GENETIC DIFFERENTIATION IN SPECIOSE VERSUS DEPAUPERATE PHYLADS: EVIDENCE FROM THE CALIFORNIA MINNOWS

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The systematics of cyprinid fishes native to California has had a chaotic history. Considerable controversy exists about the number of species, their placement in genera, and the evolutionary affinities among the genera (Uyeno, 1961; Hopkirk, 1973). About 16 species and 10 genera are recognized at present (Moyle, 1974). Five of these genera (*Orthodon*, *Mylopharodon*, *Hesperoleucus*, *Lavinia*, and *Pogonichthys*) are monotypic and restricted in distribution to a single drainage basin, the Sacramento-San Joaquin.

All cyprinids in North America are thought to belong to the subfamily Leuciscinae with a single possible exception; several subfamilies are recognized in the Old World (Miller, 1959). It is commonly accepted that relatively few cyprinid fishes migrated from their center of origin in Eurasia to North America by way of a Bering land bridge during the Miocene. Therefore, most cyprinid species in North America, including the California minnows, may share a relatively recent common ancestry. "Not only the lack of basic morphological diversity but also the readiness with which most American minnows hybridize (Hubbs, 1955) supports the evidence that the group has not been here long enough to develop strongly divergent lines" (Miller, 1959). It has, nevertheless, been suggested that close affinities exist between certain Western cyprinid genera (*Mylopharodon*, *Gila*) and those in China and Japan (see Miller, 1965).

We have analyzed patterns of genetic variability at 24 gene loci in species belonging to nine genera of cyprinids inhabiting California, using techniques of gel electrophoresis. These techniques provide considerable information to elucidate evolutionary relationships among closely related species (Avise, 1974). Our results suggest that at least four, and possibly five of the genera of California minnows are very similar in genic content and are probably of recent monophyletic origin. However, the other California minnows are less closely related, and the biochemical differences observed between these species may be typical of mean levels of divergence between other North American cyprinids.

Models are considered which describe amounts of genetic differentiation expected between species within a group. One model assumes that genetic distance between species is proportional to the time since they shared a common ancestor; the other model assumes that genetic distance is proportional to the number of cladogenetic events (speciations) in the evolutionary history of the group. Those models lead to distinct predictions of mean amounts of genetic distance between species in species-diverse versus species-depauperate phylads of equal evolutionary age. Our data on the California minnows suggest that mean time since divergence from a common ancestor is more important than mean number of cladogenetic events in the evolutionary history of a group of species as a predictor of levels of biochemical differentiation.

MATERIALS AND METHODS

Fish were collected by seine and immediately placed on dry ice. The nine species and the collection sites are listed in Table 1. The data for two species, *Hesperoleucus symmetricus* and *Lavinia exilicauda*, are taken from Avise et al. (1975).

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TABLE 1. *Samples of California minnows and estimates of genetic variability in a study of 24 gene loci coding for soluble proteins and enzymes.*

Species	Common name	Collection site	Mean % ± S.E. heterozygotes per locus	Percent polymorphic loci*	Mean no. alleles per locus	Individuals sampled
Hesperoleucus symmetricus	roach	Russian River, Mendocino Co.	6.67 ± 2.54	25	1.46	32
Lavinia exilicauda	hitch	Bass Lake, Madera Co.	4.80 ± 2.38	17	1.21	48
Mylopharodon conocephalus	hardhead	Chowchilla River, Madera Co.	0.62 ± 0.62	4	1.04	60
Ptychocheilus grandis	Sacramento squawfish	Russian River, Mendocino Co.	1.13 ± 1.13	4	1.13	28
Orthodon microlepidotus	Sacramento blackfish	Clear Lake, Lake Co.	1.50 ± 1.13	4	1.08	23
Pogonichthys macrolepidotus	splittail	San Joaquin River, Contra Costa Co.	3.57 ± 1.07	8	1.13	7
Richardsonius egregius	Lahontan redside	Sagehen Creek, Placer Co.	3.00 ± 2.39	8	1.13	22
Gila bicolor	tui chub	Lake Crowley, Mono Co.	5.92 ± 2.77	21	1.25	24
Notemigonus crysoleucus	golden shiner	Bass Lake, Madera Co.	6.80 ± 2.92	21	1.29	15

* Frequency most common allele $\leqslant 0.95$.

The electrophoretic techniques used have been previously described (Avise et al., 1975). Basically these procedures consist of placing samples of muscle or liver extract from individual fish into starch gels, and applying electric currents to the gels. Proteins migrate through the gels according to their net charge; their position is visualized with specific staining mixtures. A total of 24 loci have been studied in every species; 12 enzymes are encoded by 19 gene loci and five nonenzymatic proteins are encoded by five substitutions per locus which have accumulated since any two populations diverged from a common ancestor.

GENETIC VARIATION WITHIN SPECIES

Little or no allelic variation is found at most of the 24 loci in any species. If a locus is considered polymorphic when the most common allele has a frequency no greater than 0.95, the number of polymorphic loci ranges from one (4.2% in *Mylopharodon conocephalus*, *Orthodon microlepidotus* and *Ptychocheilus grandis*)

TABLE 2. *Allele frequencies at variable loci in nine species of California minnows. Enzyme abbreviations given by Avise et al. (1975). Alleles are designated according to differences in electrophoretic mobility, with the common allele in* Hesperoleucus *usually designated 100.*

Species	Allele (frequency)					
Hesperoleucus symmetricus	*Ldh-1*	*Ldh-2*	*Gpd-1*	*Pgi-1*	*Ipo-1*	*Est-2*
	100(0.70)	100(0.86)	300(0.95)	110(0.03)	100(0.70)	100(0.91)
	85(0.30)	0(0.14)	100(0.05)	100(0.95)	50(0.30)	97(0.09)
				10(0.02)		
	Tpi-1	*Mdh-3*	*Pgm-1*	*Got-2*		
	100(0.98)	100(0.99)	110(0.02)	−100(0.97)		
	50(0.02)	0(0.01)	100(0.98)	−20(0.03)		
Lavinia exilicauda	*Ldh-1*	*Pgi-2*	*Pgm-1*	*Ipo-1*	*Est-2*	
	100(0.80)	200(0.13)	100(0.68)	150(0.18)	103(0.01)	
	85(0.20)	100(0.87)	90(0.32)	100(0.82)	100(0.99)	
Mylopharodon conocephalus	*Gpd-1*					
	100(0.92)					
	75(0.08)					
Ptychocheilus grandis	*Adh-1*	*Pgm-1*	*Ipo-1*			
	100(0.90)	100(0.04)	150(0.02)			
	90(0.10)	90(0.96)	100(0.98)			
Orthodon microlepidotus	*Pgi-2*	*Gpd-1*				
	100(0.80)	300(0.02)				
	10(0.20)	200(0.98)				
Pogonichthys macrolepidotus	*Mdh-1*	*Pgi-2*				
	85(0.92)	98(0.43)				
	70(0.08)	0(0.50)				
		−10(0.07)				
Richardsonius egregius	*Pgi-1*	*Est-2*	*Pgm-1*			
	120(0.39)	97(0.91)	110(0.98)			
	110(0.61)	95(0.09)	00(0.02)			
Gila bicolor	*Mdh-2*	*Idh-2*	*Ipo-1*	*Est-2*	*Pt-3*	*Gpd-1*
	100(0.83)	100(0.94)	50(0.94)	100(0.27)	100(0.52)	100(0.99)
	75(0.17)	90(0.06)	10(0.06)	97(0.73)	80(0.48)	75(0.01)
Notemigonus crysoleucus	*Pgd-1*	*Pgi-1*	*Ipo-1*	*Est-1*	*Pt-3*	*Pgi-2*
	175(0.74)	120(0.20)	175(0.87)	100(0.95)	120(0.50)	100(0.04)
	160(0.23)	110(0.80)	125(0.13)	98(0.05)	100(0.50)	10(0.96)
	140(0.03)					

genetically variable loci are shown in Table 2.

The levels of genetic variation found in the California minnows are within the low part of the range characteristic of vertebrate species (Selander and Kaufman, 1973). Three species (*Mylopharodon conocephalus*, *Ptychocheilus grandis*, and *Orthodon microlepidotus*) have particularly low heterozygosities. But little significance should be attached to the different levels of genetic variation among the species. The confidence of the estimates is low since so few loci contribute to the heterozygosity estimates, and the numbers of individuals sampled are small.

Little genetic differentiation exists between populations of a given species. Avise et al. (1975) have studied geographic variation within the species *Hesperoleucus symmetricus* and *Lavinia exilicauda*, and have found little differentiation among conspecific populations even when separated by considerable distances. Moreover,

TABLE 3. *Matrix of genetic distances (above diagonal) and genetic similarities (below diagonal) between species of California minnows, based on 24 loci. Distances and similarities calculated using the method of Nei (1972).*

		1	2	3	4	5	6	7	8	9
1)	*Hesperoleucus*	—	0.055	0.095	0.194	0.518	0.705	0.432	0.251	0.901
2)	*Lavinia*	0.948	—	0.147	0.216	0.616	0.746	0.519	0.354	0.919
3)	*Mylopharodon*	0.909	0.863	—	0.131	0.546	0.600	0.453	0.174	0.790
4)	*Ptychocheilus*	0.824	0.806	0.877	—	0.541	0.600	0.526	0.333	0.989
5)	*Orthodon*	0.596	0.540	0.579	0.582	—	1.079	0.776	0.518	1.094
6)	*Pogonichthys*	0.494	0.474	0.549	0.549	0.340	—	0.519	0.679	1.118
7)	*Richardsonius*	0.649	0.595	0.636	0.591	0.460	0.595	—	0.443	0.976
8)	*Gila*	0.778	0.702	0.840	0.717	0.596	0.507	0.642	—	0.884
9)	*Notemigonus*	0.406	0.399	0.454	0.372	0.335	0.327	0.377	0.413	—

we have sampled a second population of *Ptychocheilus grandis*, from the Chowchilla River, Madera County. The two populations of this species share common alleles at all loci studied; their genetic similarity is $I = 0.99$. This situation agrees with what has been generally found in studies of allozyme variation in many vertebrate and invertebrate species: geographic populations of a given species are genetically very similar, yielding similarity coefficients usually greater than 0.95 (review in Avise, 1974). Therefore, for purposes of interspecific comparisons, a single population provides an adequate representation of a species.

DIFFERENTIATION BETWEEN SPECIES

Table 3 gives the estimates of genetic similarity, I, and genetic distance, D, (calculated according to the method of Nei, 1972) between all species pairs. They spread over a broad spectrum ranging from $D = 0.055$ (*Hesperoleucus symmetricus* versus *Lavinia exilicauda*) to $D = 1.118$ (*Pogonichthys macrolepidotus* versus *Notemigonus crysoleucus*). The average value of D for all pairwise comparisons is 0.57 ± 0.05; i.e., on the average about 0.57 electrophoretically detectable allelic substitutions per locus have occurred in the separate evolutions of any two species.

Figure 1 shows the distribution of genetic similarities among loci. Pairs of species are, on the average, essentially identical ($I \geqslant 0.95$) at nearly 60% of the loci, and completely different ($I \leqslant 0.05$) at about 30% of the loci. Few loci have genetic similarities in the broad range between 0.05 and 0.95. This U-shaped distribution of genetic similarities is often found in comparisons between closely re-

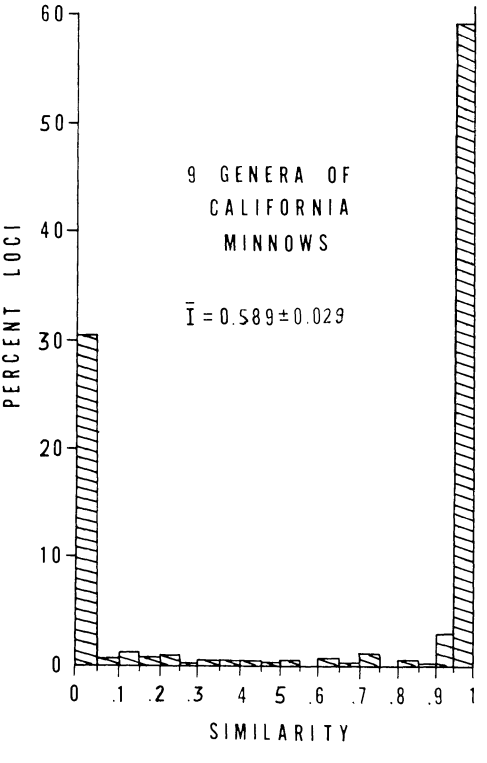

FIG. 1. Percentage of loci within a given range of genetic similarity values in the comparisons among species belonging to nine genera of California minnows.

lated species, whether they are relatively monomorphic like the California minnows, or very polymorphic like the species of the *Drosophila willistoni* group (Ayala et al., 1974*a*,*b*). This bimodal distribution of loci with respect to genetic similarity has bearing on the question of the sampling strategies to be followed when estimating genetic differentiation between closely related species. At a given locus any two species are either essentially identical in allelic configuration, or else completely distinct. Therefore, it is not essential to sample many individuals at each locus, since a few individuals provide nearly as much information as large numbers. On the other hand, the bimodal distribution of genetic similarities makes it important that as many loci as possible be sampled. To put this matter differently, with respect to genetic similarities the variance about individuals is small relative to the variance about loci; therefore, the precision of the estimates is much more dependent in the number of loci than on the numbers of individuals sampled. In our study, although several minnow species are represented by few individuals, the number of loci sampled (24) is fairly large.

To a first approximation, phylogenetic relationships can be estimated from genetic or phenotypic information. Methods exist for clustering groups of organisms when a set of measurements have been made in each group, and there is no reason to weight any measurement more than others. The genetic information provided by electrophoretic studies is of this kind. We have evaluated the information contained in Table 3 using three such methods: (1) unweighted pair group method with arithmetic means or UPGMA (Sneath and Sokal, 1973); (2) weighted pair group method with arithmetic means or WPGMA (Sneath and Sokal, 1973); and (3) a modification of Wagner's tree method (Farris, 1972). We shall briefly discuss these clustering techniques since they give somewhat different dendrograms when applied to our data.

The UPGMA and WPGMA are simple agglomerative clustering procedures, cycling repeatedly through the data matrix and admitting for membership to previously formed clusters the single entity (a species in our case) exhibiting the highest level of similarity to a cluster. In UPGMA, the average similarity of a candidate species to an extant cluster is determined by weighting equally each species in the extant cluster. WPGMA differs from UPGMA by weighting the species most recently joined to the cluster equal to all previous species in the cluster. UPGMA and WPGMA imply that the resulting dendrograms represent estimates of evolutionary trees only if overall rates of evolutionary divergence are homogeneous in different phyletic lines. Although there is no a priori reason for preferring one method over another, the relative fits of the dendrograms to the original matrix (Table 3) may be computed as "cophenetic" correlation coefficients. From our data, the cophenetic correlations for UPGMA and WPGMA are 0.96 and 0.93, respectively, indicating that both methods distort little the information in the similarity matrix.

The Wagner tree method relaxes the assumption of homogeneous evolutionary rates, and, indeed, has been used to test whether evolutionary rates are homogeneous. This method results in the formation of most parsimonious trees, that is, trees with minimum length (defined as the minimum sum of differences between all nodes forming endpoints of branches). Divergence between species estimated by the Wagner procedure are greater than or equal to observed differences between species. The cophenetic correlation of the Wagner tree with our data matrix is 0.99.

Figure 2 shows the dendrogram obtained using the UPGMA method of clustering. The dendrogram obtained by the WPGMA method is virtually identical to that, except for the positions of *Pogonichthys* and *Orthodon* which are reversed. The dendrogram obtained by the modified Wagner tree method is shown in Figure 3. All three

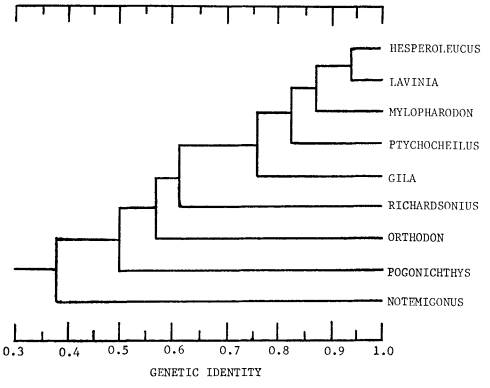

FIG. 2. Dendrogram of California minnows based on the unweighted pair-group method with arithmetic means (UPGMA). Scale is in units of genetic identity, *I*. The cophenetic correlation equals 0.96. The dendrogram based on the weighted pair-group method (WPGMA) is virtually identical, except that the positions of *Pogonichthys* and *Orthodon* are reversed.

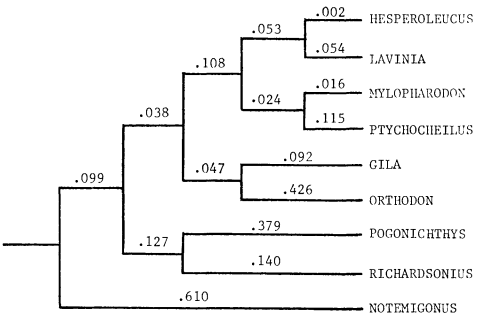

FIG. 3. Phylogenetic tree of California minnows based on the modified Wagner tree method. The tree is rotated so the root occupies the midpoint of the longest path connecting a pair of species on the tree, that connecting *Notemigonus* to *Orthodon*. Numbers indicate presumed amounts of evolution in various branches and are in units of genetic distance, *D*.

methods of clustering give similar results for the five species genetically most similar. Therefore our phylogenetic interpretations are most reliable where they involve the closely related upper clusters in the dendrograms. We now discuss the phylogenetic affinities between the minnow species based on our estimates of biochemical similarity, in relation to previous interpretations based on morphology, geographic distribution, and the fossil record.

Hesperoleucus symmetricus (*roach*) *and* Lavinia exilicauda (*hitch*).—These two species are endemic to the Sacramento-San Joaquin drainage and have no known fossil record. Adults differ in a number of apparently independent morphological features, including body size and shape, number of anal and dorsal rays, and pharyngeal tooth formula (Avise et al., 1975). Nonetheless, this morphological differentiation has occurred within the context of very little overall genetic divergence (D = 0.055). The close biochemical similarity between *Hesperoleucus* and *Lavinia* most likely evinces a relatively recent speciation, which probably occurred in the Sacramento-San Joaquin basin. These two minnows should be considered congeners. For a more complete discussion of these species, see Avise et al., 1975.

Mylopharodon conocephalus (*hardhead*) *and* Ptychocheilus grandis (*Sacramento squawfish*).—These two species are very similar in external morphology, but can be separated on the basis of head shape and presence of a frenum connecting the middle of the premaxilla to the head in *Mylopharodon*. Hardhead and Sacramento squawfish are endemic to the Sacramento-San Joaquin drainage and other minor basins formerly connected to it. *Ptychocheilus* contains three described species besides *grandis*; *oregonensis* primarily in the Columbia River drainage, *umpquae* in the Sinslaw and Umpqua drainages in Oregon, and *lucius* in the Colorado River. Fossils assigned to *Ptychocheilus* are known from the early Pliocene in Oregon (Shotwell, 1963) and Middle Pliocene in Arizona (Uyeno and Miller, 1965). *Mylopharodon* is presently a monotypic genus. Fossils assigned to "*Leucus*" from Middle Pliocene deposits in Idaho are similar to living hardhead, and may be referable to *Mylopharodon* (Uyeno and Miller, 1963; Miller, 1965).

These two genera are quite similar in genic constitution (I = 0.88). This high level of similarity seems surprising in view

of the possible age of these genera as evinced by their widespread geographic distributions and by the fossil record. Comparable levels of biochemical similarity between widespread sympatric species are unusual, but have been described in the genus *Peromyscus* (Avise et al., 1974). Nonetheless, the close morphological similarity between *Mylopharodon* and *Ptychocheilus* led Hopkirk (1973) to argue that the two are similar enough to be combined into one genus. The biochemical data support this conclusion. The hardhead and the Sacramento squawfish are genically also closely similar to *Hesperoleucus* and *Lavinia* (see Table 3 and Figs. 2 and 3).

Gila bicolor (*tui chub*).—This species is found in California in the Klamath, Lahontan, Owens Valley, and Sacramento-San Joaquin drainages. It belongs to a rather large genus with at least 17 described species distributed throughout the western United States and northern Mexico. Fish resembling *Gila* are reported from Mid-Miocene (Miller, 1965), making it the oldest known cyprinid genus in North America.

The systematic relationships of the genus *Gila* are controversial. Members of *Gila* are known to hybridize with *Lavinia* (Miller, 1963) and *Richardsonius* (Hopkirk and Benke, 1966). Hopkirk (1973) believes that *Hesperoleucus* is a creek-adapted form derived from the *Gila* phyletic line. Uyeno and Miller (1965) feel that *Pogonichthys* was also derived from a *Gila*-like ancestor, but Hopkirk (1973) disputes this claim. Uyeno (1961) examined osteological relationships among three genera and concluded that *Ptychocheilus* diverged earlier from a line leading to *Gila* and *Richardsonius*.

On the basis of biochemical information, *Gila* appears most similar to the *Lavinia-Hesperoleucus-Ptychocheilus-Mylopharodon* group. It seems possible that these four genera were derived from a *Gila*-like ancestor, but the study of other species of *Gila* appears necessary to confirm these inferences.

Richardsonius egregius (*redside*).—*Richardsonius* now contains four species in the western United States, and is thought to be closely allied to the eastern genus *Clinostomus*. As pointed out above, our confidence in determining the phylogenetic relationships among the minnows decreases as similarity values become lower. Thus, *Richardsonius* is placed in different positions by the PGMA and the Wagner tree methods (Figs. 2 and 3). In any event, *Richardsonius* appears slightly outside the range of similarities found between the five previously discussed genera (Table 2), although it is somewhat more similar to *Gila* and its derivatives than are *Orthodon* and *Pogonichthys*. Our data do not support (nor do they strongly deny) Uyeno's (1961) conclusion that *Richardsonius* diverged from the *Gila* line after *Ptychocheilus*.

Orthodon microlepidotus (*blackfish*) *and* Pogonichthys macrolepidotus (*splittail*).—These two minnows are biochemically quite distinct from each other and from the previously discussed species. Their position in the dendrograms relative to each other and to *Richardsonius* changes, depending on the method of clustering used. Both species are endemic to the Sacramento-San Joaquin drainage and have no fossil record. Hopkirk (1973) believes that the original cyprinid invader of North America was similar to *Pogonichthys* in general morphology, and that *Pogonichthys* was not derived from a *Gila*-like ancestor. Jordon and Evermann (1896) feel that species with straight teeth in one series, such as *Orthodon*, are more primitive. Our findings are consistent with other authors' interpretations that *Pogonichthys* and *Orthodon* are not closely similar to other California minnows.

Notemigonus crysoleucus (*golden shiner*).—This minnow is not native to California, but has been introduced from the eastern United States. *Notemigonus* may be the only North American member of the Abramidinae, primarily an Old World group (Miller, 1959). It is also the

biochemically most distinct minnow examined. It is well differentiated from the native California minnows, exhibiting an average of about one allelic substitution per locus relative to the other species (Table 2). If *Notemigonus* does indeed belong to the Abramidinae, its genetic differentiation from the North American Leuciscinae (such as the California minnows) should be representative of the degree of differentiation occurring between the most divergent New World minnows.

Phyletic Evolution versus Cladogenesis

As discussed earlier, most of the 250 species of North American minnows belong to the Leuciscinae, a subfamily which first appears in the fossil record of North America in the Miocene. The California minnows share geographic range but are not necessarily a monophyletic assemblage. It seems likely that at least some of the species may have their closest relatives among the eastern United States fauna (i.e., *Richardsonius* with *Clinostomus*). Furthermore, *Notemigonus* is possibly a member of a different subfamily, the Abramidinae; compared to other California minnows *Notemigonus* may be nearly as distant phylogenetically as the most separated North American minnows. As a group the California minnows may be a fairly representative sample of levels of divergence among the North American minnows.

The North American minnows are a highly species-diverse group, whose evolutionary origin has been traced to the Miocene (see above). Most of the California minnows examined occur sympatrically in at least some localities in California and still retain their identities. The species status of all species in the study is well established even for the species most closely related in every aspect, *Hesperoleucus symmetricus* and *Lavinia exilicauda* (Avise et al., 1975). How much genetic differentiation has occurred among these minnows, compared with species-depauperate groups? An important question in evolutionary genetics is whether a high degree of speciation (splitting or cladogenesis) is the result of rapid genetic change (phyletic evolution or anagenesis). To formulate the question differently: are rates of cladogenesis or splitting closely correlated with rates of anagenesis or phyletic evolution?

Avise and Smith (1974) have studied genetic differentiation at 14 loci among species of another group of fishes, the centrarchid genus *Lepomis*. This genus consists of 11 extant species, all endemic to North America. *Lepomis* has never contained many species, and appears in the fossil record at the Miocene-Pliocene border (Miller, 1965). The genus *Lepomis*, therefore, represents a species-depauperate group relative to the species-rich North American minnows. Although the phyletic evolution of both groups extends for similar lengths of time, fewer cladogenetic events have taken place in *Lepomis* than in the minnows.

Avise and Ayala (1975) have formulated theoretical models predicting relative levels of genetic differentiation in speciose versus depauperate groups, under two competing assumptions: (1) genetic differentiation is a function of time, unrelated to the number of cladogenetic events (Model 1) and (2) genetic differentiation is proportional to the number of cladogenetic events in the group (Model 2). These models provide qualitatively distinct predictions about levels of genetic divergence depending upon the relationship between rate of speciation and amount of genetic change. When genetic distance is a function of time, mean genetic distances between species in speciose and depauperate phylads of comparable evolutionary age are very similar. On the contrary, when genetic distance is a function of the number of speciations in the history of a phylad, members of speciose phylads are much more distinct than members of depauperate phylads, on the average, and the ratio of mean distances increases as the frequency

of speciation events in one group relative to the other becomes greater.

The two models are, of course, oversimplifications. We do not expect genetic distances between species to be solely a function of time since divergence, or of number of cladogenetic events in their history. Genetic distances almost certainly reflect both of these factors, as well as the particular selective regimes faced by the populations, the population sizes maintained, and other parameters (Ayala, 1974; Ayala and Gilpin, 1975). Nonetheless, if greater numbers of speciation events reflect a higher average rate of genetic evolution, we would expect to find greater mean distances between species in diversified than in depauperate phylads of comparable evolutionary age. For example, we should find mean genetic distances to be greater among the speciose Leuciscinae than among the depauperate *Lepomis*.

Using the data of Avise and Smith (1974), we have calculated genetic similarity (I) and genetic distance (D) between 10 of 11 extant species of *Lepomis* according to the method of Nei (1972). For all pairwise comparisons, the average genetic similarity is $I = 0.54$, with a range of 0.36 to 0.85; the average genetic distance is $D = 0.63$, with a range of 0.16 to 1.02. For the California minnows examined in the present study, $I = 0.59$ (range 0.33 to 0.95), and $D = 0.57$ (range 0.05 to 1.12); if we exclude comparisons among the four most closely related minnows, these statistics become $I = 0.54$, and $D = 0.65$. It is, of course, risky to compare amounts of genetic differentiation in groups as different as sunfish and minnows, particularly when different sets of loci are assayed. Nonetheless, our data certainly give no indication that representatives of the speciose minnows are genically more distinct than are members of the depauperate genus *Lepomis*. If anything, mean similarity appears slightly higher among the minnows than among *Lepomis* species. Furthermore, the maximum D observed between any two species in the two groups are very similar. Maximum $D = 1.12$ and 1.02, for the minnows and *Lepomis* respectively.

Our results do not agree with the predictions derived from model 2, i.e., that the rate of genetic evolution is proportional to the amount of cladogenesis. The mean as well as the maximum genetic distances between species are very similar in both groups, the speciose minnows, and the depauperate *Lepomis*. These results are in much better agreement with the predictions of model 1, namely that genetic divergence is approximately constant through time independently of the amount of cladogenesis.

The reliability of this test of the models rests heavily upon the assumption that levels of genetic divergence between the California minnows are representative of mean levels of divergence between North American minnows. We have earlier given arguments showing why this may be a reasonable assumption. However, we know very little about the specific evolutionary histories of the California minnows, nor can we be certain about the true number of cladogenetic events in the histories of *Lepomis* or the minnows since the Miocene, although this number is probably greater in the minnows. Although minnows comprise the largest number of known fossil species of any group of fishes in North America, the record is far from complete. The evolutionary origin of the North American minnows presently traces back to the mid-Miocene; thus they are as old or older than the genus *Lepomis* whose evolutionary origin is dated from the Miocene-Pliocene boundary. It appears that the minnows are not evolving genetically at a faster rate than *Lepomis*. Nevertheless, the predictions of the models should definitely be tested in other organisms as well. Among fishes, a particularly promising group for comparison to the depauperate *Lepomis* is the highly species-diverse minnow genus *Notropis*, apparently dating from mid-Pliocene (Miller, 1965).

The finding of lack of correlation between amount of genetic differentiation and number of speciation events is, of course, hardly surprising. There are many well known instances of high rates of anagenetic change with little cladogenesis, and vice versa. In the evolution of the genus *Homo*, a great deal of anagenesis with respect to morphological, behavioral and other traits has taken place, while no cladogenetic events seem to have occurred after that genus evolved from *Australopithecus*. Groups of sibling species, which are common in insects as well as in other organisms, are examples of the opposite situation: speciation with little or no morphological differentiation.

Discussion

An important problem currently discussed by evolutionists is "How much genetic differentiation accompanies the process of speciation?" Two general survey strategies have been employed to answer this question. The first method is most direct; it involves sampling populations at stages when reproductive isolation, and hence speciation, is being completed. The most thorough study of populations at these stages of divergence is that by Ayala and coworkers on the *Drosophila willistoni* group (review in Ayala et al., 1974*b*). The generally accepted model of geographic speciation applies to the group. Ayala et al. (1974*b*) find a substantial degree of genetic differentiation during the first stage of speciation, when isolated allopatric populations show evidence of partial reproductive isolation; some 0.23 electrophoretically detectable allelic substitutions per locus had taken place between populations representative of this stage. Similar amounts of genetic differentiation have been found in other groups of *Drosophila* flies; for example, about 0.19 electrophoretically detectable substitutions per locus exist between the subspecies *D. pseudoobscura pseudoobscura* and *D. p. bogotana* (Ayala and Dobzhansky, 1974; for other *Drosophila* species see Zouros, 1973). Hunt and Selander (1973; see also Selander et al., 1969) found that two subspecies or semispecies of *Mus musculus* differ genetically as much as the *Drosophila* subspecies.

A different situation obtains during the second stage of geographic speciation, when reproductive isolation is being completed under the influence of natural selection acting upon genetically different populations which have regained geographic contact. In the *D. willistoni* group, Ayala et al. (1974*b*) found that populations in the second stage of speciation do not differ significantly more than populations in the first stage. It appears that sexual isolation or other pre-zygotic isolating mechanisms may develop without changing a substantial proportion of structural genes.

The second survey strategy employed to answer the question, "How much genetic differentiation accompanies speciation?" involves sampling species which by other criteria appear especially closely related. Since species exhibit genetic differences which have accumulated subsequent to, as well as during the speciation process, the most telling results will be those at the low end of the distribution of genetic differences. The rationale is that if enough cases are examined, examples may be found of species which differ little in genic content, and hence, in which speciation has involved little genic change. Results of such surveys have generally indicated that species which appear closely related, such as sibling (morphologically nearly indistinguishable) species and hybridizing species, are completely distinct in allelic composition at about one third to one half of their loci (review by Avise, 1975). Such results appear compatible with the hypothesis that significant genetic divergence accompanies the development of reproductive isolation.

We have found that mean genetic differentiation between species is no greater in a species-rich group, the North American minnows, than in a depauperate group, the genus *Lepomis*. This finding is incompatible with two hypotheses: (1) that

increased degrees of cladogenesis are the result of higher rates of genetic evolution; and (2) that the process of speciation *per se* (i.e., development of reproductive isolating mechanisms) involves allelic changes at a substantial proportion of structural gene loci. If speciation *per se* were to involve much genetic change, we would expect to find on the average greater genetic differentiation in speciose groups (where the number of speciation events is greater) than in depauperate groups.

Moreover we have found that the genetic distance between the two most similar species in our study, *Hesperoleucus symmetricus* and *Lavinia exilicauda*, is fairly small, $D = 0.055$; i.e., these two species differ by about 5.5 allelic substitutions for every 100 loci. We have shown elsewhere that these two species are, indeed, "good" species (Avise et al., 1975). Although 5.5% of the genome represents a large number of loci (1,650 if we assume a genome size of 30,000 gene loci), this value is much lower than the average of 20–25 allelic substitutions per 100 loci observed during speciation in species of *Drosophila* and in *Mus*.

Other cases of speciation involving little change in structural genes have been reported. Speciation in gophers of the genus *Thomomys* has been accomplished apparently through extensive remodeling of karyotypes by Robertsonian fusions and fissions, as well as other chromosomal rearrangements with little structural gene divergence (Nevo et al., 1974). Among diploid plants, an instance of speciation involving a change in reproduction from obligate outcrossing to self-pollinating has taken place with very little structural gene change (Gottlieb, 1973).

Speciation, or cladogenetic evolution, occurs not only according to the general model of geographic speciation, but also by a variety of other processes, including chromosomal reorganization, changes in reproductive system, polyploidy and others. Some of these modes of speciation may involve little change in structural genes.

The process of geographic speciation is likely to involve changes in both structural genes and regulatory genes. Wallace (1963), Stebbins (1969), Britten and Davidson (1969; Davidson and Britten, 1973), and others have pointed out that regulatory genes may play a crucial role in evolution. Most recently, Wilson et al. (1974*a*,*b*) have suggested that adaptive evolution and speciation may depend more on changes in gene regulation than on amino acid substitutions in protein sequences. Nevertheless, a wealth of direct and inferential evidence exists showing that alleles of structural genes are subject to selection, and that they become organized in coadapted genomes (see, e.g., Dobzhansky, 1970; Prakash and Lewontin, 1968; Clegg et al., 1972; Ayala, 1972, 1974). Moreover, we should keep in mind that the genes studied by electrophoresis are for the most part involved in basic cell metabolism. Other kinds of structural genes may be equally or more important than electrophoretic loci in producing morphological and ecological differentiation and reproductive isolation. Instances of speciation without much change in genes studied by electrophoresis do not necessarily imply that few changes have taken place in structural genes. Nevertheless, gene regulation must play an important role in evolutionary change in general, and speciation in particular.

Summary

We have examined electrophoretic variation in proteins encoded by 24 gene loci in natural populations of nine genera of minnows (family Cyprinidae) endemic to waters of California. The mean proportion of polymorphic loci per population is 12.5%, and the mean frequency of heterozygotes per locus is $3.78 \pm 0.80\%$. These levels of genetic variation are within the low part of the range characteristic of vertebrate species.

Average genetic distance, D, for all pairwise comparisons among species is 0.57, i.e., about 57 allelic substitutions, on the average, are estimated to have occurred for

every 100 loci in the separate evolution of any two species. At least four genera (*Hesperoleucus, Lavinia, Mylopharodon,* and *Ptychocheilus*) are genetically very similar, and have probably evolved from a relatively recent common ancestor. The other genera are less similar; levels of genetic differentiation among them may be fairly representative for the very species-diverse North American minnows. We have also calculated the mean genetic distance among 10 of the 11 known species of the genus *Lepomis*; this is $D = 0.63$. The North American minnows and *Lepomis* are of approximately equal evolutionary age, although the minnows are highly speciose (about 250 species), while *Lepomis* is relatively depauperate (11 species).

To compare the amount of genetic differentiation in a speciose group and a depauperate group, we have considered two alternative models: (1) genetic differentiation is a function of time, unrelated to the number of cladogenetic events; (2) genetic differentiation is proportional to the number of cladogenetic events in the group. According to model 1, the values of D are approximately equal in speciose and depauperate phylads of comparable age. However, according to model 2, the value of D is substantially greater in a speciose than in a depauperate phylad. Our findings of about equal average amount of genetic differentiation in the speciose minnows and in the depauperate *Lepomis*, support the notion that time since divergence from a common ancestor is more important than the number of intermediate cladogenetic events in determining the level of genetic divergence between species. Apparently, the development of reproductive isolating mechanisms *per se* does not involve change at a substantial proportion of structural genes.

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