among six remnant populations of *Cyprinodon macularius*. Clustering was by the weighted pair group method, using averages. The values at each branch indicate the mean similarity of the population joining the cluster with all previously clustered populations.

conspecific populations of the widely distributed coastal and insular Atlantic species *Cyprinodon variegatus* (Darling, 1976) than there is among desert pupfish isolates. If allozyme techniques validly survey a representative proportion of the genome, the role of isolation per se in the evolution of the western pupfishes has been greatly overestimated. It is possible that effective population sizes of the pupfish isolates have not been consistently low enough for isolation to have had the profound effects attributed to it, or that the time intervals involved have been too short.

SUMMARY

Six remnant natural populations of the desert pupfish, *Cyprinodon macularius*, a formerly broadly distributed Colorado basin species, have been surveyed for allozyme variation at the products of 38–39 structural gene loci. The samples included four populations from the Salton Sea area (including one from an adjacent relatively pristine natural desert spring habitat), one from the Sonoyta basin (Quitobaquito Springs), and one ultimately from near the delta of the Colorado River. The comparison revealed the following: 1. Mean heterozygosity values ($\bar{H}$) are within the range reported by others for ecologically com-

TABLE 4. *Genetic similarity (above diagonal) and distance (below diagonal) measures (Nei, 1975) among six population samples of the desert pupfish.*

	Salton Sea				SCS	Qto
	1	2	3	4		
Salton Sea						
1. San Felipe Creek	X	.997	.989	.996	.992	.959
2. National Refugium Pond	.003	X	.994	.998	.986	.959
3. Salt Creek	.011	.006	X	.998	.986	.963
4. Cleveland Street Drain	.004	.002	.002	X	.985	.963
"Sta. Clara slough" (SCS)	.008	.014	.020	.015	X	.964
Quitobaquito (Qto)	.042	.042	.038	.038	.037	X

parable *Aphanius* populations, and are not strikingly low. 2. Eight loci display statistically significant differences in gene frequency. Differences are detectable among Salton Sea populations, and among all three geographic areas. The general pattern of the differentiation is temporally (and geographically) hierarchical, and is consistent with previous perceptions of the relationships of the populations based on morphology. 3. The overall level of differentiation is low. The minimum genetic similarity (I_N) measured was .959, and the average (between areas) is .970. These values are in the range of within-drainage population comparisons in other teleosts. The effect of geographic isolation per se on the divergence of pupfish populations has almost certainly been overestimated.

Acknowledgments

This research was supported by a contract from Endangered Species Program of the California Department of Fish and Game. I thank L. Courtois and G. Black of that agency for the collection of specimens and for information on the recent history of the Salton Sea populations and others from California. R. R. Miller and W. L. Minckley provided information on the Santa Clara slough and Quitobaquito populations. J. W. Hawley and especially M. F. Donnelly provided useful insights on the geochronology of the Rio Sonoyta and the Pinacate volcanic field. The manuscript was improved by comments by R. Andrews, L. Courtois, B. Wallace, and M. White. The expert technical assistance of Ms. Carolyn Harris is gratefully acknowledged.

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