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Trophic radiation through polymorphism in cichlid fishes

(genetic variation/allozymes/adaptive radiation)

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ABSTRACT **Several morphologically defined species of cichlid fishes (*Cichlasoma*) endemic to the Cuatro Cienegas basin of Mexico and differing in tooth structure, body shape, and diet are allelically identical at 27 gene loci. The presence of only one Mendelian population in each of three drainage systems studied and the occurrence of two of the morphotypes in the same broods indicate that the supposed species are morphs. That trophic radiation in the Cuatro Cienegas cichlids has been achieved through ecological polymorphism rather than speciation raises questions regarding the genetic basis for the extensive intralacustrine radiation of cichlids in Africa and elsewhere.**

The discovery of a flock of endemic species of cichlid fishes (*Cichlasoma*) in the isolated Cuatro Cienegas basin of Coahuila, Mexico, was reported by Taylor and Minckley (1–3), who analogized the situation to the extensive adaptive radiation of cichlids in the rift valley lakes of Africa (4–6). Three species were recognized: a snail-eating type with molariform (crushing) teeth and a short gut; a detritus- or alga-feeding form with papilliform teeth and a long gut; and a fish-eating species with a fusiform body. La Bounty (7) recently distinguished two piscivorous species, one having molariform and the other papilliform teeth.

The genetic structure of populations of the snail- and detritus-eating types was studied by Kornfield and Koehn (8), who examined proteins electrophoretically in samples from three localities. Failing to demonstrate either interspecific or interlocality variation, they concluded that speciation has occurred without genic differentiation. However, another interpretation is that the morphological "species" are morphs of a single species.

To determine the genetic basis for adaptive radiation in the Cuatro Cienegas cichlids, we have re-examined their morphology, ecology, and genetic structure. Our analysis indicates that radiation has been achieved through polymorphism rather than speciation.

MATERIALS AND METHODS

Samples. Collections were made in January and May, 1974, in three of the seven drainage systems at Cuatro Cienegas (2): (*a*) a 100-m stretch of the Rio Churince, ½ km from its origin at Laguna Churince; (*b*) Poso de la Becerra; and (*c*) Laguna El Mojarral. A sample of the widely distributed species *Cichlasoma cyanoguttatum* was collected at Socavon Spring near Musquiz, Coahuila, in May, 1974.

Analysis of Gut Contents and Morphology. The stomach and intestine and the ventral pharyngeal jaw plate (9) were fixed in 10% formalin. Contents of the guts of 128 of the larger Cuatro Cienegas fishes were scored for the presence or absence of (*1*) snails; (2) plant leaves, stems, or flowers; (3) fishes; and (*4*) arthropods.

Eight measurements were made on specimens of a standard body length (10) greater than 50 mm: body depth; gut length; head length; angle of fore part of head; angle subtending arc from center of eye to mid-body line; and, on pharyngeal plate, basal angle of toothed surface, number of teeth in medial row from apex to base, and number of molariform teeth. Body depth, head length, and gut length are expressed as ratios to standard length.

Electrophoresis of Protein Extracts. Extracts of kidney, liver, muscle, and eye were electrophoresed and stained according to methods described by Selander *et al.* (11). Proteins encoded by 27 loci were scored (see Table 2), and alleles were designated numerically according to relative mobility.

RESULTS

Trophic analysis

Most material in the digestive tracts was particulate matter composed of sand grains and fragments of snail shells, in which algae, bacteria, minute snails, and decayed plant parts probably are the chief nutritive components. Other material, apparently representing starch granules from seeds, was interspersed with ground-up seed coats. Pieces of plant stems, leaves, flower heads, and grass awns were sometimes present. Thirty-six fish had snails in the gut, including representatives of all dominant aquatic snail genera occurring at Cuatro Cienegas (12). Arthropods included ostracods, branchiopods, centipedes, spiders, mites, and insects. Remains of small fishes were present in the tracts of one individual from Churince, one from Becerra, and four from Mojarral.

Of the 128 digestive tracts examined, 83 contained one or more of the four major food types; the numbers of tracts with one, two, or three types were 59, 22, and 2, respectively, for a total of 109 food type records. Numbers (and percentages) of tracts in which each type occurred (either singly or in association with other types) were as follows: snails, 36 (33%); plants, 40 (37%); arthropods, 27 (25%); and fishes, 6 (5%). Specificity in diet, as reflected by the presence of only one type of food in a tract, was high for individuals eating fishes, plants, or snails (67, 65, and 56%, respectively), but relatively low (33%) for those eating insects. Both snails and plants were found together in the tracts of seven individuals, whereas 10 such associations were expected on the basis of the frequencies of occurrence of these food types in all tracts examined; comparable observed and expected numbers of associations for other pairs of food types were as follows: snails–arthropods, 11/7; plants–arthropods, 8/8; snails–fishes, 0/1; plants–fishes 1/2; and arthropods–fishes, 1/1. Although these data suggest that food types are not randomly associated in individual tracts, the sample sizes are not sufficient for meaningful statistical analysis.

Morphological variation

In *C. cyanoguttatum*, the distributions of number of molars and of number of teeth are unimodal, with relatively small variances (Fig. 1); but the Cuatro Cienegas populations are

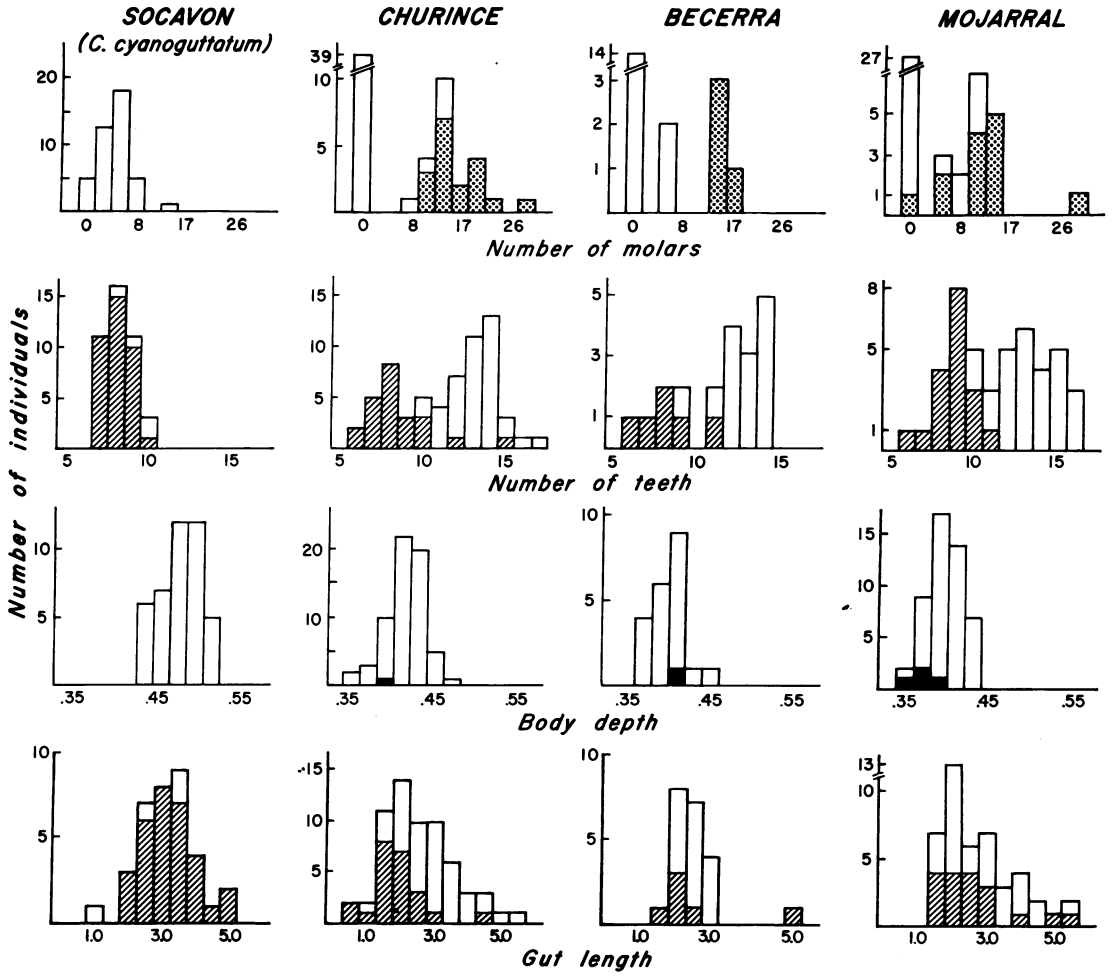

FIG. 1. Variation in four morphological characters in *Cichlasoma cyanoguttatum* and three samples of *Cichlasoma* from Cuatro Cienegas. Stippled pattern, individuals with snails in digestive tract; solid rectangles and squares, specimens with fish in digestive tract; and diagonal lines, individuals with molar teeth. Body depth is body depth/body length; gut length is gut length/body length.

dimorphic in molar number (Fig. 2), individuals having either none or from 4 to 31 of these teeth, and tooth number is bimodally distributed. Molar number and body size are correlated (Fig. 3), but the occurrence of large individuals without molars demonstrates the existence of distinctive developmental patterns. The dentitional polymorphism is independent of sex. Individuals with molars tend to have short guts but are not otherwise distinguishable. Apart from molar number and tooth number, character variances are little, if any, greater in the Cuatro Cienegas populations than in *C. cyanoguttatum*.

With one exception, all individuals with snails in the gut have molar teeth (top row of histograms in Fig. 1). Herbivorous individuals are similar to snail-eaters in body and head shape but have more teeth, few if any molars, and longer digestive tracts. Individuals eating arthropods are not morphologically distinguishable from molluscivores or herbivores. Those few individuals with fish in their tracts tend to have shallow bodies, elongated heads (reflected by the jaw angle), and short guts, but they are not a discrete morphotype (Table 1).

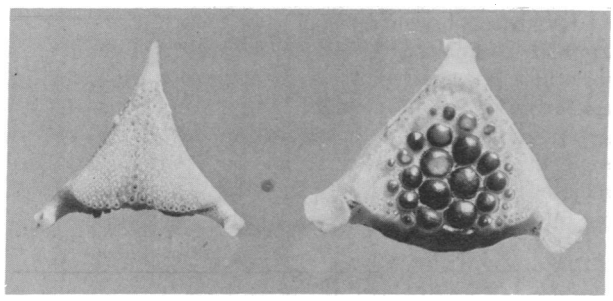

FIG. 2. Toothed surface of lower pharyngeal plate from two large specimens of Cuatro Cienegas cichlids, showing papilliform (*left*) and molariform (*right*) tooth morphs.

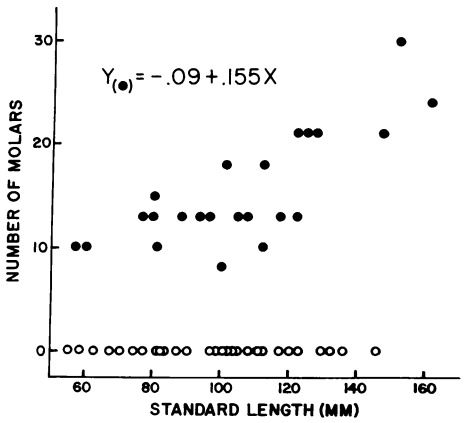

FIG. 3. Relationship between number of molars on pharyngeal plate and body size (standard length) in cichlids from Churince, Cuatro Cienegas.

Table 1. Mean measurements for subgroups of Cuatro Cienegas cichlids defined by food type in digestive tract

Character	Subgroup†				
	Snails (N = 36)	Plants (N = 40)	Arthropods (N = 27)	Fishes (N = 6)	Total (N = 83)
Number of molars	13.9***	3.4***	6.5	1.3	7.05
Number of teeth	7.9***	11.7***	9.5	12.8	10.18
Body depth	0.41	0.41	0.40	0.38*	0.405
Gut length	2.5*	3.0	2.7	2.5	2.81
Head length	0.19	0.19	0.18	0.20	0.187
Head angle	0.99	0.99	0.98	0.94	0.986
Jaw angle	0.49	0.49	0.46	0.44**	0.485
Pharyngeal plate angle	1.02	1.02	1.02	1.02	1.018

Asterisks indicate level of significance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) for Chi-square or t-tests of subgroup means versus means for remainder of total sample.

† Individuals were classified in one or more subgroups.

To establish nonarbitrary criteria for distinguishing a piscivorous morphotype, we did a stepwise discriminate function analysis of eight morphological characters on three subgroups of individuals from Mojarral defined on the basis of the presence of fishes, snails, or plants in the digestive tract. Molar number provided an almost perfect discrimination between molluscivorous and herbivorous individuals, whereas the four piscivorous individuals were characterized by body depth and head length. On the basis of the coefficients derived from this analysis, all 128 individuals from the three localities were assigned to one of three trophic-morphotype subgroups.

Genetic analysis

Of the 27 loci surveyed, 20 were monomorphic in all individuals from Cuatro Cienegas, and 7 were polymorphic. Allele frequencies are presented in Table 2 for total samples and for the three subsamples of individuals over 50 mm in standard length defined by the discriminant function classification. These data provide two convincing lines of evidence that all cichlids in each drainage system are members of a single Mendelian population. First, there are no significant departures of observed genotypic proportions from Hardy–Weinberg expectations; and at no locus at any locality is there significant heterogeneity in allele frequencies among subgroups. Second, *interlocality variation is concordant among subgroups at all loci.* For example, all three Churince subgroups are polymorphic for four alleles at *Pgi-1*, but one allele is fixed in all subgroups at Becerra and Mojarral.

Support for the hypothesis that individuals with either papilliform or molariform teeth are morphs is provided by their occurrence in the same broods. Samples of three broods collected at Mojarral were raised from a length of 3 to about 70 mm standard length in aquaria on a diet of liquid fry food, brine shrimps, and Purina trout chow. Because each brood was tightly schooled and closely guarded by a female, it is highly probable that all members were sibs. All seven individuals from one brood had papilliform teeth; in a second brood, one individual had papilliform and four had molariform teeth. Of four individuals from a third brood, two had papilliform and two had molariform teeth. It is apparent that molars can develop in the absence of snails or other hard food.

DISCUSSION

Trophic radiation of cichlids at Cuatro Cienegas apparently has been achieved through phenotypic diversification within one species. The molariform–papilliform tooth dichotomy is a polymorphism differentially adapting individuals to feed on plants and detritus or molluscs, but piscivorous individuals are not a discrete morph. Although the basis for phenotypic polymorphism in dentition may be a sharp threshold of developmental reactivity to stimuli impinging on the pharyngeal plate (13), the dimorphism of individuals raised in the laboratory on soft food suggests genetic control independent of experience. If gut length also is genetically controlled, its association with tooth type points to the existence of a supergene (14), but it may be only a secondary response to diet. The simplest explanation for differences in feeding behavior is that individuals become conditioned to take types of food for which their teeth are adapted. Similarly, the piscivorous morphotype may merely consist of unusually elongated individuals of either tooth morph that can swim relatively fast and therefore are successful in catching fishes. It is noteworthy that the dentition of the piscivorous morphotype is not distinctive.

We presume that an ancestral cichlid not unlike *C. cyanoguttatum*, with generalized dentition and diet (15, 16), became isolated in the Cuatro Cienegas basin, where a rich snail fauna was unexploited by predators. In this circumstance, a proliferation of species specializing on various food resources might have occurred. That disruptive selection led instead to phenotypic polymorphism may have two explanations. First and most importantly, populations probably have not become geographically isolated within the basin for periods sufficiently long for isolating mechanisms to evolve (see ref. 17). In the long history of the Cuatro Cienegas basin (18), there undoubtedly has been a recurrent temporal pattern of isolation and fusion of drainage systems and, thus, of cichlid populations, as a consequence of the hydrographic cycle producing the springs, which are collapsed caverns of solution chambers in porous limestone. This hypothesis is supported by the close genic similarity of populations in the three drainage systems we sampled. For paired populations, average genetic similarity (19) over 27 loci is 0.9904 (range 0.987–0.995). Average individual heterozygosity is 0.064, a value similar to means reported for other species of fishes and for tetrapods having large continental ranges (20). Inasmuch as populations in the separate drainages consist of only a few thousand individuals (ref. 8 and personal observation), and effective population sizes probably are in the hundreds, the maintenance of "normal" levels of heterozygosity in these endemic cichlids also suggests periodic contact among populations. Second, populations of molluscivores or piscivores that managed to reach the species level may have ex-

Table 2. Allele frequencies at polymorphic loci in Cuatro Cienegas cichlids*

Locality and Sample	N	Allele a	b	c	d	Genotypic ratios χ^2(df)	Heterogeneity χ^2(df)
Esterase-3 (1.00, 0.97)							
Churince							
Total	74	0.53	0.47			0.20(1)	
Snails	22	0.55	0.45			0.25(1)	
Fishes	4	0.50	0.50			0.08(1)	0.14(2)
Plants	37	0.51	0.49			1.15(1)	
Becerra							
Total	59	0.36	0.64			4.38†(1)	
Snails	5	0.50	0.50			0.04(1)	
Fishes	3	0.17	0.83			0	1.81(2)
Plants	12	0.42	0.58			1.30(1)	
Mojarral							
Total	52	0.66	0.34			0.48(1)	
Snails	15	0.67	0.33			0.42(1)	
Fishes	10	0.75	0.25			0.69(1)	0.98(2)
Plants	20	0.63	0.37			1.57(1)	
Lactate dehydrogenase-1 (1.08, 1.00)							
Churince							
Total	55	0.06	0.94			0.22(1)	
Snails	18	0.06	0.94			0.03(1)	
Fishes	4	0.12	0.88			0	0.58(2)
Plants	33	0.06	0.94			0.10(1)	
Becerra							
Total	22	0.23	0.77			1.68(1)	
Snails	5	0.20	0.80			0.14(1)	
Fishes	3	0.17	0.83			0	0.59(2)
Plants	12	0.29	0.71			1.70(1)	
Mojarral							
Total	51	0.06	0.94			0.16(1)	
Snails	15	0.03	0.97			0	
Fishes	10	0.15	0.85			0.20(1)	3.10(2)
Plants	20	0.05	0.95			0.03(1)	
Lactate dehydrogenase-2 (1.31, 1.13, 1.00)							
Churince							
Total	52	0.04	0.13	0.83		1.51(3)	
Snails	15	0.07	0.10	0.83		0.47(3)	
Fishes	4		0.12	0.88		0	0.18(2)‡
Plants	33	0.03	0.15	0.82		2.59(3)	
Becerra							
Total	21	0.02	0.12	0.86		0.48(3)	
Snails	5		0.20	0.80		0.14(1)	
Fishes	3		0.17	0.83		0	0.82(2)‡
Plants	11		0.09	0.91		0.05(1)	
Mojarral							
Total	50	0.22	0.42	0.36		4.13(3)	
Snails	15	0.27	0.27	0.47		3.56(3)	
Fishes	10	0.15	0.65	0.20		4.37(3)	7.34(4)
Plants	20	0.22	0.40	0.38		2.14(3)	

Locality and sample	N	Allele a	b	c	d	Genotypic ratios χ^2(df)	Heterogeneity χ^2(df)
Lactate dehydrogenase-3 (1.00, 0.62)							
Churince							
Total	55	0.98	0.02			0.01(1)	
Snails	18	1.00				—	
Fishes	4	0.88	0.12			0	—
Plants	33	0.98	0.02			0	
Becerra							
Total	59	0.97	0.03			0.05(1)	
Snails	5	1.00				—	
Fishes	3	1.00				—	—
Plants	12	0.96	0.04			0	
Mojarral							
Total	51	1.00				—	
Phosphoglucose isomerase-1 (1.11, 1.00, 0.85, 0.73)							
Churince							
Total	74	0.04	0.70	0.21	0.05	4.44(6)	
Snails	22	0.02	0.73	0.23	0.02	3.67(6)	
Fishes	4	0.12	0.50	0.38		1.83(3)	1.06(2)§
Plants	37	0.05	0.73	0.19	0.03	4.78(6)	
Becerra							
Total	59		1.00			—	
Mojarral							
Total	51		1.00			—	
Phosphoglucose isomerase-2 (2.94, 1.00)							
Churince							
Total	73	0.41	0.59			0.34(1)	
Snails	21	0.33	0.67			0.05(1)	
Fishes	4	0.50	0.50			0.08(1)	1.70(2)
Plants	37	0.45	0.55			0.26(1)	
Becerra							
Total	59	0.51	0.49			0.21(1)	
Snails	5	0.40	0.60			0.40(1)	
Fishes	3	0.83	0.17			0	3.22(2)
Plants	12	0.46	0.54			2.63(1)	
Mojarral							
Total	52	0.37	0.63			1.50(1)	
Snails	15	0.43	0.57			0.11(1)	
Fishes	10	0.35	0.65			0.02(1)	1.29(2)
Plants	20	0.30	0.70			1.98(1)	
Indophenol oxidase (1.00, 0.39)							
Churince							
Total	74	1.00				—	
Becerra							
Total	59	0.97	0.03			0.05(1)	
Snails	5	1.00				—	
Fishes	3	0.83	0.17			0	—
Plants	12	0.88	0.12			0.16(1)	
Mojarral							
Total	51	1.00				—	

* The following 20 proteins were monomorphic: esterase-2, acid phosphatase, peptidase (leucine-alanine), leucine aminopeptidase [aminopeptidase (cytosol)], fumarase (fumarate hydratase), aldolase (fructose-bisphosphate aldolase), two phosphoglucomutases, two malate dehydrogenases, malic enzyme, phosphogluconate dehydrogenase, two isocitrate dehydrogenases, two glutamic oxaloacetic transaminases (aspartate aminotransferase), two hemoglobin components, and two non-enzymatic proteins.

† $P < 0.05$. In a second sample of 115 individuals collected at Becerra in March, 1975, $Est\text{-}3^{1.00} = 0.343$, $Est\text{-}3^{0.97} = 0.657$; $F = 1 - (H_o/H_E) = 0.058$, $\chi^2_{(1)} = 0.39$.

‡ Combining alleles a and b.

§ Combining alleles a and b, and c and d.

perienced high extinction rates through reduction in population size as a result of over-utilization of their food supply. However, when snails are exploited through polymorphism, the proportion of molluscivores can be adaptively adjusted

through frequency-dependent selection (21). A similar argument can be advanced to explain the independence of the polymorphism from sex. If the tooth dimorphism is in fact genetically controlled by two alleles at a single locus (e.g., a switch-gene), we predict that expression of the molariform pattern is recessive to the papilliform, since, by this arrangement, the snail-eating morph would not be lost from a population even in periods when snail densities became too low to support molluscivores.

In the populations we studied, there probably is little if any spatial restriction of morphs, but some degree of associative mating may occur as a consequence of temporal variation in availability of major food resources, especially if reproduction is opportunistic and closely tied to food abundance. In this context, our observations on the physical and reproductive condition of individuals in our samples may be relevant. Many appeared to be emaciated, and, in May, only 14 females guarding broods were seen at three localities, although we specifically searched for such females (they are conspicuous due to the white coloration they assume). In addition, only a few adult females had enlarged ova or showed signs of recent breeding. Gonads were generally small and clearly in nonbreeding condition, at a time of the year when most Temperate Zone fishes are breeding.

Sexual dimorphism associated with trophic diversification has been abundantly documented in vertebrates (22–25), but apparently the only previous consideration of non-sexual trophic polymorphism is that of Roberts (26), who suggested that five taxonomic species of freshwater fishes of the genus *Saccodon*, also based on dentition, are morphs. The demonstration of trophic polymorphism in the Cuatro Cienegas cichlids has immediate importance with respect to evolutionary interpretations of the extensive and well known morphological differentiation and trophic radiation of cichlids in the rift valley and other lakes in Africa (4). In Lake Victoria, for example, from 150 to 170 species are believed to have evolved in a period of 750,000 years, and numerous theories relating to mechanisms of speciation have been proposed (review in ref. 5). Our findings for the Cuatro Cienegas cichlids raise the possibility that much of the adaptive radiation in the African cichlids similarly has been achieved through polymorphism rather than by speciation, and emphasize the need for a genetic approach to the problem.

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