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Genic heterozygosity and rate of speciation

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Abstract.—The hypothesis is proposed that mean level of heterozygosity is functionally related to rate of speciation in evolutionary phylads. Under this hypothesis, phylads which speciate more rapidly do so because of increased level of within-species genetic variability which is then available to conversion to species differences under appropriate ecological or environmental conditions. An important corollary is that rate of speciation could be limited in phylads with low genetic variability, irrespective of environmental considerations.

This hypothesis has been tested with respect to electrophoretically detectable variation in products of structural genes in two families of North American fishes characterized by grossly different rates of speciation. Totals of 69 species of the highly speciose Cyprinidae, and 19 species of the relatively depauperate Centrarchidae, were assayed for mean level of heterozygosity at 11–24 genetic loci. Since Cyprinidae and Centrarchidae exhibit on the average nearly identical levels of genic variation ($\hat{H} = 0.052 \pm 0.004$, and $\hat{H} = 0.049 \pm 0.009$, respectively), the hypothesis that level of heterozygosity affects rate of speciation in these fishes is not supported.

Nonetheless, the amount of genic variability in both Cyprinidae and Centrarchidae is large, comparable to mean levels in previously studied vertebrates. The great wealth of genome variability, reflected in the electrophoretic variation present in virtually all outcrossing organisms, apparently can accommodate considerable flexibility in rate and pattern of evolutionary response to the various environmental regimes challenging organisms.

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Introduction

Rates of appearance of new kinds of organisms (such as species, genera, or families) are clearly heterogeneous thru evolutionary time and across evolutionary phylads (Simpson 1944). To many evolutionists in the early part of this century, mutation rates were an important limiting factor in the origin of new taxa. Hence, "At first thought it might seem obvious that periods of explosive radiation should be attributed to an increased rate of mutation" (Rensch 1959, p. 103). This hypothesis was gradually abandoned (though never strongly tested and falsified) as evidence accumulated that stores of genetic variability were normally great in most populations, and in any generation of sexual reproducers, the variability generated by recombination far surpassed novel variation introduced by mutations. As expressed by Huxley (1942, p. 517): "limits (to evolution) are relative to the environmental situation: if this is radically altered, evolutionary radiation may again set in, showing that the previous standstill was not due to lack of genetic variability"; and by Rensch (1959, p. 104): "mutation at normal rates provides such a wealth of variants that we need assume no further increase of mutation, but only a more intense selection, for . . . rapid adaptive radiation."

Recent views emphasize organism-environment relationships as the principal factors controlling species proliferation (Grant 1963; Hutchinson 1959). Thus adaptive radiations often occur when a species enters an unoccupied habitat with diverse "open niches," or when a population acquires a new complex of adaptive characters enabling it to exploit an available environment more efficiently (Stebbins 1971). The possibility that general genetic factors, apart from historically unique acquisitions, might exert a profound influence on rate of speciation has been largely ignored. Notable exceptions are the suggestions of Mayr (1963) that evolutionary rates are strongly affected by degree of genetic buffering or homeostasis and Carson's (1959, 1968) early hypothesis that genetic systems with open or free recombination under certain conditions promote species formation.

TABLE 1. Taxonomic and evolutionary information on minnows (Cyprinidae) and sunfish (Centrarchidae). Information from Branson and Moore (1962), Gosline (1971, 1974), Miller (1959, 1965), and Romer (1966).

Attribute	Cyprinidae	Centrarchidae
1) # living species worldwide	≈ 2000	≈ 30
2) # living genera worldwide	≈ 250	≈ 9
3) # living N. Amer. species	≈ 200	≈ 30
4) # living N. Amer. genera	≈ 40	≈ 9
5) # known fossil species in N. Amer.	$\geqslant 28$	$\geqslant 8$
6) # known fossil genera in N. Amer.	$\geqslant 16$	$\geqslant 6$
7) earliest known N. Amer. fossils	Miocene	Eocene (?)–Miocene
8) probable ancestors of N. Amer. forms	one or a few members of the subfamily Leuciscinae from Eurasia	forms similar to Serranidae (sea basses)

There is at least a minimal relationship between level of genetic variability within a species and its potential for speciation: rates of divergence among populations lacking genetic variability are no greater than rates of appearance of new mutations. Is the relationship between genetic variability and rate of speciation stronger than this? Lewontin (1974, p. 186) argues "This last point, that considerable evolutionary change (including speciation and divergence of new full species) occurs without being limited by the rate of appearance of novel genes is the chief consequence, for the process of speciation, of the immense array of genetic variation that exists in populations of sexually reproducing organisms." Evidence gathered in the last ten years, primarily through electrophoretic techniques, suggests that not all evolutionary phylads have similar levels of genic variation (Selander 1976; Powell 1976). The null hypothesis erected and tested in this paper is that there is no correlation between rate of speciation in an evolutionary phylad and the mean level of genic variability within its member species.

Two families of fishes characterized by greatly different rates of speciation were chosen for study: the highly speciose North American minnows (Cyprinidae) and the comparatively depauperate sunfish (Centrarchidae). Ancestors of both families entered the North American continent in the mid-Cenozoic and radiated into species which now occupy nearly all bodies of water on the continent. But the minnows are represented by many more living species than are the sunfish, and there are more fossil species of minnows as well. Evidence for the greater rate of speciation among the minnows is discussed in more detail elsewhere (Avise and Ayala 1976) and is summarized in Table 1. Since species of minnows (and sunfish) have been described primarily by morphological criteria, the conclusion of a higher rate of speciation among minnows is really a statement about a higher perceived rate of diversification into morphologically recognizable types of organisms. Biological species criteria may not be met in all cases. For purposes of the present study, we must simply regard North American Cyprinidae as an unusually rapidly speciating phylad. This should be true, even if the group has been "oversplit" somewhat by classical taxonomists because there is nearly an order of magnitude more recognized species of minnows than sunfish.

Using standard techniques of starch-gel electrophoresis, we have assayed levels of genic heterozygosity in populations belonging to a total of 69 species of North American Cyprinidae and in populations of an additional 19 species of Centrarchidae. A finding of significantly higher levels of heterozygosity among the minnows would support hypotheses causally linking genetic variability to rate of speciation. Conversely, a finding of comparable levels of heterozygosity in the two families would be consistent with modern interpretations that ecological factors are primarily responsible for varying rates of speciation.

Materials and Methods

Fish were frozen on dry ice immediately after capture and stored at –60°C until they could be processed and run, which was almost invariably within six months of time of collection. The horizontal starch-gel electrophoretic procedures are similar to those now routinely employed by many laboratories to assay levels of genic variation in a wide variety of organisms (for detailed procedures see Selander et al. 1971 and Ayala et al. 1972). Only those systems were scored which showed exceptionally clear banding patterns.

Different types of loci characteristically differ in mean levels of heterozygosity across species (Selander 1976). For example, esterase loci encode an unusually variable class of proteins. In order to obtain unbiased relative estimates of heterozygosity in sunfish versus minnows, an attempt was made to assay analogous (and often presumably homologous) proteins in the two groups. A total of 1429 specimens of 19 species of Centrarchidae was assayed at 11–15 genetic loci: lactate dehydrogenases (*Ldh*, 2 loci), isocitrate dehydrogenase (*Idh*), 6-phosphogluconate dehydrogenase (*6Pgd*), esterases (*Es*, 2 loci), malate dehydrogenase (*Mdh*), glutamate-oxalate transaminases (*Got*, 2 loci), tetrazolium oxidase (*To*), phosphoglucomutase (*Pgm*), phosphoglucose isomerases (*Pgi*, 2 loci), peptidase (*Pep*), and one non-enzymatic protein (*Pt*). A total of 499 specimens of 69 species of Cyprinidae was assayed at 14–24 genetic loci: *Ldh* (2 loci), *Idh*, *6Pgd*, *Es* (3 loci), *Mdh* (3 loci), *Got* (2 loci), *To*, *Pgm*, *Pgi* (2 loci), alcohol dehydrogenase (*Adh*), α-glycerophosphate dehydrogenase (*Gpd*), triosephosphate isomerase (*Tpi*), and five loci encoding nonenzymatic proteins. For more complete descriptions of these systems in sunfish see Avise and Smith (1974), and in minnows see Avise and Ayala (1976). Ten to eleven homologous loci were consistently scored in both the cyprinids and centrarchids (Fig. 1).

Several statistics may be employed to summarize levels of genic variability within a population. The *mean number of alleles per locus* suffers the major bias of being strongly dependent upon sample size; as more individuals are sampled, more rare alleles are found. The *proportion of polymorphic loci*

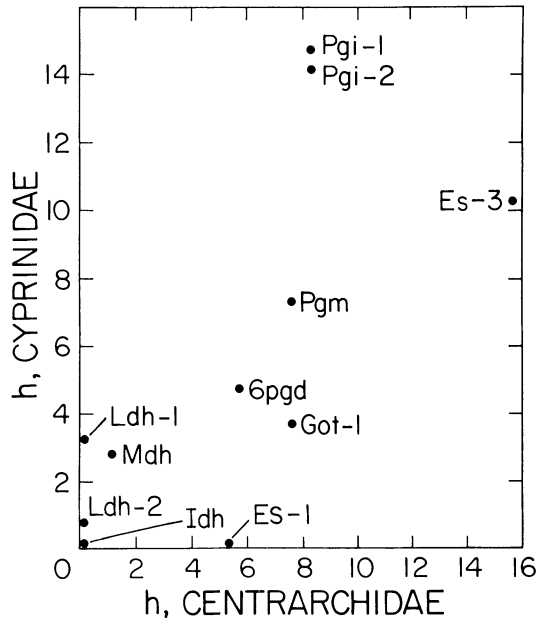

FIGURE 1. Heterozygosities at homologous loci assayed in both the Cyprinidae and Centrarchidae. The correlation is significant ($r = 0.69$, $df = 9$, $0.01 < P < 0.05$).

also has weaknesses: if a locus is considered polymorphic when two or more alleles are present, the proportion of polymorphic loci increases with increasing sample size; if more stringent criteria for polymorphism are used (i.e., frequency of common allele $\leqslant 0.95$), the proportion of polymorphic loci becomes partly dependent upon the particular criterion chosen. *Average heterozygosity*, for a population, $\overline{H}$, is defined as

$$\frac{1}{l}\sum_{i=1}^{i=l} h_i, \qquad (1)$$

where h_i is the proportion of individuals heterozygous at the ith locus and l is the number of loci. Since estimates of $\overline{H}$ are not dependent upon arbitrary criteria and are relatively unbiased with respect to sample size, they provide for purposes of the present study the most appropriate summaries of levels of genic variability (see Nei 1975).

Only eight genomes per species were examined in most of the 60 sampled species of Cyprinidae inhabiting the eastern United States, including members of the large genus *Notropis*, although sample sizes for most centrarchids and western cyprinids were con-

TABLE 2. Estimates of genic variability in North American Centrarchidae. Polymorphic loci defined by frequency most common allele $\leqslant 0.95$. Intra-locus and inter-locus variances calculated according to procedure of Nei and Roychoudhury (1974).

genus	species	# loci sampled	mean # alleles per locus	percent polymorphic loci	mean ± S.E. heterozygosity per locus	intra-locus variance	inter-locus variance
Lepomis	auritus	14	1.71	36	0.082 ± 0.036	.00129	.01685
Lepomis	cyanellus	14	1.21	21	0.083 ± 0.046	.00049	.02913
Lepomis	gibbosus	14	1.14	14	0.056 ± 0.039	.00038	.02091
Lepomis	gulosus	14	1.14	14	0.030 ± 0.022	.00080	.00598
Lepomis	humilis	14	1.28	14	0.046 ± 0.029	.00056	.01122
Lepomis	macrochirus	15	1.40	27	0.056 ± 0.040	.00076	.02164
Lepomis	marginatus	14	1.21	14	0.069 ± 0.049	.00027	.03334
Lepomis	megalotis	14	1.78	50	0.122 ± 0.051	.00175	.03467
Lepomis	microlophus	14	1.28	14	0.033 ± 0.017	.00040	.00365
Lepomis	punctatus	14	1.21	21	0.082 ± 0.045	.00056	.02779
Acantharchus	pomotis	11	1.09	9	0.029 ± 0.029	.00186	.00739
Ambloplites	rupestris	11	1.36	36	0.129 ± 0.061	.00180	.03913
Archoplites	interruptus	11	1.09	0	0.004 ± 0.004	.00011	.00007
Centrarchus	macropterus	11	1.00	0	0.000	–	–
Elassoma	evergladei	11	1.00	0	0.000	–	–
Elassoma	okefenokee	11	1.09	9	0.027 ± 0.027	.00053	.00750
Enneacanthus	obesus	11	1.00	0	0.000	–	–
Micropterus	salmoides	11	1.18	18	0.082 ± 0.055	.00046	.03281
Pomoxis	nigromaculatus	11	1.09	9	0.010 ± 0.010	.00042	.00068

siderably larger (up to 760 individual bluegill, *Lepomis macrochirus*). Single locus heterozygosities were calculated from allele frequencies using Hardy-Weinberg probabilities ($h_i = 1 - \Sigma\chi^2_j$, where χ_j is the frequency of the jth allele at the locus).

The variance of h, given by

$$V(h) = \frac{1}{l-1} \sum_{i=1}^{i=l} (h_i - \overline{H})^2, \qquad (2)$$

was partitioned into the inter-locus and intra-locus components according to procedures developed by Nei and Roychoudhury (1974). For 73 of the 80 species exhibiting variability, the variance due to inter-locus heterogeneity was greater, usually many times greater, than that due to intra-locus variation attributable to sample size and allele frequencies (Tables 2 and 3). As emphasized by Fuerst et al. (1977) and Nei and Roychoudhury (1974), for purposes of estimating average heterozygosity per population, it is far preferable to examine a large number of loci rather than a large number of individuals.

Genetic variability within a species has two components: heterozygosity within local populations and differences between populations. For those species represented by samples from two or more populations (*Lepomis auritus*, *L. macrochirus*, *L. microlophus*, and *L. gulosus*), average values of the statistics $\overline{H}$ and $V(h)$ are reported. For the majority of species a single population was assayed, so conclusions of this study apply strictly to possible correspondence of rate of speciation with mean population heterozygosity. Since an overwhelming result from electrophoretic studies suggests that conspecific populations are typically very similar in overall allelic composition, this restriction is probably not too severe (Avise 1974; Ayala 1975).

Results

Levels of genic variability in the centrarchid species examined in this study are summarized in Table 2. Estimates of heterozygosity ($\overline{H}$) range from a low of 0.000 in *Enneacanthus obesus*, *Elassoma evergladei*, and *Centrarchus macropterus* to a high of 0.129 ± 0.061 in *Ambloplites rupestris*. Heterozygosity estimates for the 69 minnow species are presented in Table 3 and exhibit a similar range from 0.000 (in *Notropis coccogenis*, *Notropis dorsalis*, *Notropis spilopterus*, *Hybopsis lineapunctata*, and *Semotilus atromaculatus*) to 0.154 ± 0.052 (in *Notropis texanus*). Since heterozygosity levels can be influenced by a variety of evolutionary forces, both stochastic and deterministic, the apparent differences among particular species of minnows or sunfish must be interpreted with extreme caution. The confidence for any single heterozygosity

TABLE 3. Estimates of genic variability in North American Cyprinidae. Polymorphic loci defined and variances calculated as for Table 2.

genus	species	# loci sampled	mean # alleles per locus	percent polymorphic loci	Mean ± S.E. heterozygosity per locus	intra-locus variance	inter-locus variance
Notropis	atherinoides	17	1.24	24	0.079 ± 0.037	.00531	.01796
Notropis	atropiculus	17	1.23	18	0.070 ± 0.041	.00323	.02535
Notropis	baileyi	17	1.12	12	0.026 ± 0.018	.00292	.00259
Notropis	bellus	17	1.23	24	0.074 ± 0.035	.00490	.01592
Notropis	boops	17	1.12	12	0.040 ± 0.029	.00312	.01118
Notropis	buchanani	16	1.25	19	0.080 ± 0.046	.00299	.03087
Notropis	callisema	16	1.06	6	0.015 ± 0.015	.00146	.00214
Notropis	chalybaeus	17	1.41	35	0.114 ± 0.045	.00720	.02722
Notropis	chrosomus	17	1.29	24	0.101 ± 0.048	.00297	.03620
Notropis	chrysocephalus	17	1.23	24	0.096 ± 0.045	.00351	.03091
Notropis	coccogenis	17	1.00	0	0.000	–	–
Notropis	cornutus	17	1.18	12	0.044 ± 0.033	.00309	.01542
Notropis	cummingsae	16	1.25	19	0.082 ± 0.048	.00273	.03413
Notropis	dorsalis	16	1.00	0	0.000	–	–
Notropis	euryzonus	16	1.06	6	0.029 ± 0.029	.00073	.01273
Notropis	fumeus	17	1.24	18	0.050 ± 0.028	.00473	.00860
Notropis	galacturus	17	1.18	18	0.048 ± 0.027	.00402	.00837
Notropis	gibbsi	17	1.06	6	0.013 ± 0.013	.00146	.00141
Notropis	greenei	17	1.35	29	0.096 ± 0.039	.00781	.01805
Notropis	hudsonius	16	1.06	6	0.017 ± 0.017	.00201	.00261
Notropis	hypselopterus	17	1.06	6	0.013 ± 0.013	.00146	.00141
Notropis	leedsi	16	1.12	12	0.043 ± 0.032	.00228	.01410
Notropis	longirostris	17	1.24	18	0.050 ± 0.028	.00473	.00860
Notropis	lutipinnis	15	1.20	13	0.058 ± 0.045	.00213	.02824
Notropis	lutrensis	16	1.38	31	0.103 ± 0.044	.00670	.02428
Notropis	maculatus	16	1.50	38	0.135 ± 0.048	.00839	.02847
Notropis	niveus	16	1.12	12	0.041 ± 0.030	.00228	.01212
Notropis	ozarcanus	16	1.06	6	0.031 ± 0.031	.00146	.01392
Notropis	petersoni	17	1.35	29	0.130 ± 0.052	.00814	.03783
Notropis	pilsbryi	17	1.12	12	0.059 ± 0.040	.00080	.02640
Notropis	rubellus	16	1.12	12	0.043 ± 0.032	.00228	.01410
Notropis	signipinnis	16	1.06	6	0.014 ± 0.014	.00155	.00159
Notropis	spilopterus	14	1.00	0	0.000	–	–
Notropis	sp. (undescribed)	17	1.23	24	0.092 ± 0.044	.00383	.02908
Notropis	stramineus	17	1.24	35	0.130 ± 0.047	.00626	.03129
Notropis	telescopus	17	1.18	12	0.037 ± 0.026	.00326	.00824
Notropis	texanus	17	1.41	41	0.154 ± 0.052	.00755	.03842
Notropis	topeka	17	1.23	24	0.093 ± 0.044	.00383	.02908
Notropis	trichroistius	17	1.12	12	0.035 ± 0.025	.00274	.00788
Notropis	umbratilis	17	1.12	12	0.040 ± 0.030	.00214	.01316
Notropis	uranoscopus	16	1.25	25	0.066 ± 0.030	.00803	.00637
Notropis	venustus	16	1.19	19	0.078 ± 0.043	.00315	.02643
Notropis	volucellus	17	1.24	18	0.081 ± 0.045	.00629	.02813
Notropis	whipplei	17	1.23	24	0.069 ± 0.034	.00479	.01486
Notropis	xaenurus	15	1.07	7	0.025 ± 0.025	.00264	.00674
Notropis	zonatus	16	1.12	12	0.028 ± 0.019	.00292	.00286
Notropis	zonistius	17	1.18	18	0.053 ± 0.031	.00361	.01273
Campostoma	anomalum	16	1.06	6	0.014 ± 0.014	.00155	.00159
Dionda	nubila	16	1.31	25	0.076 ± 0.036	.00620	.01454
Ericymba	buccata	16	1.12	12	0.043 ± 0.032	.00228	.01410
Gila	bicolor	24	1.25	21	0.059 ± 0.028	.00043	.01839
Hesperoleucus	symmetricus	24	1.46	25	0.067 ± 0.025	.00085	.01415
Hybopsis	lineapunctata	16	1.00	0	0.000	–	–
Hybopsis	sp. (undescribed)	15	1.20	13	0.050 ± 0.037	.00351	.01703
Hybopsis	storeriana	15	1.07	7	0.018 ± 0.018	.00214	.00272
Lavinia	exilicauda	24	1.21	17	0.048 ± 0.024	.00037	.01345
Mylopharodon	conocephalus	24	1.04	4	0.006 ± 0.006	.00005	.00081
Nocomis	leptocephalus	16	1.06	6	0.015 ± 0.015	.00155	.00205
Nocomis	micropogon	16	1.06	6	0.029 ± 0.029	.00073	.01273

TABLE 3.—(Continued).

genus	species	# loci sampled	mean # alleles per locus	per cent polymorphic loci	mean ± S.E. heterozygosity per locus	intra-locus variance	inter-locus variance
Notemigonus	crysoleucas	24	1.29	21	0.068 ± 0.029	.00145	.01873
Orthodon	microlepidotus	24	1.08	4	0.015 ± 0.011	.00032	.00258
Phoxinus	erythrogaster	14	1.21	21	0.078 ± 0.044	.00382	.02328
Pimephales	notatus	14	1.21	14	0.045 ± 0.032	.00396	.01038
Pimephales	vigilax	16	1.31	25	0.084 ± 0.041	.00620	.02070
Pogonichthys	macrolepidotus	24	1.13	8	0.036 ± 0.011	.00020	.00270
Ptychocheilus	grandis	24	1.13	4	0.011 ± 0.011	.00030	.00260
Rhinichthys	cataractae	16	1.12	12	0.015 ± 0.015	.00228	.00132
Richardsonius	egregius	24	1.13	8	0.030 ± 0.024	.00034	.01348
Semotilus	atromaculatus	16	1.00	0	0.000	–	–

estimate is low since (1) relatively few loci contribute to the genic variability, (2) the number of individuals sampled per species is generally small, and (3) in most cases only a single population of a species was sampled. The fact that several species of sunfish and minnows displayed no variation at the loci examined does not imply that their genomes totally lack genetic variability.

There is considerable heterogeneity in mean level of variability across loci in both the minnows and sunfish (Table 4). The most consistently variable loci in Cyprinidae are *Es-2*, *Pgi-1*, and *Pgi-2*, with mean heterozygosities across species equal to 0.183, 0.148, and 0.142, respectively. Among Centrarchidae, the *Es-3* locus is most variable: mean heterozygosity equals 0.157. Eleven of the loci examined in the majority of Cyprinidae and Centrarchidae were judged likely to be homologous on the basis of zymogram patterns and tissue specificities. For these loci, there is a significant correlation between mean level of variability in sunfish and minnows (Fig. 1). Selander (1976) has emphasized the desirability of assaying homologous enzymes when close comparisons among species are attempted.

Notwithstanding the fact that individual values of $\bar{H}$ are not precise, overall estimates of levels of genic variation in Cyprinidae and Centrarchidae should reflect real differences between the two groups if they do indeed exist. Mean heterozygosities across species ($\hat{H}$) in minnows and sunfish are summarized in Table 5. Cyprinidae and Centrarchidae appear remarkably similar in *amount* of genetic variability: mean heterozygosities per species in the two groups are 0.052 ± 0.004 and 0.049 ± 0.009, respectively. The *patterns* of genetic variability, reflected in the frequency distributions of single locus heterozygosities across all assayed species, are also virtually identical in Cyprinidae and Centrarchidae (Fig. 2). Thus we cannot falsify the hypothesis that there is no relationship of within-species variability and rate of speciation in these two families of fishes.

However, within each family an interesting pattern emerges. Of the 200 species of Cyprinidae inhabiting North America, roughly one-half are currently placed in a single genus *Notropis*. On the average, the *Notropis* species appear significantly more heterozygous, $\hat{H} = 0.059 \pm 0.006$, than do members of other cyprinid genera, $\hat{H} = 0.037 \pm 0.006$ ($t_{67} = 2.46$, $P < 0.01$, one-tailed test). Similarly, *Lepomis* is the most diverse of centrarchid genera (comprising 11 of the 30 species), and species of *Lepomis* appear more heterozygous on the average, $\hat{H} = 0.066 \pm 0.009$, than do representatives of the other genera, $\hat{H} = 0.031 \pm 0.015$ ($t_{17} = 2.04$, $P < 0.05$, one-tailed test). If we accept the current belief that rates of speciation in *Notropis* and *Lepomis* have been particularly rapid for their respective families, then *within* Centrarchidae and Cyprinidae a positive correlation between rate of speciation and heterozygosity may exist.

Members of *Lepomis* and *Notropis* are renowned for propensity of interspecies hybridization (Hubbs 1955). Thus the higher mean heterozygosities of species in these genera could conceivably result from present or past introgression of alleles from one species to another. Roberts (1964) hypothesizes that hybridization and introgression provided

TABLE 4. Mean heterozygosities at various loci in species of Cyprinidae and Centrarchidae. Note that homologies for Pt-0 locus in minnows and sunfish are uncertain.

	mean heterozygosity per species	
locus	Cyprinidae (# species)	Centrarchidae (# species)
Ldh-1	0.032 (69)	0.000 (19)
Ldh-2	0.007 (69)	0.000 (19)
Mdh	0.029 (69)	0.011 (19)
Es-1	0.000 (69)	0.054 (10)
Es-3	0.103 (69)	0.157 (10)
Idh	0.000 (69)	0.000 (19)
Pgi-1	0.148 (69)	0.084 (19)
Pgi-2	0.142 (69)	0.083 (19)
Pgm	0.073 (69)	0.076 (19)
6Pgd	0.047 (69)	0.058 (19)
Got-1	0.037 (69)	0.077 (19)
Got-2	0.003 (9)	0.056 (19)
To	0.122 (9)	0.047 (10)
Pep	–	0.009 (19)
Es-2	0.183 (55)	–
Adh	0.021 (9)	–
Gpd	0.036 (9)	–
Tpi	0.002 (9)	–
Pt-0	0.000 (69)	0.000 (1)
Pt-1	0.006 (65)	–
Pt-2	0.000 (9)	–
Pt-3	0.114 (9)	–
Pt-4	0.044 (69)	–

the genetic and phenotypic variability necessary for adaptation of many centrarchids to changing environmental conditions during the Pleistocene. However, this thesis should be reevaluated in view of recent evidence that despite ability to hybridize, centrarchids are very different in allelic composition (Avise and Smith 1974). The extent and evolutionary significance, if any, of introgression among species of *Notropis*, is unknown. Thus in broad perspective, the most reliable comparison between rate of speciation and genic heterozygosity involves Centrarchidae versus Cyprinidae, although results for these families are moderated somewhat by results of comparisons of genera within each family.

Discussion

A plethora of hypotheses has been advanced to account for apparent differences in levels of heterozygosity among different organisms (Ayala 1976; Lewontin 1974; Berger 1976). Prominent have been attempts to relate the genetic system itself with population features such as patterns of reproduction, recombination, or functional diversity which might be

TABLE 5. Summary of levels of heterozygosity per species in North American Cyprinidae and Centrarchidae.

Group	# species assayed	Mean ± S.E. heterozygosity per species
Centrarchidae		
Lepomis	10	0.066 ± 0.009
other genera	9	0.031 ± 0.015
total	19	0.049 ± 0.009
Cyprinidae		
Notropis	47	0.059 ± 0.006
other genera (eastern U.S.)	13	0.036 ± 0.008
other genera (western U.S.)	9	0.038 ± 0.008
total	69	0.052 ± 0.004

favored in particular environmental regimes (Allard 1975). For example, environmental heterogeneity per se may select for greater variability by favoring different genetic variants in different environmental niches (Powell 1971; McDonald and Ayala 1974). Levins' (1968) theory of adaptive strategy led to a suggestion that organisms which perceive their environment as patchy (a *coarse-grained* environment) respond by maintaining greater levels of variability (Selander and Kaufman 1973). More recently, the suggestion has been made that trophic resource stability increases genic heterozygosity by permitting specialization to habitats which are then perceived by the organisms as coarse-grained (Valentine 1976; Valentine and Ayala 1974; Ayala et al. 1974). Although some data exist to support these hypotheses, the sum of all available evidence suggests that genetic variability is remarkably similar among species, independent of environmental considerations or lifestyle (Lewontin 1974).

In this paper the hypothesis is proposed that phylads which speciate more rapidly do so because of increased level of within-species genetic variability which is then available to conversion to species differences under appropriate environmental circumstances. This hypothesis has the corollary that rate of speciation could be limited in phylads with low genetic variability, irrespective of environmental considerations. This hypothesis has been tested with respect to electrophoretically detectable variation in products of structural

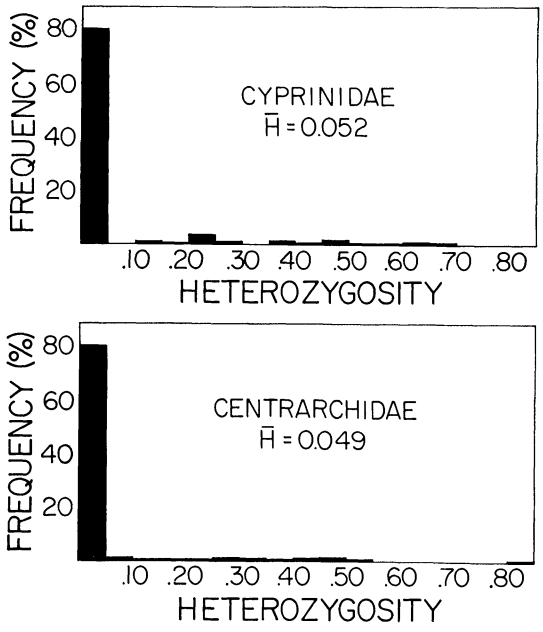

FIGURE 2. Frequency distributions of single locus heterozygosity across all assayed species of Cyprinidae and Centrarchidae.

genes in two families of fishes characterized by grossly different rates of speciation. Since Cyprinidae and Centrarchidae exhibit on the average nearly identical levels of genic variation ($\hat{H} = 0.052 \pm 0.004$ for 69 species of cyprinids, and $\hat{H} = 0.049 \pm 0.009$ for 19 species of centrarchids), the hypothesis that rate of speciation and level of heterozygosity are related is not supported.

These results are inconsistent with the idea that level of variability in structural genes affects rate of speciation. The levels of heterozygosity in Cyprinidae and Centrarchidae are nearly identical to the mean values previously reported for other vertebrates (Selander and Kaufman 1973). "Normal" levels of heterozygosity in most phylads appear sufficient to support widely varying rates of population differentiation and speciation. Hence these results are not inconsistent with prevailing hypotheses relegating a major role to ecological considerations in determining rates of species proliferation. Of course, the minnows and sunfish constitute only a single test of the original hypothesis. Despite the availability of heterozygosity estimates in more than 250 species (Powell 1976), I am not aware of any other appropriate sets of data to examine this question. Because it is possible that a subtle positive correlation between $\hat{H}$ and speciation rate does exist, but was overridden in this test by other phenomena, additional tests with other types of organisms would be welcome.

One such phenomenon leading to a negative theoretical correlation between rate of speciation and genic heterozygosity has been proposed by Soulé (1971, 1976), who argued that younger species should be less polymorphic than older species, when speciation is accomplished by population bottlenecks and loss of heterozygosity. The time course of change in heterozygosity depends not only on the size of the population bottleneck, but also on the subsequent rate of population increase (Nei et al. 1975). Once heterozygosity is reduced to a low level, the number of generations necessary to reestablish equilibrium heterozygosity levels for neutral alleles is very roughly the reciprocal of the mutation rate (Nei et al. 1975) or perhaps ten million years for Centrarchidae or Cyprinidae (assuming $\mu = 10^{-7}$, generation length = one year, heterozygosity severely reduced during speciation). If we assume the North American centrarchids and cyprinids are 50 million years old, and speciation events have occurred at regular time intervals in all lineages, the average durations of centrarchid and cyprinid species are about 10 million and 6 million years, respectively; mean heterozygosity among cyprinids should be reduced below equilibrium levels. If $\mu \leqslant 10^{-8}$, $\hat{H}$ should be below equilibrium in both families, and if $\mu \geqslant 10^{-6}$, $\hat{H}$ should be at equilibrium in both families. Gross uncertainties about μ, equilibrium heterozygosity levels, and specific evolutionary histories of minnows and sunfish preclude more definitive analysis of the "time-divergence" model. However, the finding of increased $\hat{H}$ in *Notropis* and *Lepomis* (many of whose members are thought to be particularly young compared to most species in other cyprinid and centrarchid genera—Avise and Smith 1977; Avise, in prep.) is exactly the opposite of what would be predicted if age of species is positively related to heterozygosity. Either speciations in Centrarchidae and Cyprinidae do not normally entail severe loss of heterozygosity, and/or mean durations of species are sufficient to permit recovery of lost variability.

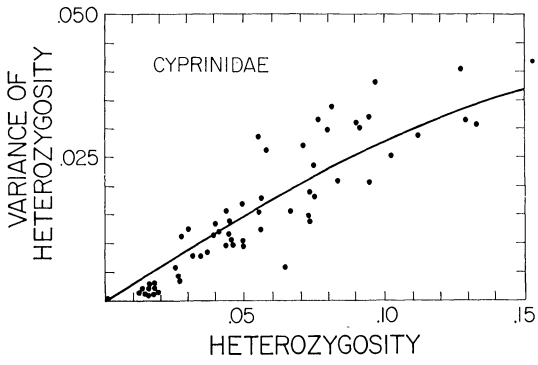

FIGURE 3. Mean heterozygosities and interlocus variances of heterozygosity for species of North American Cyprinidae. The solid line indicates the theoretical relationship under the neutrality hypothesis for an infinite allele model (the theoretical curve for a stepwise mutation model is very similar over the range of heterozygosities plotted).

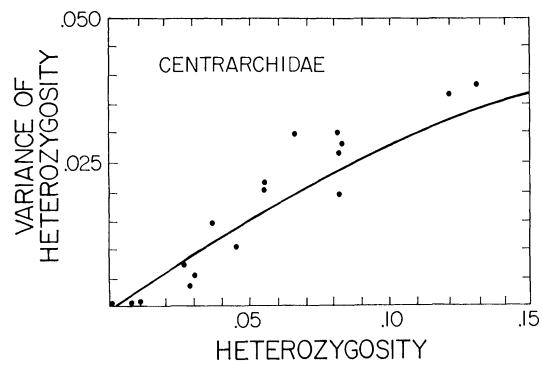

FIGURE 4. Mean heterozygosities and interlocus variances of heterozygosity for species of North American Centrarchidae. The solid line indicates the theoretical relationship under the neutrality hypothesis for an infinite allele model (the theoretical curve for a stepwise mutation model is very similar over the range of heterozygosities plotted).

Another related attempt to account for observed heterozygosity levels in natural populations constitutes the mutation-drift hypothesis, which holds that the majority of protein variation is neutral with respect to fitness (Kimura and Ohta 1971). This hypothesis is particularly valuable, since neutral mutation rates and population sizes account for changes in genic composition, and specific predictions can be generated about characteristics of genetic variation in populations. Nei (1975) and Fuerst et al. (1977) determined the theoretical relationship between mean and variance of heterozygosity, and found a close fit with empirical data taken from 129 species of vertebrates and invertebrates. Figures 3 and 4 indicate that the fit of mean and interlocus variance of heterozygosities to the theoretical relationship under the neutral mutation model is also very good for both the Cyprinidae and Centrarchidae. If variation in the structural genes assayed in this study is indeed invisible to natural selection, by definition it could not play a role in determining rates of speciation. In the future it will be valuable to propose additional hypotheses about possible genetic factors affecting speciation and to test these hypotheses with a broader class of genes.

One final possibility concerning the lack of apparent correlation between $\hat{H}$ and speciation rate should be considered. Although improbable, it is conceivable that the difference in numbers of living minnows and sunfish is attributable solely to differential extinction rather than to differential speciation. In this case, our present study would in fact have tested the null hypothesis that there is no correlation between rate of extinction and mean level of heterozygosity in an evolutionary phylad. Neither could this hypothesis be falsified with the present data. However, a strong bias might be operating against a falsification—those sunfish species which did survive to be assayed were those with higher heterozygosities.

Results of this study complement and extend those of two earlier reports which attempted to relate genic heterozygosity to evolutionary rates. Ayala et al. (1973) concluded from a study of *Tridacna maxima* that the massive marine extinctions registered in the fossil record were not due to a general scarcity of genetic variability in populations inhabiting stable environments. Similarly, Selander et al. (1970) found that the slow rate of morphological evolution in "living fossils" such as *Limulus polyphemus* was not due to a lack of variation at the genic level. The present study shows that the slow rate of speciation in sunfish relative to minnows is not attributable to a lower level of within-species variation in structural genes. Attempts to find a positive relationship between rate of evolutionary change (either anagenetic or cladogenetic) and level of genic variation as measured electrophoretically have been unsuccessful. The wealth of electrophoretic

variation present in virtually all outcrossing organisms appears to reflect levels of genome variability sufficient to account for wide flexibility in rate and pattern of evolutionary response to environmental challenges. Explanations for differing rates of speciation apparently must be sought in terms of specific organism-environment relationships, and/or other types of genetic influences.

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