ROLE OF GENOME SIZE VARIATION IN SPECIATION OF NORTH AMERICAN CYPRINID FISHES

by

Laura Jo Schliesing

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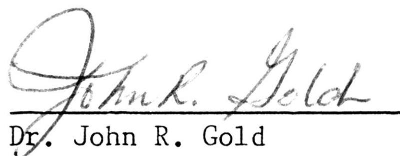

Dr. John R. Gold

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ABSTRACT

An important question in evolutionary biology today is exactly how and when do the genetic changes which accompany evolutionary divergence occur? This study examines two competing hypotheses, with the major difference between the two being the tempo in which organismal and or genetic changes occur. The traditional view that most evolutionary change is gradual and cummulative within lineages - phyletic gradualism - is challenged by the more recent theory that the majority of evolutionary change is concentrated within speciation episodes - rectangular evolution. These two models lead to distinct predictions of mean amounts of genetic distance between species in species-rich versus species-poor phylads of equal evolutionary age. Genetic distance may be measured with any of a number of quantifiable parameters, i.e. gross karyotype, structural genes, morphologies, or DNA. In this study, the DNA of North American cyprinid fishes (minnows) was examined quantitatively. Results were consistent with earlier work done with these fishes using different parameters of measurement. Despite the extensive speciation of Cyprinidae, changes in nuclear genome size, as well as karyotypic, structural gene, and morphological changes do not support a rectangular mode of evolution.

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INTRODUCTION:

An important problem in evolutionary biology is how and when the organismal or genetic changes which accompany evolutionary divergence occur. Currently, there exist two conflicting hypotheses. The first is phyletic gradualism (Eldridge and Gould, 1972), which holds that most evolutionary change is gradual and occurs by the slow and even transformation of populations within established species into reproductively isolated units. Genetic change under the hypothesis of phyletic gradualism is essentially Darwinian and is thought to occur primarily as a result of natural selection. The second and more recent hypothesis is that of rectangular evolution (Stanley, 1975), or punctuated equilibria (Eldridge and Gould, 1972), which proposes that most evolutionary change in both morphology and genotype occurs during speciation episodes with a relatively slow rate of change in intervening periods. The resulting phyletic patterns appear stepwise, or rectangular. Schematic representations of gradual versus rectangular models of evolutionary change are shown in Figure 1.

The two models above, which describe amounts of genetic differentiation expected between species within a group, lead to distinct predictions of mean amounts of genetic distance between species in species-rich versus species-poor phylads of equal evolutionary age (Avise and Ayala, 1975). Tests of these two models can be carried out utilizing the logic of theoretical models devised by Avise and Ayala (1975, 1976) and Avise (1977,

1978) which compare expected means and variances of genetic distance among living members of rapidly versus slowly speciating taxa. Briefly, if genetic distance between species is a function of time (gradual evolution), the ratio of mean genetic distances separating species in species-rich versus species-poor taxa should be very nearly one, and the ratio of variances should be less than one. If genetic distance between species is a function of speciation episodes (rectangular evolution), the ratio of both means and variances of genetic distance in species-rich versus species-poor taxa should be considerably greater than one (Gold, 1980). This test can be carried out using any of a number of quantifiable measures of evolutionary change. Avise et. al. (1977) have carried out this test using electrophoretic analyses of structural genes, Gold (1981) has used karyotypic comparisons, and Douglas (1982) has used a set of morphological characters. The use of this type of approach is important in that comparisons of living taxa can be made without having to rely completely on the fossil record to resolve the rectangular evolution - phyletic gradualism controversy. "We can now make reliable inferences directly from the morphologies, gene products, chromosomes, or DNA of organisms alive today" (Avise, 1977).

Finally, it is important to note that phyletic gradualism and rectangular evolution represent extreme hypotheses with a large range of intermediates; however, the two models are important because if rectangular patterns do exist, then the theory of phyletic gradualism becomes less important to the

process of evolution (Avise, 1977; Gold, 1980).

The principle objective of this research effort was to assess the role of change in nuclear genome size in the speciation of North American cyprinid fishes. The family Cyprinidae (minnows) is the largest family of North American freshwater fishes containing some 35 genera and about 250 species. Most of these species are thought to have originated from one or a few ancestors which migrated to North America from Eurasia during mid- to late- Miocene times (Miller, 1975). Of these 250 species, about 125 belong in the single genus Notropis making it by far the most diverse of minnow genera. Thus, the North American cyprinid fishes represent a group with an extremely rapid rate of speciation; however, Avise's (1977), Gold's (1981), and Douglas' (1982) studies of genic, chromosomal, and morphological change, respectively, in North American cyprinids did not support a rectangular mode of evolution in these fishes.

Nuclear genome size, or DNA content has been correlated with the speciation process (Price, 1980; Cavalier-Smith, 1982), and is another measurable parameter of genetic change. Whether variations in genome size are causal, or merely consequence of speciation is not known. This study includes an assessment of variations in genome size among 20 cyprinid species. The same logic applied in comparison of other parameters was used to compare the species-rich genus Notropis to other species-poor genera. In this manner the rate of evolutionary change in genome

size within Notropis could be compared to that within other genera.

MATERIALS AND METHODS:

There are numerous ways in which genome size can be quantified. Densitometric measurement was utilized in this study. For each cyprinid species examined (Table 1), 10 specimens were collected by seine and slides prepared in the manner used in Gold's laboratory. Fish blood was collected in 50 mcl heparinized microhematocrit capillary tubes via heart puncture. Chicken blood, used as an internal standard to test validity of comparison in slides prepared at different times, was obtained by venipuncture of full sibs from an inbred line of domestic chicken. One drop of fish blood and one drop of chicken blood were placed on opposite ends of a microscope slide and wedge smears of the two (using different coverslips) were made. Two slides were made from each individual. After allowing the blood to dry, slides were stored in the dark under dessicated conditions at 4 degrees C for 24 hours.

Fuelgen hydrolysis was carried out *en bloc* at 37 degrees C in 3.5N HCl for 35 min. (35 min. was found in preliminary studies to be the time at which optimal fuelgen staining for both fish and chicken was obtained). Following hydrolysis, slides were rinsed for 5 sec. in distilled water and stained with Schiff's reagent for 2 hr. at room temperature. After 2 hr., the slides were bleached twice in SO_2-water (10 min. each), rinsed 10 min. in distilled water, air-dryed, cleared in xylenes for 10 min., and

then mounted in Permount. Slides were stored in the dark until analyzed to prevent fading. Fuelgen-stained erythrocytes were measured using a Zeiss Universal II scanning microdensitometer. Fifteen fish nuclei and five chicken nuclei were measured per slide at a wavelength of 560 nm. Nuclear genome size of fish erythrocytes was estimated via absorbancy comparisons with the internal standard (there is approximately 2.5 picograms of DNA in chicken RBC nuclei). Table 2 gives the picograms of DNA determined for each of the species examined.

RESULTS:

The Statistical Analysis System (SAS) was used for data analyses. Average genome sizes were computed as a percentage of the chicken standard and multiplied by 20. This coded data is designated Fuelgen Absorbancy Units (FAU). Averages were computed by slides, individuals, and species, with coefficients of variation at each level about 4 or less for all species. Frequency distributions were plotted at each level, and g1 and g2 tests run on these distributions to test for deviations from normality. The majority (12 of 20) of species distributions were normal; a few were either skewed or kurtotic. The latter may be due to small sample size, i.e., these distributions might be normal given a larger sample size since tests for skewness and kurtosis are unduly sensitive to small sample size. Deviations from normality can mean several things. If the distribution is drawn out in one direction (skewed), it indicates that there may

be selection for or against organisms falling in one of the tails of the distribution, or possibly that the scale of measurement chosen is such as to bring about a distortion of the distribution (Sokal and Rohlf, 1969). The distributions of genome size of most of the species were normal. This suggests that changes in genome size occur in small incremental steps - a gradualistic pattern. Analysis of variance showed there was significant heterogeneity between population means; a Duncan's multiple range test was used to discriminate among significantly different species means. Table 3 shows descriptive statistics for 20 cyprinid species and includes the results of the multiple range tests. Normal distributions are designated N, skewed right - SR, skewed left - SL, and leptokurtic - L. A nested analysis of variance was carried out to assess the percentage of the variation occurring at each hierarchical level (Table 4).

Distance matrices were constructed using the mean FAU for each species for the calculations of average distance and variance. The first matrix was constructed with the 12 *Notropis* species (species-rich group), and the second matrix was constructed with the 8 non-*Notropis* species (species-poor group). Average distances and variances were used in ratio comparisons and the results are shown in Figure 2. Since the average distance ratio is near one, and the variance ratio is less than one, they are consistent with the predictions of phyletic gradualism, and inconsistent with a rectangular model of evolution. The richly speciated *Notropis* does not appear to be

any more differentiated in terms of genome size than less speciated genera.

A phenogram (Figure 3) showing possible evolutionary relationships among the species studied was constructed from the species by species distance matrix using the UPGMA algorithm (Sneath and Sokal, 1973). The cophenetic correlation coefficient is indicated in the lower right-hand corner of the phenogram. The species by species distance matrix for the 15 cyprinid species included in the phenogram is given in Table 5. Distances between pairs of species were computed by taking the difference between mean genome sizes in pair-wise coombinations. There does appear to be some phyletic component. As noted earlier, the distributions of genome size in each species overlap, but certain groups do pull out at lower levels (taxons) as shown in the phenogram. *Pimephales notatus*, *Pimephales promelas*, and *Pimephales vigilax* (not shown in the phenogram, but falls just below *P. promelas*) all have similar mean genome sizes. *Campostoma oligolepis* and *Campostoma anomalum* (not shown, falls just above *C. oligolepis*) are very close in genome size. *Notropis nubilus* and *Notropis rubellus* are very closely related being in the same subgenus *Hydrophlox*, and clump together at the first level of the phenogram. *Notropis stramineus* and *N. girardi* are at opposite extremes in their subgenus *Alburnops*, and are separated in the phenogram. The same can be said for *N. chrysocephalus* and *N. pilsbryi* both of the subgenus *Luxilus*.

DISCUSSION:

The distributions of genome size in most of the species examined were normal, and there were no consistent patterns to those that were not normal. The distribution of all nuclei measured from all species also was normal. This pattern of distribution normality suggests normalizing selection for genome sizes where extremes are removed and most individuals are at or near an adaptive norm (= the mean). It also suggests that the variation in genome size follows the assumptions of the normal probability density function, i.e., contributing factors are small, frequently occurring, independent and cumulative in effect. This is interpreted to mean that both gains and losses of DNA occur, and that all such events are independent and cumulative (Gold, personal communication); they occur in small incremental steps resulting in a gradualistic evolutionary pattern.

It does not appear that speciation in these fishes is concurrent with large changes in genome size. The mean genome size of a certain species does not indicate its place in taxonomic order. Although closely related species may share similar mean genome sizes, very distantly related species may also have similar mean genome sizes, and more closely related species may be separated by larger differences in mean genome size. Genome size does not dictate membership in a particular species, genus, etc.

This study does not support a rectangular mode of evolution.

These data will be increasingly important as they are meshed with genome size data to be collected in the future on other cyprinids. A larger data base will lead to greater ease in examining phylogenetic relationships, and questions concerning modes of speciation in these fishes.

Table 1. Collection Sites for Species Examined.

Taxon	Site	County,State	Drainage	Date
Campostoma anomalum	Boardhouse Cr.	Hays,TX	Blanco	7/24/81
Campostoma oligolepis	White R. (West fork)	Washington,ARK	White R.	7/13/82
Notemigonus crysoleucas	Bill's Bait Shop	Brazos,TX		
Notropis boops	Briar Cr.	Marshall,OKLA	Red R.	7/12/82
Notropis chrysocephalus	Blue R.	Ponotoc,OKLA	Blue R.	7/11/82
Notropis girardi	S. Canadian R.	Gift from B. Mathews		
Notropis lutrensis	Little Brazos R.	Brazos,TX	Brazos	4/08/83
Notropis nubilus	White R. (West fork)	Washington,ARK	White R.	7/13/82
Notropis pilsbryi	White R. (West fork)	Washington,ARK	White R.	7/13/82
Notropis rubellus	Blue R.	Ponotoc,OKLA	Blue R.	7/11/82
Notropis shumardi	Arkansas R.	Crawford,ARK	Arkansas R.	7/14/82
Notropis stramineus	Caddo Cr.	OKLA		7/17/82
Notropis umbratilis	Blue R.	Ponotoc,OKLA	Blue R.	7/11/82
Notropis venustus	Bull Cr.	Travis,TX	Colorado	3/20/83
Notropis whipplei	Clear Cr.	Crawford,ARK	Arkansas R.	7/13/82
Phoxinus erythrogaster	Bob Jordan Spring	Washington,OKLA	Illinois R	7/13/82
Pimephales notatus	Blue R.	Ponotoc,OKLA	Blue R.	7/11/82
Pimephales promelas	Briar Cr.	Marshall,OKLA	Red R.	7/12/82
Pimephales vigilax	Little Brazos R.	Brazos,TX	Brazos	2/01/81
Semotilus atromaculatus	Osake Cr.	Benton,ARK	Illinois R.	7/13/82

Table 2. Nuclear Genome Size Measured in Picograms (10^{-12} g)

Taxon	Mean±S.E.
Campostoma anomalum	2.29±0.02
Campostoma oligolepis	2.26±0.02
Notimegonus crysoleucas	2.28±0.02
Notropis boops	2.19±0.01
Notropis chrysocephalus	2.31±0.01
Notropis girardi	2.35±0.02
Notropis lutrensis	2.37±0.01
Notropis nubilus	2.38±0.01
Notropis pilsbryi	2.48±0.01
Notropis rubellus	2.38±0.01
Notropis shumardi	2.70±0.02
Notropis stramineus	2.51±0.01
Notropis umbratilis	2.65±0.01
Notropis venustus	2.42±0.01
Notropis whipplei	2.50±0.01
Phoxinus erythrogaster	2.63±0.02
Pimephales notatus	2.24±0.01
Pimephales promelas	2.22±0.01
Pimephales vigilax	2.21±0.01
Semotilus atromaculatus	2.51±0.01

Table 3. Descriptive Statistics for 20 Cyprinid Species

Taxon	Mean FAU ± S.E.* (n = 300)	Range	σ^2	C.V.	Shape of Distribution*
Campostoma anomalum	18.28 ± 0.04	16.76 - 20.21	0.41	3.51	N
Campostoma oligolepis	18.11 ± 0.04	16.71 - 20.24	0.53	4.01	SR
Notemigonus crysoleucas	18.23 ± 0.04	16.50 - 20.40	0.56	4.12	SR
Notropis boops	17.54 ± 0.04	15.90 - 19.07	0.44	3.80	N
Notropis chrysocephalus	18.45 ± 0.04	16.89 - 21.04	0.42	3.52	SR, L
Notropis girardi	18.77 ± 0.04	10.00 - 23.60	0.55	3.97	SR, L
Notropis lutrensis	18.99 ± 0.03	17.53 - 20.49	0.32	2.96	N
Notropis nubilus	[a] 19.02 ± 0.04	17.47 - 20.95	0.46	3.52	N
Notropis pilsbryi	19.81 ± 0.04	17.93 - 21.79	0.56	3.78	N
Notropis rubellus	[a] 19.01 ± 0.04	17.19 - 22.59	0.57	3.97	SR, L

*See text for explanation.

[a-c] Means not significantly different indicated with identical superscripts.

Table 3. Descriptive Statistics for 20 Cyprinid Species (continued)

Taxon	Mean FAU$\pm$S.E.[*] (n = 300)	Range	σ^2	C.V.	Shape of Distribution[*]
Notropis shumardi	21.76 $\pm$ 0.05	19.20 - 24.19	0.77	4.04	N
Notropis stramineus	[b]20.11 $\pm$ 0.04	18.56 - 22.05	0.43	3.27	N
Notropis umbratilis	[c]21.18 $\pm$ 0.04	19.47 - 22.99	0.43	3.10	N
Notropis venustus	19.32 $\pm$ 0.03	18.02 - 20.71	0.30	2.84	N
Notropis whipplei	[b]20.00 $\pm$ 0.04	18.44 - 22.32	0.41	3.21	SR
Phoxinus erythrogaster	[c]21.07 $\pm$ 0.05	18.79 - 23.33	0.70	3.96	N
Pimephales notatus	17.93 $\pm$ 0.04	14.52 - 21.09	0.55	4.15	SL, L
Pimephales promelas	17.75 $\pm$ 0.03	15.89 - 19.24	0.34	3.28	N
Pimephales vigilax	17.63 $\pm$ 0.03	16.23 - 19.25	0.28	3.02	N
Semotilus atromaculatus	[b]20.10 $\pm$ 0.05	18.20 - 29.24	0.86	4.62	SR, L

[*] See text for explanation.
[a-c] Means not significantly different indicated with identical superscripts.

Table 4. Nested Analysis of Variance of Variable FAU[1]

Variance Source	D.F.	S.S.	M.S.	F	%
Total	4499	9793.360	2.177		100.00
Species	14	7390.944	527.925	163.698[2]	76.26
Individual	135	435.325	3.225	1.096	0.41
Slide	150	441.451	2.943	8.107[2]	7.50
Error	4200	1525.641	0.363		15.84

[1] See text for explanation.
[2] $p < 0.05$

Table 5. Distance Matrix for 15 Cyprinid Species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. <u>Notropis</u> <u>shumardi</u>	-	0.58	0.69	1.65	1.66	1.76	1.95	2.74	2.75	2.99	3.31	3.64	3.83	4.01	4.22
2. <u>Notropis</u> <u>umbratilis</u>		-	0.11	1.07	1.08	1.18	1.37	2.16	2.17	2.41	2.73	3.06	3.25	3.43	3.64
3. <u>Phoxinus</u> <u>erythrogaster</u>			-	0.96	0.97	1.07	1.26	2.05	2.06	2.30	2.62	2.95	3.14	3.32	3.53
4. <u>Notropis</u> <u>stramineus</u>				-	0.01	0.11	0.30	1.09	1.10	1.34	1.66	1.99	2.18	2.36	2.57
5. <u>Semotilus</u> <u>atromaculatus</u>					-	0.10	0.29	1.08	1.09	1.33	1.65	1.98	2.17	2.35	2.56
6. <u>Notropis</u> <u>whipplei</u>						-	0.19	0.98	0.99	1.23	1.55	1.88	2.07	2.25	2.46
7. <u>Notropis</u> <u>pilsbryi</u>							-	0.79	0.80	1.04	1.36	1.69	1.88	2.06	2.27
8. <u>Notropis</u> <u>nubilus</u>								-	0.10	0.25	0.57	0.90	1.09	1.27	1.48
9. <u>Notropis</u> <u>rubellus</u>									-	0.24	0.56	0.89	1.08	1.26	1.47
10. <u>Notropis</u> <u>girardi</u>										-	0.32	0.65	0.84	1.02	1.23
11. <u>Notropis</u> <u>chrysocephalus</u>											-	0.33	0.52	0.70	0.91
12. <u>Campostoma</u> <u>oligolepis</u>												-	0.19	0.37	0.58
13. <u>Pimephales</u> <u>notatus</u>													-	0.18	0.39
14. <u>Pimephales</u> <u>promelas</u>														-	0.21
15. <u>Notropis</u> <u>boops</u>															-

Figure 1. Schematic Representation of Gradual Versus Rectangular Evolution.

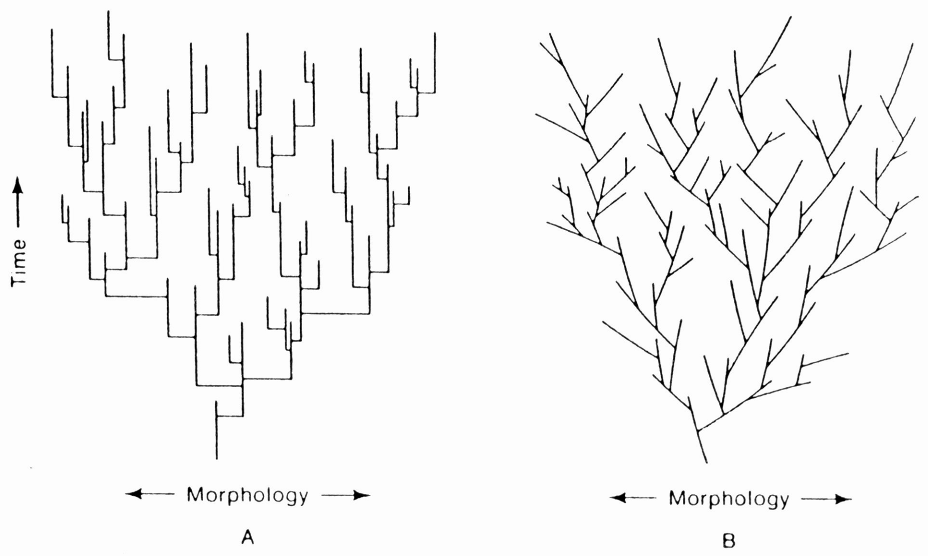


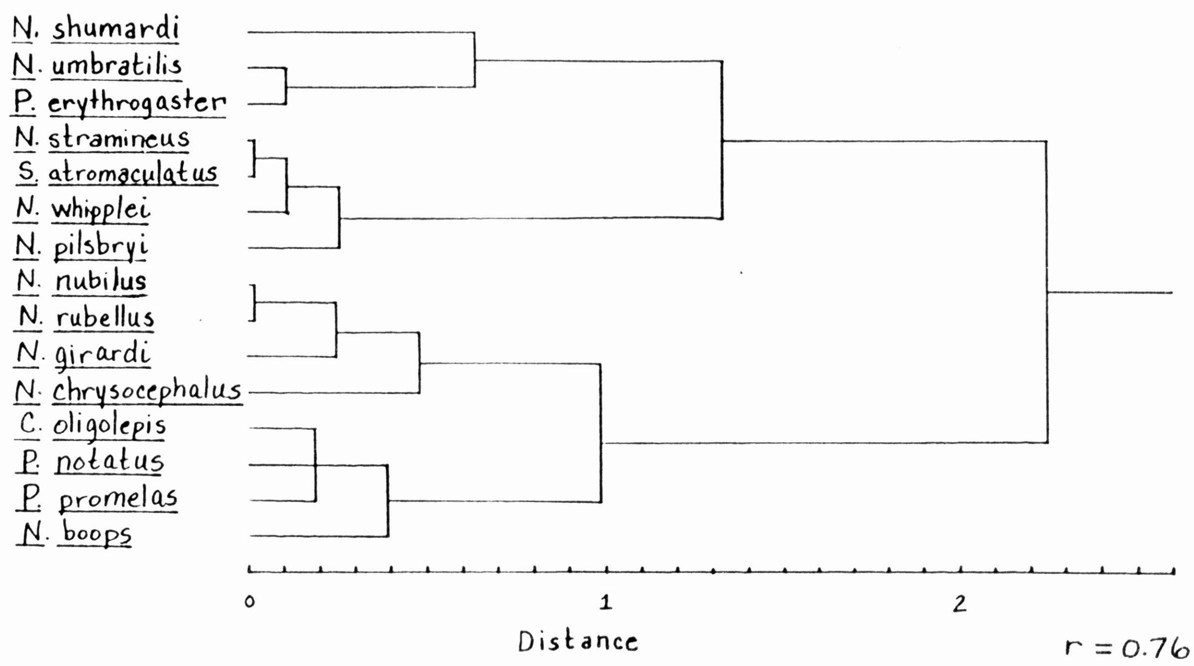
Figure 2. Average Distance and Variance Ratios Using 12 *Notropis* Species for Rich Phylad and 8 Non-*Notropis* Species for Poor Phylad

$$\frac{\text{Avg. Distance (Rich)}}{\text{Avg. Distance (Poor)}} = \frac{1.3389}{1.3203} = 1.0141$$

$$\frac{\text{Variance (Rich)}}{\text{Variance (Poor)}} = \frac{0.9298}{1.4250} = 0.6525$$

Figure 3.

Phenogram of 15 Cyprinid species Based on UPGMA Cluster Analysis. Species Shown at Left are Arranged in Order of Decreasing Amounts of DNA.



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