GENE FLOW AND POPULATION DIFFERENTIATION IN TWO SPECIES OF GOODEID FISHES (CYPRINIDONTIFORMES: GOODEIDAE)

by

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(ABSTRACT)

The role of gene flow in population differentiation was examined by electrophoretic analysis of populations of two species of goodeid fishes, Goodea atripinnis and Chapalichthys encaustus, from lakes and streams on the Mesa Central of Mexico. Microgeographic differentiation was observed among continuous stream populations of Goodea. Highly significant genic heterogeneity was exhibited among continuous lacustrine populations of both species. Levels of differentiation (based on a genetic distance coefficient) among populations of G. atripinnis in Lake Chapala were similar to levels among populations from a number of isolated drainages.

These results suggested that population continuity and gene flow do not necessarily imply genetic continuity and allele frequency homogeneity. Neighborhood effects (population subdivisions due to behavioral constraints such as homing or low vagility) were proposed as contributing to

reductions in gene flow among populations from lakes and streams, but at least in the case of Goodea were not of major importance. Data from Lake Chapala for both species lended support to intralacustrine or sympatric models of lacustrine species flock evolution.

Population comparisons of Goodea from a number of drainage systems suggested that a simple time-since-divergence model was insufficient to explain the observed patterns of genetic variation. Local effects (drift, bottlenecks, selection) were proposed as important mediators of genetic variation and population differentiation. It is suggested that levels of gene flow much greater than the "one migrant" rule would still permit differentiation of populations in the absence of selection.

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Chapter 1

General Introduction

Since the advent of gel electrophoresis as a tool in population genetics (Lewontin & Hubby, 1966; Harris, 1966), the number of studies of allozyme variation in natural populations has been enormous. Although this technique has been used to address aspects of geographic variation and systematics (Selander & Johnson, 1973; Avise, 1975; Nevo, 1978), an important question in evolutionary biology, that of gene flow and its effect on population differentiation, has yet to be critically examined.

Controversies concerning the frequency of gene flow (Mayr, 1963; Erlich & Raven, 1969), the size of the migrant population and its effect on population differentiation (Spieth, 1974; Allendorf & Phelps, 1981) have yet to be adequately resolved. Unfortunately, there are few data that directly address these questions (Jackson & Pounds, 1979) though a few studies have suggested that lack of gene flow may have a significant effect on population differentiation (Echelle et al., 1975, 1976). Jackson & Pounds (1979) proposed that comparisons of continuous and isolated populations under similar habitat regimes are needed to determine the effect of gene flow (or its lack) on population differentiation.

The goodeid fish, Goodea atripinnis, a ubiquitous and extremely common species from the Mesa Central region of Mexico, was chosen to address, by allozyme analysis, three aspects of gene flow and population differentiation.

1. The extent of microgeographic differentiation (Chapter 2). Previous studies of allozyme variation in fishes have dealt primarily with populations over a wide geographic range (e.g., Avise & Smith, 1974; Powers & Place, 1978; Winans, 1980). Relatively few have addressed differentiation among populations over quite small areas (Yardley & Hubbs, 1976; Ferguson & Noakes, 1981). Such analyses are clearly needed to determine over what geographic range continuous populations may differentiate, and to allow an understanding of the behavioral and genetic dynamics of continuous populations.

2. Intralacustrine differentiation (Chapter 3). Despite the considerable interest in the evolutionary origins of lacustrine fish species flocks (e.g., The African Rift Lake cichlids, the Lake Baikal cottids) the models proposed for these origins have yet to be tested. Specifically, there is little genetic data to assess the potential for continuous lake populations to undergo differentiation (a prerequisite for a sympatric or intralacustrine mode of speciation).

3. Comparative differentiation within and between drainages

(Chapter 4). In order to assess the effect of gene flow on population differentiation, genetic distances within and between drainages, can be compared. Most studies to date (Echelle et al., 1975; Buth & Burr, 1978; Brett, 1982) demonstrated greater differentiation between drainages than within them and therefore imply a homogenizing role for gene flow.

This dissertation is divided into three major chapters which discuss these aspects of gene flow and population differentiation for two species of fish from the Central Plateau of Mexico. Chapter Two has been accepted for publication in Environmental Biology of Fishes and will appear during the summer of 1983. Chapter Three has been submitted to Copeia and is being reviewed at this time. Chapter Four has recently been submitted to Copeia.

Chapter 2

Microgeographic differentiation in a stream population of *Goodea atripinnis* (Goodeidae) from the Mexican plateau

Introduction

The recent literature contains numerous studies of allozyme variation within and among fish populations, but microgeographic variation has received limited attention. A number of authors have found considerable differentiation among populations over large geographic areas (Avise & Smith, 1974; Powers & Place, 1978; Winans, 1980). Similar levels of differentiation have been observed within relatively limited areas (Echelle et al., 1975; Avise & Felley, 1978; Christiansen et al., 1981) but even these studies have dealt with populations separated by considerable distances (e.g., greater than 50 km). Yardley & Hubbs (1976) found considerable differentiation at two esterase loci in populations of *Gambusia affinis* inhabiting several hundred meters of a stream, however, the biological basis of this differentiation was not addressed. Ferguson & Noakes(1981) hypothesized that differences at an esterase locus among schooling groups of *Notropis cornutus* might be due to a small number of dominant males siring young at different breeding areas. Such social influences on genetic

structure have been documented in populations of the house mouse (Selander, 1970).

This study examines allozyme variation among stream populations of Goodea atripinnis, a ubiquitous goodeid fish from central Mexico, along a 1 km transect. The data reveal a high level of heterogeneity over relatively short distances; the observed differentiation would certainly not have been predicted from current knowledge of the biology of the species.

Though a few authors have dealt with the genus in passing (Hubbs & Turner, 1939; Nelson, 1976; Kingston, 1980), no aspect of the biology of Goodea has been studied in detail. Goodea atripinnis (Fig. 1) is the most ubiquitous goodeid and possibly the most widespread fish of any family on the Mexican plateau. It occurs throughout the Rio Ameca and Rio Lerma-Santiago river systems, including associated endorheic basins, and in all aquatic habitats from small streams and ponds to the largest lakes. In the latter it occurs in enormous schools, frequently far from shore, and is a large part of the commercial catch (though it is not highly desirable). In lakes, diurnal migrations to and from shore are evident but have not been studied in detail. Juveniles appear restricted to inshore habitats. Maximum size of the species is about 150 mm standard length. Preliminary observation indicate that at least in shallow

lakes (like Chapala and Cuitzeo) G. atripinnis is a benthic detritivore, but in some habitats it has been observed grazing on lithophilic algae. Like other goodeids, G. atripinnis is viviparous. Brood sizes average about 30 but are frequently greater in large females. There is considerable variation between populations in some aspects of color pattern and several morphological features, including jaw shape and body depth. Alvarez (1970) who seemed to have been unaware of the existence of Goodea in the Rio Ameca, recognized four subspecies, but field collected material is far more variable than his scheme suggests. The systematic limits of G. atripinnis and related taxa such as G. luitpoldi and G. gracilis remain to be convincingly established.

Materials and Methods

Three samples of G. atripinnis were collected by seine from the Rio Teuchitlan, a small spring fed tributary of the Rio Ameca, Estado Jalisco, Mexico (Fig 2). Fish were frozen on dry ice and stored at -90 C. Extracts of eye, liver and muscle were subjected to standard electrophortic techniques. The products of 36 structural gene loci were surveyed (details in Turner, 1983) and were analysed with the BIOSYS-1 computer program of Swofford & Selander (1981). The vast majority of the fish collected were approximately

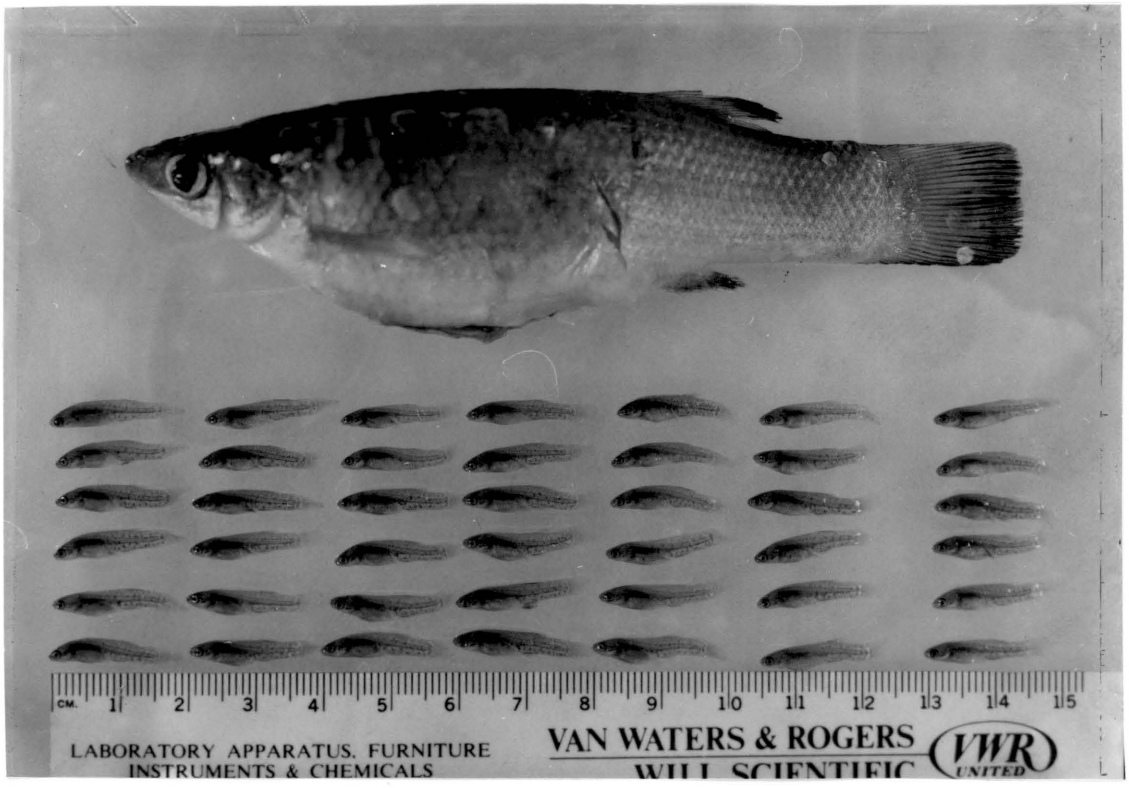


Figure 1: Female Goodea atripinnis and young removed from ovary.

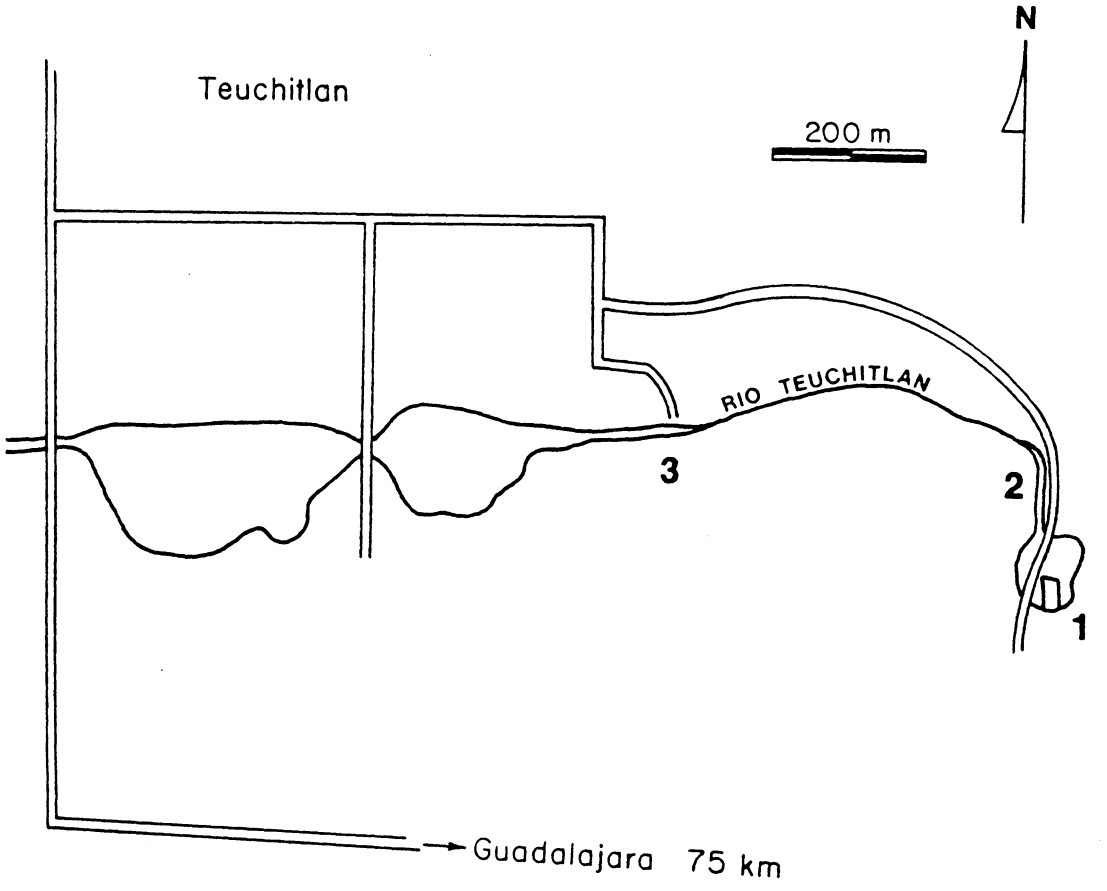


Figure 2: Map of the Rio Teuchitlan (Estado Jalisco, Mexico) showing collection localities.

40-60 mm standard length and at all three localities, the sex ratio did not deviate significantly from 1:1.

Results

Percent polymorphic loci (P_{95}) values increased with distance from the spring (6.1%-12.1%), but P_{99} values indicated a greater number of rare alleles in the spring population (P_{99}=21.7). Average heterozygosities are similar among the three samples and are lower than the average for 82 species of fish reported by Winans(1980)(H=0.048 versus 0.020-0.033 in Goodea) Only two loci exhibit significant departures from Hardy-Weinberg equilibrium, Mec at locality 1(see Fig 2) and Xdh at locality 2. In both cases the departure was due to a deficiency of heterozygotes.

x^2 analysis (Table 1) demonstrates significant genic heterogeneity at 2 of the 7 polymorphic loci, Agp and Mpi. The combined x^2 value is also significant (p=.01). Standardized genetic variances (F_{st}) among samples were calculated for each locus (Table 2). The values ranged from 0.008 for Phi-2 to 0.041 for Agp. Three of these variances(Agp, Mec, Mpi) were significantly different from zero. Nei genetic identities among the three populations were extremely high (I>0.997).

Table 1: Allele frequencies and heterogeneity x^2 values among three samples of *Goodea atripinnis* from the Rio Teuchitlan, Estado Jalisco, Mexico.

	El Rincon N=44	Ditch N=32	River N=35	x^2	df	p
Locus						
Adh-100	0.987	1.000	1.000	1.77	2	0.412
-300	0.013	0.000	0.000			
Agp-100	0.961	0.828	0.939	8.51	2	0.014*
-110	0.039	0.172	0.061			
Mec-100	0.961	1.000	1.000	5.36	2	0.068
- 95	0.039	0.000	0.000			
Mpi-100	0.961	1.000	0.914	7.15	2	0.028*
-110	0.039	0.000	0.086			
Phi-100	0.989	1.000	1.000	1.53	2	0.465
- 80	0.011	0.000	0.000			
Sod-100	0.842	0.922	0.943	5.68	4	0.224
-150	0.013	0.000	0.014			
- 50	0.145	0.078	0.043			
Xdh-100	0.330	0.250	0.386	4.57	4	0.333
- 85	0.523	0.656	0.500			
- 70	0.148	0.094	0.114			
			Total	34.58	18	0.010*

Table 2: Standardized genetic variances and significance levels for Goodea atripinnis from the Rio Teuchitlan.

Locus	F_{ST}
Adh	0.009
Agp	0.041**
Mec	0.027
Mpi	0.036**
Phi	0.008
Sod	0.021
Xdh	0.015

Discussion

The significant heterogeneity in allele frequencies among the three localities from the Rio Teuchitlan indicates a level of microgeographic differentiation previously undetected in presumably continuous fish populations. Environmental gradients sufficient to account for this differentiation among these populations are not at all apparent. Tests of pH (7.2), T^0 (23-26^0 C), NO_3, and PO_4 levels (<1ppm) at each locality suggested quite homogeneous conditions over the section of stream sampled. I therefore suspect that the variation is related to stochastic forces. If this is the case, it follows that the 'neighborhood' size (sensu Wright, 1946) in stream populations of Goodea is probably small.

Even small amounts of gene flow supposidly renders differentiation due to drift unlikely (Spieth, 1974). However, Allendorf and Phelps (1981) have shown that, assuming neutrality, differentiation among populations is possible even in the face of moderate amounts of gene flow, especially if the effective population size is small. They argue that such exchange will maintain the same alleles in each population but not necessarily identical allele frequencies. Though the Rio Teuchitlan is spring-fed and apparently continuous throughout the year, seasonal draw for irrigation may drastically reduce flow at times. The low

water level may result in some degree of population bottlenecking, or at least reduce normal fish movements. If these restrictions were to occur during the breeding season (March-June) small neighborhoods with reduced effective population sizes could result. Unfortunately, little is known of the seasonal flow patterns of the river or the vagility of Goodea in streams. However, Gerking (1953) has suggested that many stream fish species may show restricted movements. Dominance relations among male Goodea may also contribute to reductions in effective population size.

The degree of microgeographic differentiation observed in Goodea from the Rio Teuchitlan may be more characteristic of fish populations than has been previously supposed. Similarly high levels of variation among stream populations have been documented for Poeciliopsis monacha (Vrijenhoek, 1979) and Ilyodon, another goodeid fish (Grudzien & Turner, 1984). Bell (1982) has documented considerable within-stream variation in the morphology of Gasterosteus populations. Collectively, our data and these works suggest that genetic differentiation of ostensibly continuous stream populations may be a general phenomenon. The differentiation emphasizes the need for further work on the behavioral and genetic dynamics of stream fish populations.

Chapter 3

Intralacustrine differentiation in two species of goodeid fishes.

Introduction

The fish faunas of many of the world's largest lakes are characterized by assemblages of related species or "species flocks" (Brooks, 1950). Despite considerable interest in these assemblages, their modes of origin and evolution are both controversial and poorly understood. Several models have been proposed. A multiple invasion hypothesis was proposed by Hubbs (1961) but was later abandoned in favor of other models (Greenwood, 1974). Allopatric models suggest that species arose in small, isolated lakes and came into contact when these lakes were united into a larger basin (Lake Victoria Haplochromis, Greenwood, 1965, 1974 and Lake Baikal cottoids, Brooks, 1950, and Kozhov, 1963). Two other models suggest that species arose through differentiation of local populations within a single lake basin. The "intralacustrine allopatric" model (Fryer & Iles, 1972) is based on isolation of populations in specific habitats (e.g., the Cichlidae of lakes Malawi and Tanganyika). The "intralacustrine sympatric" model, isolation by behavioral modification, has

been suggested for certain African cichlids (Kosswig, 1947), salmonid populations of Lake Ohrid (Stankovic, 1960) and Lake Windemeere (Frost, 1965). Surprisingly, little genetic data are available that allow a genetic assessment of the potential for intralacustrine differentiation of populations.

I address this issue by examining the extent of intralacustrine differentiation among populations of two species of lake-dwelling fish, Goodea atripinnis and Chapalichthys encaustus, from Lake Chapala, Mexico. Both are members of the family Goodeidae, a viviparous family of cyprinodontoid fishes essentially endemic to the central plateau of Mexico. Aspects of the distribution, reproduction and ecology of Goodea have been described elsewhere (Chapter 2). The geographic distribution of C. encaustus centers on Lake Chapala but this species also occurs in streams and lakes of the nearby Rio Grande de Santiago and Rio Ameca drainages. Both species are extremely abundant and apparently continuously distributed around the lake. Adult Goodea exhibit a diurnal migration to deeper water while adult Chapalichthys apparently remain near shore

Lake Chapala is a large (1.1×10^6 ha), shallow (average depth 6-8 m), and highly productive lake. It has a low diversity of habitats, mostly rocky shores but with some

silt-sand shoreline to the west and south. The shallow areas often contain beds of submergent vegetation (Potamogeton). The hydrographic history of Lake Chapala was discussed by Mitchell & Toscano (1965) and Barbour (1973).

Materials and methods

Samples of both species were obtained during June and July of 1980, either by seining or by purchase from local fishermen as they pulled in their nets. These nets were usually set no more than 300 m from shore. Collections were made at the following localities (Figure 3). Numbers in parentheses refer to sample sizes of Goodea and Chapalichthys respectively.

1. Beach 0.5 km west of the town of Chapala (131, 56).

2. Fishing camp 17 km east of the town of Chapala (72, 40).

3. Mouth of the Rio Grande de Santiago in the town of Ocotlan (65, 40).

4. Northeast shore, 3 km east of the town of El Fuerte (56 Chapalichthys only).

5. Rio Lerma at the town of Concuea, approximately 25 km upstream from Lake Chapala (45, 56).

6. Dike east of the town of La Palma, 9 km north of Hwy 15

(36 Goodea only).

7. Beach 1 km west of the town of Palo Alto (54, 56).

8. Beach at the end of Calle Emiliano Zapata in the town of Tizapan el Alto (38, 40).

9. Town of San Luis Soyatlan (79, 52).

10. Beach near the town of Jocotepec (64 Goodea only).

Fish were frozen on dry ice and subsequently stored in the laboratory at -90 C. Eye, liver and muscle extracts were subjected to standard electrophoresis techniques (Turner, 1983). Products of 36 presumptive structural gene loci were surveyed. The survey revealed the following polymorphic loci in both species: adenosine deaminase(Ada), alcohol dehydrogenase(Adh), alpha-glycerophosphate dehydrogenase(Agp), creatine phosphokinase(Cpk-2), dipeptidase(Dip-2), glutamate-oxaloacetate transaminase(Got-1,2), general muscle protein(Gp) (not a creatine kinase or parvalbumin), major eye carboxylesterase(Mec), manose phosphate isomerase(Mpi), 6-phosphogluconate dehydrogenase(6Pg), phosphoglucomutase(Pgm), phosphohexose isomerase(Phi-1,2), superoxide dismutase(Sod), tripeptidase(Trp), Xanthine dehydrogenase(Xdh). Portions of the data were analysed using the BIOSYS-1 computer program of Swofford & Selander

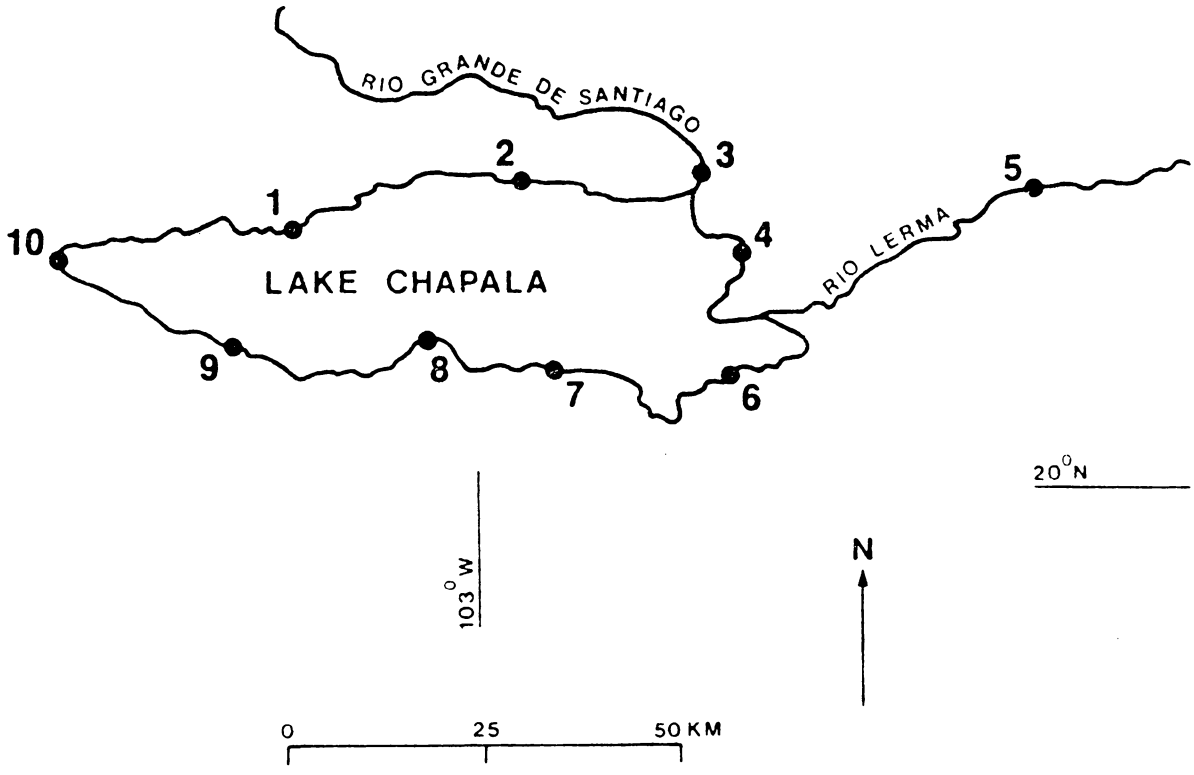


Figure 3: Map of Lake Chapala, Mexico, showing the collection localities of Goodea atripinnis and Chapalichthys encaustus. Refer to the text for number designations.

(1981). Genic heterogeneity was determined by the formula of Workman and Niswander (1970):

$$x^2 = 2N\sigma^2_p/pq$$

where N is the total number of individuals, σ^2_p is the variance in allele frequencies, p and q are mean allele frequencies. Standardized variances (F_{st}) were computed from the equation

$$F_{st} = x^2/2N$$

and adjusted for sampling variance.

Results

Genetic variability estimates for nine populations of Goodea and eight populations of Chapalichthys were uniform within each species but Chapalichthys was approximately half as polymorphic and half as heterozygous as Goodea (Table 3). The estimates for Goodea were conservative because of the loss of resolution of three highly polymorphic loci, Aco, Idh, and Xdh, due to deterioration of enzyme activity in storage.

Of 91 comparisons of observed genotypes with Hardy-Weinberg expectations for Goodea, four deviated significantly ($p<.005$) from equilibrium, based on a x^2 test where rare genotype classes were pooled; Mpi at locality 1,

Cpk-2 at locality 3, Agp at locality 5, Got-1 at locality 9. In all cases, the deviation was due to a deficiency of heterozygotes. Of 50 comparisons for Chapalichthys, four deviated significantly from random mating expectations; Xdh at localities 1, 3, 7, and 8. As in Goodea, heterozygote deficiency was responsible.

Allele frequencies for all populations are presented in Table 4. F statistics and heterogeneity x^2 statistics for each species for Lake Chapala populations only are given in Tables 5 and 6. For Goodea, 6 loci exhibit significant heterogeneity (Adh, Agp, Dip-2, Mpi, Pgm and Trp). The overall x^2 was highly significant ($p<.001$). The standardized variances (F_{st}, adjusted for sampling variance) for these loci are also significant (since the significance of F_{st} is based on the significance of the heterogeneity x^2, Workman & Niswander, 1970). Chapalichthys (Table 4) exhibits significant heterogeneity at five loci (Agp, Dip-2, 6-Pgd, Trp and Xdh). Again, the overall heterogeneity was highly significant ($p<.001$). The standardized variances for these five loci were significant. Although these F_{st} values were statistically significant, they were small (F_{st} for Goodea = .019, for Chapalichthys = .020). F_{st} values are similar to those obtained for bluegill (Lepomis) populations within reservoirs (Avise & Felley, 1979), Helix populations within city blocks, and intrafarm Mus musculus populations

(Selander & Kaufman, 1975).

Nei (1978) genetic identities between populations of both Goodea and Chapalichthys were quite high (I>0.994). These are similar to values obtained for whitefish populations (Imhof et al., 1980). An analysis of correlation between Rogers genetic distance and geographic distance was performed. Goodea exhibited a positive and significant correlation ($r=.43$, 26 df, $p<.05$). For Chapalichthys, the correlation was positive but not significant ($r=.16$, 19 df, $p>.05$).

Discussion

Although Nei similarities and standardized variances suggest considerable homogeneity among populations of Goodea and Chapalichthys in and near Lake Chapala, the number of significantly heterogeneous loci and overall heterogeneity indicate some degree of population differentiation. Although the majority of loci in both species indicate a small heterozygote excess, all significant departures from random mating expectations were due to a heterozygote deficit.

If our samples were taken from a single, large panmictic population, the expected heterogeneity may be estimated by the equation,

Table 3: Genetic variability estimates for populations of *Goodea atripinnis* and *Chapalichthys encaustus* from Lake Chapala, Mexico.

	P_{95a}	P_{99b}	H_c
Goodea	12.5-15.6	18.7-34.4	0.041-0.050
Chapalichthys	5.7-11.4	8.5-22.8	0.014-0.024

[a]percent of loci polymorphic where q>.05

[b]percent of loci polymorphic where q>.01

[c]average heterozygosity

Table 4: Allele frequencies for populations of *Goodea atripinnis* and *Chapalichthys encaustus* from Lake Chapala.
See text for location designations.

Goodea atripinnis

	LOCATION								
LOCUS	1	2	3	5a	6	7	8	9	10
Ada-a	.981	.993	.985	.911	.986	.981	1.00	.981	.969
-b	.019	.000	.008	.089	.014	.009	.000	.000	.023
-c	.000	.007	.008	.000	.000	.009	.000	.019	.008
Adh-a	1.00	1.00	.976	1.00	1.00	1.00	1.00	1.00	1.00
-b	.000	.000	.024	.000	.000	.000	.000	.000	.000
Agp-a	.346	.281	.283	.167	.292	.292	.276	.338	.325
-b	.591	.688	.700	.756	.694	.688	.711	.649	.643
-c	.063	.031	.017	.078	.014	.021	.013	.013	.032
Cpk2-a	.985	1.00	.977	.989	.972	.981	.987	.987	.977
-b	.015	.000	.023	.011	.028	.019	.013	.013	.023
Dip-a	1.00	1.00	.977	.989	.972	.957	.987	.987	1.00
-b	.000	.000	.023	.011	.028	.043	.013	.013	.000
Got1-a	.995	1.00	1.00	1.00	1.00	1.00	1.00	.981	.992
-b	.005	.000	.000	.000	.000	.000	.000	.019	.000
-c	.000	.000	.000	.000	.000	.000	.000	.000	.008
Got2-a	1.00	.993	.992	.933	1.00	1.00	1.00	.987	.992
-b	.000	.007	.008	.067	.000	.000	.000	.013	.008
G3p-a	.995	1.00	.992	.989	1.00	1.00	1.00	1.00	1.00
-b	.005	.000	.008	.011	.000	.000	.000	.000	.000
Mec-a	.817	.809	.746	.591	.694	.778	.803	.709	.711
-b	.099	.127	.146	.239	.222	.093	.132	.165	.133
-c	.042	.015	.054	.091	.028	.037	.013	.019	.047
-d	.042	.049	.054	.080	.056	.093	.053	.108	.109
Mpi-a	.940	.951	.944	.935	.985	.941	.947	.943	1.00
-b	.055	.049	.016	.065	.015	.044	.053	.051	.000
-c	.005	.000	.040	.000	.000	.015	.000	.006	.000

Table 4 continued

Pgm-a	.985	.986	1.00	.956	.986	.963	.974	.961	.945
-b	.015	.014	.000	.044	.014	.028	.026	.039	.031
-c	.000	.000	.000	.000	.000	.009	.000	.000	.023
Phi1-a	1.00	.993	1.00	1.00	1.00	1.00	1.00	.994	1.00
-b	.000	.007	.000	.000	.000	.000	.000	.006	.000
Phi2-a	.905	.910	.954	.922	.917	.972	.921	.930	.898
-b	.017	.014	.000	.022	.000	.000	.013	.000	.031
-c	.074	.076	.046	.056	.083	.028	.066	.070	.070
-d	.004	.000	.000	.000	.000	.000	.000	.000	.000
Sod-a	.902	.903	.927	.913	.897	.897	.934	.943	.929
-b	.013	.014	.016	.000	.000	.015	.000	.019	.008
-c	.000	.021	.008	.065	.015	.015	.039	.013	.024
-d	.085	.063	.048	.022	.088	.074	.026	.025	.040
Trp-a	1.00	.993	.992	1.00	1.00	1.00	.974	1.00	1.00
-b	.000	.007	.008	.000	.000	.000	.026	.000	.000

Chapalichthys encaustus

Location

Locus	1	2	3	4	5a	7	8	9
Adh-a	.955	1.00	.975	.982	.964	.946	.962	.990
-b	.045	.000	.025	.018	.036	.054	.038	.010
Agp-a	.925	1.00	1.00	.955	1.00	1.00	1.00	.933
-b	.075	.000	.000	.045	.000	.000	.000	.067
Dip-a	.982	1.00	.962	.991	1.00	1.00	1.00	1.00
-b	.018	.000	.038	.009	.000	.000	.000	.000
Got2-a	1.00	1.00	.987	1.00	1.00	.991	1.00	.981
-b	.000	.000	.000	.000	.000	.009	.000	.019
-c	.000	.000	.012	.000	.000	.000	.000	.000
Gp-a	1.00	1.00	1.00	.991	1.00	1.00	1.00	.981
-b	.000	.000	.000	.009	.000	.000	.000	.019

Table 4 continued

G3p-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	.990
-b	.000	.000	.000	.000	.000	.000	.000	.010
Mpi-a	1.00	1.00	.987	1.00	1.00	1.00	1.00	1.00
-b	.000	.000	.013	.000	.000	.000	.000	.000
6Pg-a	.982	.925	.962	.973	.955	.920	.987	1.00
-b	.018	.075	.038	.027	.045	.080	.013	.000
Pgm-a	1.00	1.00	.975	.991	1.00	.991	1.00	.990
-b	.000	.000	.025	.009	.000	.009	.000	.010
Phi1-a	.982	1.00	1.00	.982	1.00	1.00	1.00	.990
-b	.018	.000	.000	.018	.000	.000	.000	.010
Phi2-a	.982	1.00	1.00	1.00	1.00	1.00	1.00	.990
-b	.018	.000	.000	.000	.000	.000	.000	.010
Trp-a	.660	.912	.737	.764	.787	.839	.825	.769
-b	.300	.087	.237	.217	.212	.125	.175	.221
-c	.040	.000	.025	.019	.000	.036	.000	.010
Xdh-a	.795	.875	.825	.688	.950	.912	.825	.857
-b	.205	.125	.175	.312	.050	.088	.175	.143

a Population from the Rio Lerma

Table 5: Genic heterogeneity and standardized variances for populations of Goodea atripinnis from Lake Chapala.

Locus	x^2	df	p	F_{st}
Ada	14.80	14	.50	.015
Adh	23.60	7	.001	.023
Agp	28.71	14	.025	.028
Cpk-2	4.49	7	.90	.004
Dip-2	16.57	7	.025	.016
Got-1	23.56	14	.10	.023
Got-2	5.26	7	.90	.005
G3p-2	4.89	7	.90	.005
Mec	31.82	21	.10	.032
Mpi	37.73	14	.005	.037
Pgm	25.16	14	.05	.025
Phi-1	5.05	7	.90	.005
Phi-2	21.58	14	.10	.021
Sod	24.00	21	.50	.024
Trp	16.75	7	.025	.016
Total	283.97	175	.001	

Table 6: Genic heterogeneity and standardized variances for populations of Chapalichthys encaustus from Lake Chapala.

Locus	x^2	df	p	F_{st}
Adh	9.93	6	.10	.014
Agp	30.88	6	.005	.045
Dip-2	13.65	6	.05	.020
Got-2	15.28	12	.10	.013
Gp-1	9.33	6	.50	.014
G3p-2	3.38	6	.90	.005
Mpi	6.76	6	.50	.010
6pg	17.53	6	.01	.026
Pgm	6.81	6	.50	.010
Phi-1	6.81	6	.50	.010
Phi-2	8.28	6	.50	.013
Trp	31.20	12	.01	.046
Xdh	23.87	6	.001	.035
Total	184.25	90	.001	

$$x^2 = nr$$

where n is the number of independent x^2 comparisons made (the number of loci) and r is the number of populations (Workman & Niswander, 1970). This number is compared to the observed x^2 value by an F-ratio test. For Goodea,

$$F = 283.97/120 = 2.37 \quad p<.001$$

and for Chapalichthys,

$$F = 184.25/91 = 2.02 \quad p<.005$$

These results indicate that the amount of heterogeneity is significantly greater than that expected by random sampling from a single population.

Avise & Felley (1979) found significant heterogeneity in allelic frequencies among populations of bluegill within reservoirs, yet concluded that the overall differentiation was small. They felt that the bluegill breeding system was conducive to large outbreeding populations. Studies on whitefish populations in northern Lake Michigan (Imhof et al., 1980) suggested the presence of at least four populations based on allele frequency data and tagging returns. Spatial and temporal differences in spawning grounds and homing as well as behavioral differences were suggested as possible isolating factors.

The differentiation noted among populations of Goodea

and Chapalichthys may be the result of either selection or genetic drift. There is some evidence to suggest the existence of two somewhat distict physiochemical regions (east and west) in Lake Chapala (J. Batha, personal communication), but the genetic differentiation does not seem to reflect an east-west selection gradient and shows no apparent pattern. If the allelic frequencies are mediated by stochastic forces, then the population differentiation may be described by "isolation by distance" or "neighborhood" models (Wright, 1946). These models assume that motility of individuals is small in relation to population density.

An additional analysis included a sample from the Rio Lerma (Fig. 1, locality 5) as well as the data discussed above. Inclusion of this population for Chapalichthys did not affect either the identity of the heterogeneous loci or the overall significance. In the case of Goodea the number of heterogeneous loci did not change but there was a change in the identity of three of these loci (Ada, Got-2, and Mec instead of Dip-2, Pgm, and Trp). The overall heterogeneity also did not change, suggesting that genic differentiation among the lake populations is as great as that between lake and river populations. If the differentiation was due solely to selection, the considerable differences between the lake and river environments would be expected to result

in much higher levels of heterogeneity between these. This result, together with those from the correlation analysis reported earlier suggest that the neighborhood model, and not an isolation by distance model, may accurately describe the population differentiation observed, and that the differentiation we have detected is largely due to stochastic forces within neighborhoods.

The proposal that Goodea and Chapalichthys live in neighborhoods around the perimeter of the lake suggests that there is low vagility or some degree of homing. Reports of fish movements within lakes and streams suggest that many species have fairly precise home ranges (Kudrna, 1965; Gerking, 1953), though young may be more mobile (Werner, 1967). In Lake Chapala, young were usually captured in shallow water or in areas of submergent vegetation, and unlike the adults, apparently remained there throughout the day. Thus young goodeids may not disperse far and may contribute little to population mixing.

Speith (1974), citing Wright (1943), stated that even low levels of gene flow will be sufficient to remove the differentiating effects of drift, though Allendorf and Phelps (1981), however, found that differentiation due to drift is possible, even in the face of moderate amounts of gene flow. Such gene flow will ensure that the same alleles are in each population but not necessarily at the same

frequencies. An additional argument that we cannot now address fully is that, as Speith (1974) suggests, the population structuring we observe may be a non-equilibrium situation due to some recent historical phenomenon. However, samples from two consecutive years for locality 5 for both species indicated no change in allelic frequencies (Appendix Table 2).

There is considerable similarity in the standardized variances of both species (Tables 3 and 4), which, in general, are similar to values obtained for lacustrine bluegill populations (Avise & Felley, 1979). These variances are also similar to those calculated for riverine populations of Goodea (Chapter 2), suggesting a consistent level of heterogeneity among both lake and small stream populations. The survey of riverine populations showed highly significant heterogeneity between populations separated by distances of less than 1 km.

The levels of heterogeneity are similar for Goodea and Chapalichthys, even though the former displays a diurnal migration pattern (information obtained from local fishermen). Though one might assume that this movement would promote mixing, apparently it has no measureable dampening effect on genic differentiation.

These results suggest that even continuously distributed fish species may show a surprising level of

population structuring. Though neither of the species studied is a precise analog of those involved in the species flocks mentioned above, our data clearly show that neighborhood effects or isolation by distance models are likely to be of some significance in the evolution of lacustrine faunas. The data are most consistent with the intralacustrine models of species flock evolution.

Chapter 4

Components of genetic variation in *Goodea atripinnis* (Goodeidae)

Introduction

The role of gene flow in population differentiation has been the subject of considerable debate but little research has been conducted on the topic. While some authors feel that even small amounts of gene exchange will be sufficient to obscure the effects of drift (e.g. Speith, 1974), others have concluded that differentiation may occur in the face of moderate amounts of gene flow (e.g. Allendorf & Phelps, 1981). Erlich & Raven (1969) asserted that levels of gene flow among populations may be lower than had been presumed and may play only a small role in evolution. Mayr (1963) suggested that gene flow is common enough to insure considerable similarity among populations of mobile, sexual organisms. However, Jackson & Pounds (1979) noted that there are little data to assess the magnitude and effect of gene flow in natural populations.

In this study I examined the geographic pattern of allozyme variation in the goodeid fish *Goodea atripinnis*, one of the most widespread and ubiquitous fish species from the Mesa Central of Mexico. This species is found in a wide

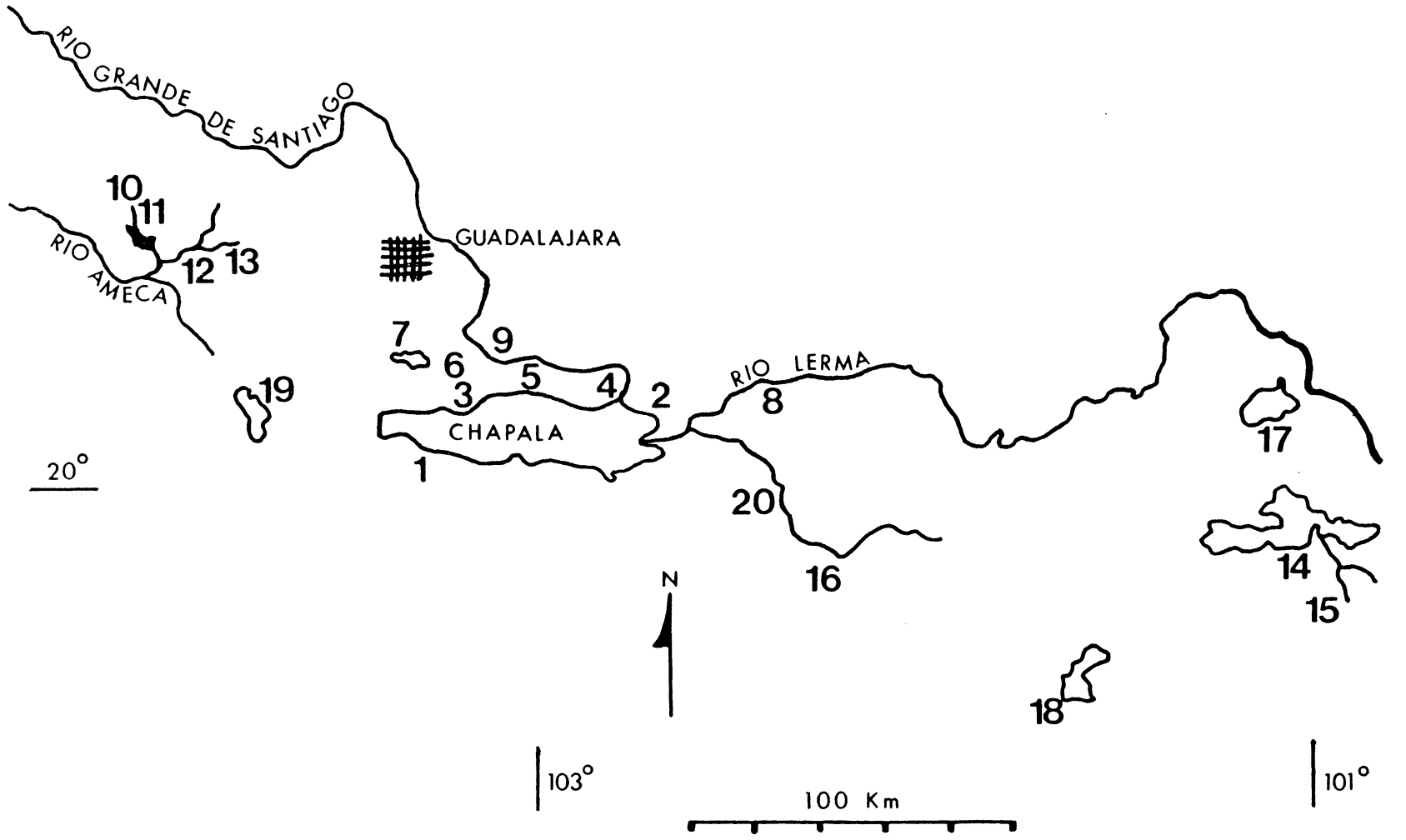
variety of habitats, from small streams to large lakes. In the previous sections, considerable genic heterogeneity was observed in populations from certain lakes and streams. The objective in this study was to examine a broader array of populations to assess the degree of variation within and differentiation among them and estimate by several analytical methods the components of this variation. I addressed the effect of gene flow by comparing differentiation among continuous and isolated populations, and evaluated the level of differentiation among some of the currently recognized subspecies of G. atripinnis.

Materials and Methods

Twenty samples of Goodea atripinnis were collected from localities in the Rios Ameca and Lerma-Santiago and in a number of endorheic basins in this region during 1980 and 1981 (see Figure 4). Sample sizes are given in the allele frequency table (Appendix). Specimens were frozen on dry ice and stored at -90 C until dissection. Thirty-four presumptive loci were surveyed using sample preparation and electrophoresis techniques described by Turner (1983).

Data were analyzed with the BIOSYS-1 computer package of Swofford & Selander (1981). Analyses used in this study were: genic heterogeneity x^2 (Workman and Niswander, 1970),

Fig. 4: Collection sites of Goodea atripinnis. The following abbreviations refer to the state: Jal., Jalisco; Mich., Michoacan; Gto., Guanajuato. 1) Soyatlan, Lago Chapala, Jal. 2) Jamay, Lago Chapala, Jal. 3) Chapala, Lago Chapala, Jal. 4) Ocotlan, Lago Chapala, Jal. 5) North shore, Lago Chapala, Jal. 6) Leech Pit, Jal. 7) Laguna Cajititlan, Jal. 8) Rio Lerma at Concuea, Mich. 9) Rio Grande de Santiago at Atontonoquillo, Jal. 10) Rio Teuchitlan, Jal. 11) El Rincon, Teuchitlan, Jal. 12) Rio Ahuizula, Jal. 13) Presa Huaxtla, Jal. 14) Lago Cuitzeo, Mich. 15) Tsintsimeo, Mich. 16) Lago Camecuaro, Mich. 17) Lago Yurriria, Gto. 18) Lago Patzcuaro, Mich. 19) Lago Atontonilco, Jal. 20) Ixtlan, Mich.


RIO GRANDE DE SANTIAGO
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RIO AMECA
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the Rogers (1972) genetic distance and the Nei (1978) unbiased genetic distance, a cluster analysis (UPGMA) based on the Roger's distance measure, and the Nei (1973, 1975) gene diversity analysis.

The Nei gene diversity analysis partitions the gene diversity into within population diversity (H_c, Nei's within colony), within drainage diversity (D_{cs}, Nei's within subpopulation), and diversity among drainages (D_{st}). These components sum to H_t, the total gene diversity. G_{st}, related to the more familiar F_{st}, is the relative genic differentiation among drainages and is computed from the equation,

$$G_{st} = D_{st}/H_t.$$

Although this is a good measure of relative genetic differentiation, it is dependant on the value of H_t, and when H_t is small, G_{st} may be large, even though there is little differentiation (Nei, 1975). D_m, a better measure, is the absolute degree of genic differentiation and is computed by

$$D_m = sD_{st}/(s-1)$$

where s is the number of subpopulations (drainages).

Results

Of the 34 loci scored for all populations, 14 loci were polymorphic: adenosine deaminase (Ada), alcohol

dehydrogenase (Adh), alpha-glycerolphosphate dehydrogenase (Agp), creatine phosphokinase (Cpk2) major eye carboxylesterase (Mec), glutamate-oxaloacetate transaminase (Got 1,2) glycerol-3-phosphate dehydrogenase (Gpd), mannose phosphate isomerase (Mpi), 6-phosphogluconate dehydrogenase (6Pgd), phosphoglucomutase (Pgm), phosphohexose isomerase (Phi 1,2) superoxide dismutase (Sod). Allele frequencies for all populations are found in the Appendix.

Genetic variability estimates (Table 7) varied considerably among populations. Percent of loci polymorphic (P_{99}) ranged from a low of 6% in Lake Cuitzeo to a high of 34.38% at Soyatlan in Lake Chapala. Most populations were in the range of 15 to 30%. Average heterozygosities ranged from 1.7 to 7.4% with a mean of 3.4% which are consistent with values found for a wide range of fish species (Nevo, 1978; Winans, 1980).

Genic heterogeneity x^2 analyses were performed where possible on populations grouped by drainage system. Four drainages were examined: Chapala (localities 1-5,9), Lerma (8,16,17,20), Ameca (10-13), and Cuitzeo (14,15)(Fig. 3). All drainages except Cuitzeo ($p=.096$) exhibited highly significant within drainage heterogeneity ($p<.001$).

The Nei (1975) gene diversity analysis (Table 8) was performed on the above drainage systems to determine components of the overall genetic variation. The values of

Table 7: Genetic variability estimates for populations of Goodea atripinnis.

Locality	P_{99a}	H_b
Soyatlan	34.38	0.050
Jamay	28.13	0.043
Chapala	25.00	0.043
Ocotlan	28.13	0.040
North Shore	18.75	0.043
Leech Pit	28.13	0.026
L. Cajititlan	18.18	0.037
Concuea	34.38	0.047
Atontonoquillo	31.25	0.045
Rio Teuchitlan	12.12	0.029
El Rincon	21.21	0.033
Rio Ahuizula	18.18	0.032
Huaxtla	15.15	0.017
Lago Cuitzeo	6.06	0.017
Tsintsimeo	6.06	0.017
Lago Camecuaro	18.18	0.074
Lago Yurriria	6.06	0.027
Lago Patzcuaro	15.15	0.030
Lago Atontonilco	9.09	0.026
Ixtlan	6.06	0.030

[a]criterion: q>.01

[b]average heterozygosity

Table 8: Components of gene diversity (Nei, 1975) for *Goodea atripinnis*.

Locus	Hc	Dcs	Dst	Ht	Gst	Dm
Ada	.045	.004	.001	.049	.020	.001
Adh	.050	.009	.002	.057	.035	.002
Agp	.226	.023	.017	.266	.064	.020
Cpk-2	.038	.035	.004	.070	.057	.005
Mec	.177	.027	.002	.207	.009	.002
Got-1	.026	.000	.001	.027	.037	.001
Got-2	.008	.000	.000	.009	.000	.000
Gpd-2	.028	.002	.000	.030	.000	.000
Mpi	.061	.001	.000	.062	.000	.000
6Pgd	.006	.000	.001	.006	.167	.001
Pgm	.022	.000	.000	.022	.000	.000
Phi-1	.004	.000	.000	.004	.000	.000
Phi-2	.050	.001	.001	.051	.019	.001
Sod	.172	.020	.001	.194	.005	.001
Mean	.061	.008	.002	.075	.029	.003

D_m (Table 8) are small, indicating little genic differentiation among drainages. An examination of the components of total gene diversity reveals that in all cases, except Cpk2, the bulk of the diversity (H_c/H_t = 81.3%) is due to within population variation. Only about 2.6% (D_{st}/H_t) is related to among drainage differentiation.

Rogers genetic distances (summarized in Table 9), with the exception of comparisons with Lake Camecuaro, were low (range=0.012 - 0.091) and similar to values expected for continuously distributed, conspecific populations (Selander & Johnson, 1973). Within drainage comparisons averaged 0.032±0.010 and 0.019±0.006 for Chapala and Ameca respectively. These are similar to observed values for Lepomis (Avise & Smith, 1974). Comparisons of Chapala and Ameca populations yielded considerably higher genetic distances (0.052±0.011). Distances between Chapala populations and all other populations, excluding Ameca, Lake Camecuaro and the Ixtlan spring (see discussion), were larger still (0.061±0.013). However, comparisons among the populations from the endorheic basins (localities 6,7,14,15,17,18,19 in Fig. 1) yielded distances of 0.031±0.013, similar to those observed within Lake Chapala.

Table 9: Summary of Rogers genetic measures for within and between drainage comparisons of populations of Goodea atripinnis.

Comparison	Mean±S.E.
Within Chapala	0.032±0.010
Within Ameca	0.019±0.006
Chapala vs. Ameca	0.052±0.011
Among isolated basins[1]	0.031±0.013
Chapala vs. All	0.061±0.013
Camecuaro vs. All	0.128±0.017
Ixtlan vs. All	0.076±0.023

[1]Excluding Chapala, Camecuaro and Ixtlan

Discussion

Avise & Smith (1974) suggested that the discontinuous nature of freshwater habitats may lower the genetic variability within fish populations and increase allelic heterogeneity between drainage systems. Genetic continuity of populations within drainages and high heterogeneity among drainages was reported in other studies of fish species (Echelle et al., 1975; Buth & Burr, 1978; Buth & Mayden, 1981; Brett, 1981). In contrast, our results for Goodea suggest a pattern in which levels of between drainage and within drainage differentiation are comparable.

Only 10 drainage specific alleles were detected among all populations, and all may be considered as rare alleles ($p<.09$). Six of these were encountered in Chapala, where large population size might allow rare alleles to accumulate.

The degree of differentiation between drainage systems, based on the Nei gene diversity analysis (Table 8), is small (D_m values of 0.00-0.02, 2.6% of the variance). Although the genic heterogeneity analysis indicated extensive differentiation among populations within drainages, this component of the gene diversity (D_{cs}) accounted for only 10.6% of the total variance. The most important component was H_c, the within population (i.e. among individuals at a locality) gene diversity which suggests that local effects

based on selection, drift or population structure are of overriding influence on genetic variation in Goodea.

Components of gene diversity for populations of Salmo clarki (Loudenslager & Gall, 1980) exhibit a different pattern from that observed in Goodea. Populations of S. clarki were characterized by low within population gene diversity (29.7%) and a higher total variability (H_t=0.128) with approximately 70% (G_{st}=0.703) of the variation attributed to between subpopulation (subspecies) differentiation. Polymorphic loci were frequently fixed for different alleles in different subspecies, a condition not found in Goodea. Within a single subspecies, contributions of the components of gene diversity varied (H_c=55% for S.c.henshawi to H_c=91% for S.c.bouvieri). These results suggest that differentiation in Goodea over a large part of its range is comparable to that exhibited by a single subspecies of S. clarki.

Jackson & Pounds (1979) suggested that in order to assess effects of gene flow among populations, comparisons between continuous and isolated populations (in this case, within and among drainages) need to be evaluated. They also suggested that comparisons need to be made from relatively similar habitats. We have made such comparisons using the Rogers distance measure. Genetic distance coefficients suggested considerable homogeneity among all populations

with the possible exception of Lake Camecuaro (locality 16). Comparisons with this population yielded distance measures which were generally twice as large as all other comparisons (Table 3). The reason for the uniqueness of the Lake Camecuaro population is unknown. The Ixtlan warm mineral spring (locality 20), a rather different habitat from the others, was not included in the analysis.

The genetic distance comparisons suggest greater differentiation among than within drainages when Chapala is compared to other systems. This is not surprising since comparisons of populations with different levels of genetic variability are likely to yield higher distances than comparisons of populations with similar levels.

The most surprising aspect of the data was the relatively low level of differentiation among the isolated drainage systems. These comparisons yielded distances slightly less than distances obtained within the Chapala system. This suggests that differentiation among drainages is on the average no greater than that within drainages. This can be seen more clearly in the cluster analysis based on the Rogers distance (Fig. 5). This analysis differentiated two major groups of populations, excluding Lake Camecuaro and the Ixtlan spring (localities 16 and 20, Fig. 1). Differentiation within the Chapala system was similar to that evident among all other populations. Thus,

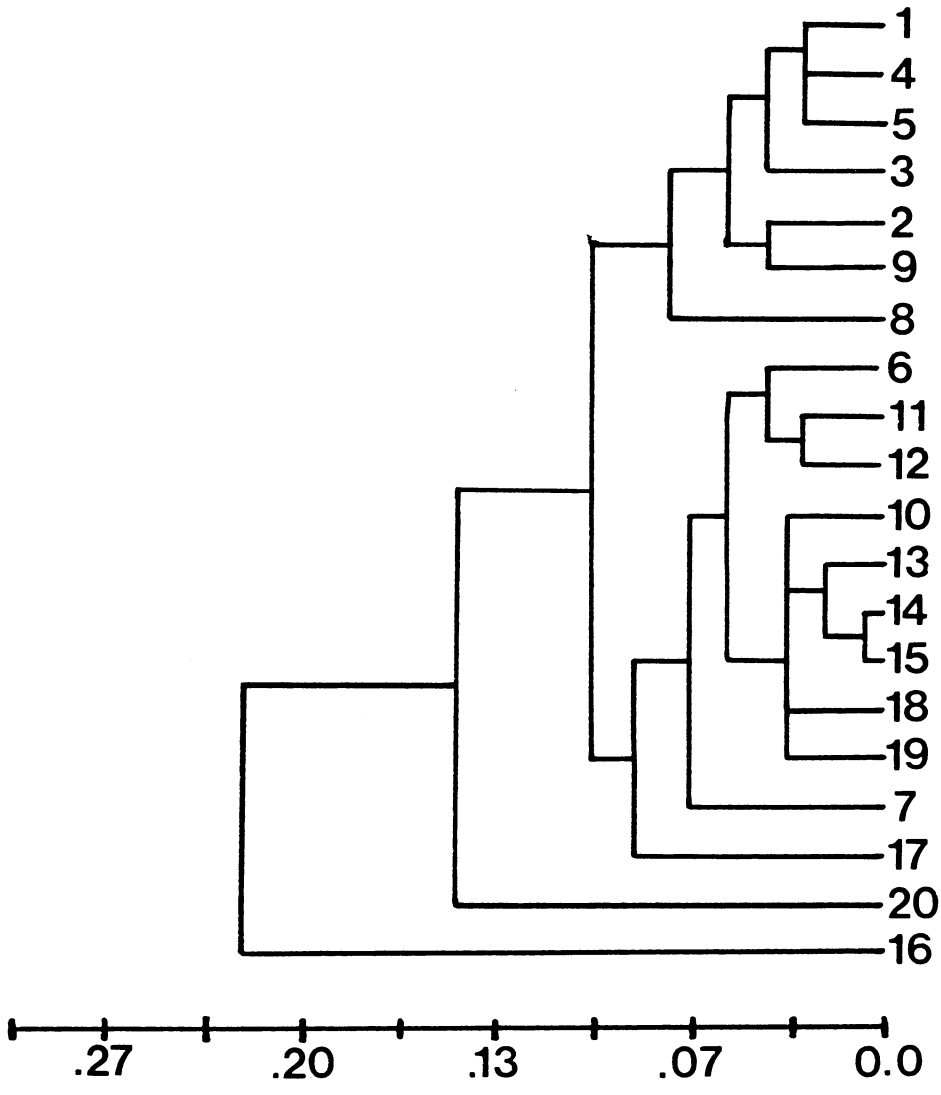

Fig. 5: A dendrogram, based on UPGMA clustering of Goodea atripinnis populations using the Rogers genetic distance.

within the Chapala system gene flow has not had a significant effect on population differentiation. Populations that are completely isolated have similar levels of differentiation. The differentiation within Lake Chapala was attributed to neighborhood effects (Chapter 3), suggesting that vagility of individuals was low relative to population density. Selection differentials sufficient to account for the heterogeneity observed were not readily apparent. Allendorf & Phelps (1981) demonstrated that differentiation among populations is possible, even in the face of moderate amounts of gene flow. My results appear to be consistent with their suggestion.

Use of genetic distance measures to describe population differentiation is common. However, restrictions to gene flow may be obscured by use of distance measures when the pattern of allelic differentiation is quantitative (frequency differences) rather than qualitative (allele substitutions) (Buth & Crabtree, 1983). Although the genetic distance coefficients for within drainage population comparisons are quite low, the genic heterogeneity analysis suggests considerable population structuring. Buth (1984) suggested that sampling one locality per major drainage within a species range may be adequate for detailed studies of fish population differentiation. Given the extent of microgeographic differentiation we have noted here and in

other studies, it is clear that such studies should include more than a single population per drainage.

Three of four recognized subspecies of G. atripinnis, (Alvarez, 1970) were encountered in this study: G. a. xalicone (Lake Chapala and nearby drainages), G. a. martini (Lake Cuitzeo) and G. a. luitpoldi (Lake Patzcuaro). Rogers distance coefficients among the three subspecies were small (0.02 to 0.07) relative to those observed among subspecies of Lepomis (0.12 to 0.22) (Avise & Smith, 1974). Nei distances (0.00-0.014) were also small relative to other comparisons (Campostoma, Buth & Burr, 1978, 0.15-0.19 Salmo clarki, Loudenslager & Gall, 1980, 0.082-0.282), and suggest that either genetic differentiation among subspecies has progressed slower than morphological differentiation or that subspecific rank is not warrented. Our observations of field collected Goodea suggest that there is a considerable amount of local morphological variation (possibly of an ecophenotypic nature) and that subspecific designations are not appropriate for these populations.

An assumed prerequisite to genetic divergence are isolation by geographic barriers and the concomitant restriction of gene exchange (Avise, 1976). The isolated populations then accumulate genetic differences by adaptation to local environment and/or genetic drift. Does the pattern of differentiation exhibited by Goodea reflect

the hydrologic history of this region and can it be described by a time-since-divergence model?

The geologic and hydrologic history of this region is somewhat obscure (Barbour, 1973). The drainages were apparently connected in a large continuous system as recently as the Pleistocene. Geologic and volcanic events sometime in the mid-Pleistocene resulted in the fragmentation and compartmentalization of this system and the isolation of the Rio Ameca and the endorheic basins of Lakes Atontonilco, Cajititlan, Cuitzeo and Patzcuaro. The antiquity of these drainages does not seem to have decisively affected gene diversity. Although the geologic and climatic conditions of the western United States have apparently had considerable impact on differentiation among populations of S. clarki (Loudenslager & Gall, 1980). Potentially quite similar conditions (geologic activity, dessication) in the Mesa Central of Mexico do not seem to have had a similar effect on differentiation in Goodea. Thus, a simple time-since-divergence model of genic differentiation cannot adequately explain the data. Though other effects (e.g., a reduction in mutation rate and therefore in that of allele substitution) cannot be formally excluded, our data clearly suggest that local effects and not antiquity are the primary mediators of genetic differentiation in Goodea. Our data suggest that the

presence or absence of barriers to gene flow (i.e., the physical contiguity of populations) appears to be a minor factor in determining the extent of differentiation among populations.

Chapter 5

Summary

The results of the analysis of genetic variation in populations of two species of goodeid fishes are summarized as follows. 1. Microgeographic (distances of several hundred meters) differentiation is possible in ostensibly continuous stream populations (Chapter 2). 2. Continuous lake-dwelling populations of both species exhibit highly significant genic heterogeneity. This lends support to an intralacustrine or sympatric model of fish species flock evolution (Chapter 3). 3. Differentiation among populations of G. atripinnis within a single drainage, the Chapala system, was similar to levels of differentiation among populations from other drainages which have been isolated since the mid-Pleistocene. Thus, a simple time-since-divergence model of population differentiation is inadequate to explain these results. Local effects (drift, bottlenecks, selection) appear to be the major force mediating genetic variation (Chapter 4).

The results of Chapters Two and Three, significant genic heterogeneity among continuous populations, suggests two possible explanations. The first, an isolation model, would propose that there is no gene flow among populations. This population structuring or neighborhood effect may be a

result of homing behavior, low vagility or reduced effective population sizes as a result of dominance relations among breeding males. This hypothesis is unlikely from what we know of the biology of Goodea which is known to undergo a diurnal migration and thus make population mixing quite likely. However, components of each of these may play a role in population differentiation.

The second explanation, a selection model, suggests that differentiation among populations is a result of differential selection pressures on a very fine geographic scale. Although it is not possible to rule this out completely, such selective regimes were not readily apparent, especially along the several hundred meters sampled in the Rio Teuchitlan (Chapter 2).

The analysis of genetic variation among populations of Goodea from the isolated systems revealed levels of differentiation no greater than that observed within Lake Chapala. Although the levels of differentiation within Lake Chapala were average or slightly above levels observed for within drainage differentiation in other species, levels between the isolated systems were lower than observed elsewhere. These results suggest that geographic isolation (and therefore lack of gene flow) is potentially unimportant in determing levels of genetic differentiation. It also appears that gene flow may play but a minor role as a

dedifferentiating force among continuous populations. The data suggest that local effects (drift, bottlenecks, selection, and population structuring) are the primary mediators of patterns of genetic variation and population differentiation.

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APPENDIX

Allele frequencies for 20 populations of *Goodea atripinnis*. Refer to Figure 1 for population designations.

	Population						
Locus	1	2	3	4	5	6	7
N	79	16	103	65	72	41	16
ADA							
A	0.981	1.000	0.981	0.985	0.993	1.000	0.906
B	0.000	0.000	0.019	0.008	0.000	0.000	0.094
C	0.019	0.000	0.000	0.008	0.007	0.000	0.000
ADH							
A	1.000	1.000	1.000	0.976	1.000	1.000	1.000
B	0.000	0.000	0.000	0.024	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CPK-2							
A	0.987	0.969	0.985	0.977	1.000	0.988	1.000
B	0.013	0.031	0.015	0.023	0.000	0.012	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MEC							
A	0.781	0.875	0.817	0.746	0.809	1.000	1.000
B	0.167	0.063	0.099	0.146	0.132	0.000	0.000
C	0.019	0.031	0.042	0.054	0.015	0.000	0.000
D	0.096	0.031	0.042	0.054	0.044	0.000	0.000
GOT-1							
A	0.981	0.969	0.995	1.000	1.000	0.988	0.875
B	0.019	0.031	0.005	0.000	0.000	0.012	0.125
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GOT-2							
A	0.987	1.000	1.000	0.992	0.993	1.000	1.000
B	0.013	0.000	0.000	0.008	0.007	0.000	0.000
GPD-2							
A	1.000	1.000	0.990	0.992	1.000	0.890	0.875
B	0.000	0.000	0.010	0.008	0.000	0.110	0.125

table continued

MPI							
A	0.943	1.000	0.940	0.944	0.951	0.963	1.000
B	0.051	0.000	0.055	0.016	0.049	0.037	0.000
C	0.006	0.000	0.005	0.040	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6PGD							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM							
A	0.961	1.000	0.985	1.000	0.986	0.988	0.969
B	0.039	0.000	0.015	0.000	0.014	0.012	0.031
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PHI-1							
A	0.994	1.000	1.000	1.000	0.993	1.000	1.000
B	0.006	0.000	0.000	0.000	0.007	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PHI-2							
A	0.930	0.969	0.905	0.954	0.910	1.000	1.000
B	0.000	0.000	0.017	0.000	0.014	0.000	0.000
C	0.070	0.031	0.074	0.046	0.076	0.000	0.000
D	0.000	0.000	0.004	0.000	0.000	0.000	0.000
SOD							
A	0.943	0.938	0.833	0.927	0.903	0.837	0.875
B	0.019	0.000	0.011	0.016	0.014	0.000	0.000
C	0.013	0.031	0.071	0.008	0.021	0.063	0.125
D	0.025	0.031	0.085	0.048	0.063	0.100	0.000
AGP							
A	0.338	0.219	0.346	0.287	0.281	0.049	0.063
B	0.649	0.781	0.591	0.689	0.688	0.951	0.938
C	0.013	0.000	0.063	0.025	0.031	0.000	0.000

	Population						
Locus	8	9	10	11	12	13	14
N	45	78	35	38	40	70	72
ADA							
A	0.991	0.955	1.000	1.000	1.000	1.000	1.000
B	0.089	0.038	0.000	0.000	0.000	0.000	0.000
C	0.000	0.006	0.000	0.000	0.000	0.000	0.000
ADH							
A	1.000	0.875	1.000	1.000	0.987	0.975	1.000
B	0.000	0.125	0.000	0.000	0.013	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.025	0.000
CPK-2							
A	0.989	1.000	1.000	1.000	1.000	1.000	1.000
B	0.011	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
MEC							
A	0.578	0.865	1.000	0.961	0.987	0.979	0.965
B	0.244	0.083	0.000	0.039	0.012	0.021	0.035
C	0.100	0.026	0.000	0.000	0.000	0.000	0.000
D	0.078	0.026	0.000	0.000	0.000	0.000	0.000
GOT-1							
A	1.000	0.981	1.000	1.000	1.000	1.000	1.000
B	0.000	0.013	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
GOT-2							
A	0.933	1.000	1.000	1.000	1.000	1.000	1.000
B	0.067	0.000	0.000	0.000	0.000	0.000	0.000
GPD-2							
A	0.989	0.987	0.986	0.986	1.000	1.000	0.986
B	0.011	0.013	0.014	0.014	0.000	0.000	0.014
MPI							
A	0.935	0.963	0.914	0.974	1.000	1.000	1.000
B	0.065	0.019	0.086	0.026	0.000	0.000	0.000
C	0.000	0.019	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000

table continued

6PGD							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM							
A	0.956	1.000	1.000	1.000	0.938	1.000	1.000
B	0.044	0.000	0.000	0.000	0.025	0.000	0.000
C	0.000	0.000	0.000	0.000	0.037	0.000	0.000
PHI-1							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PHI-2							
A	0.922	0.923	1.000	1.000	1.000	1.000	1.000
B	0.022	0.006	0.000	0.000	0.000	0.000	0.000
C	0.056	0.058	0.000	0.000	0.000	0.000	0.000
D	0.000	0.013	0.000	0.000	0.000	0.000	0.000
SOD							
A	0.913	0.865	0.943	0.842	0.800	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.065	0.077	0.014	0.013	0.000	0.000	0.000
D	0.022	0.058	0.043	0.145	0.200	0.000	0.000
AGP							
A	0.167	0.197	0.061	0.039	0.000	0.068	0.000
B	0.756	0.789	0.939	0.961	1.000	0.932	1.000
C	0.078	0.014	0.000	0.000	0.000	0.000	0.000

Population

Locus	15	16	17	18	19	20
ADA						
A	1.000	0.781	1.000	1.000	1.000	1.000
B	0.000	0.188	0.000	0.000	0.000	0.000
C	0.000	0.031	0.000	0.000	0.000	0.000
ADH						
A	1.000	1.000	1.000	1.000	0.923	0.667
B	0.000	0.000	0.000	0.000	0.067	0.333
C	0.000	0.000	0.000	0.000	0.000	0.000
CPK-2						
A	1.000	0.406	0.972	1.000	1.000	1.000
B	0.000	0.594	0.000	0.000	0.000	0.000
B	0.000	0.000	0.028	0.000	0.000	0.000
MEC						
A	0.979	0.500	1.000	0.967	1.000	1.000
B	0.007	0.375	0.000	0.033	0.000	0.000
C	0.014	0.125	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000
GOT-1						
A	1.000	1.000	1.000	0.967	0.967	1.000
B	0.000	0.000	0.000	0.033	0.033	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000
GOT-2						
A	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000
G2D-2						
A	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000
MPI						
A	1.000	1.000	1.000	0.933	0.900	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.067	0.000	0.000
D	0.000	0.000	0.000	0.000	0.100	0.000

table continued

6PGD						
A	1.000	1.000	1.000	0.933	1.000	1.000
B	0.000	0.000	0.000	0.067	0.000	0.000
PGM						
A	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000
PHI-1						
A	1.000	1.000	1.000	0.967	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.033	0.000	0.000
PHI-2						
A	1.000	0.969	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.031	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000
SOD						
A	1.000	0.625	0.639	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.375	0.361	0.000	0.000	0.000
AGP						
A	0.000	0.281	0.000	0.000	0.000	0.500
B	1.000	0.719	1.000	1.000	1.000	0.500
C	0.000	0.000	0.000	0.000	0.000	0.000

APPENDIX TABLE 2

Allele frequencies for locality 5a for *Goodea atripinnis* from 1980 and 1981

Locus	1980	1981	x^2	df	p
Ada-a	0.935	0.852	1.42	1	.50
-b	0.065	0.148			
Agp-a	0.195	0.136	0.90	2	.90
-b	0.717	0.795			
-c	0.086	0.068			
Cpk2-a	0.989	1.000	1.89	1	.50
-b	0.011	0.000			
Mec-a	0.500	0.659	4.64	3	.50
-b	0.305	0.182			
-c	0.108	0.091			
-d	0.087	0.068			
Got2-a	0.935	0.932	0.04	1	.90
-b	0.065	0.068			
Gpd2-a	0.979	1.000	1.65	1	.90
-b	0.021	0.000			
Mpi-a	0.957	0.977	2.13	1	.50
-b	0.043	0.023			
Pgm-a	0.957	0.955	0.04	1	.90
-b	0.043	0.045			
Phi2-a	0.914	0.932	2.75	2	.50
-b	0.000	0.023			
-c	0.086	0.045			
Sod-a	0.936	0.978	2.44	2	.50
-c	0.043	0.022			
-d	0.021	0.000			
Combined			19.74	15	.50

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