Genome size variation in North American minnows (Cyprinidae). II. Variation among 20 species

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Genome sizes (nuclear DNA contents) from 200 individuals representing 20 species of North American cyprinid fishes (minnows) were examined spectrophotometrically. The distributions of DNA values of individuals within populations of the 20 species were essentially continuous and normal; the distribution of DNA values among species was continuous and overlapping. These observations suggest that changes in DNA quantity in cyprinids are small in amount, involve both gains and losses of DNA, and are cumulative and independent in effect. Significant heterogeneity in mean genome size occurs both between individuals within populations of species and among species. The former averages maximally around 6% of the cyprinid genome and is nearly the same as the amount of DNA theoretically needed for the entire cyprinid structural gene component. The majority of the DNA content variation among the 20 species is distributed above the level of individuals within populations. Comparisons of average genome size difference or distance between individuals drawn from different levels of taxonomic organization indicate that considerably greater divergence in genome size has occurred in the extremely speciose cyprinid genus *Notropis* as compared with other North American cyprinid genera. This may suggest that genome size change is concentrated in speciation episodes. Finally, no associations were found between interspecific variation in genome size and five life-history characters. This suggests that much of the variation in genome size within and among the 20 species may be phenotypically inconsequential.

Key words: genome size, North American cyprinid fishes, evolution.

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La dimension des génomes (teneur en ADN nucléaire) de 200 individus représentant 20 espèces de ciprinidés (goujons) de l'Amérique du Nord a été étudiée par spectrophotométrie. La distribution des valeurs individuelles d'ADN au sein des populations de chacune des 20 espèces fut essentiellement continue et normale, alors qu'entre les espèces, cette distribution fut aussi continue, mais avec superpositions partielles. Ces observations suggèrent que les changements en quantité d'ADN sont faibles, impliquant des gains et des pertes, et qu'ils sont cumulatifs et indépendants dans leurs effets. Les moyennes de dimensions des génomes ont présenté une hétérogénéité significative, tant entre les individus au sein de chaque population d'espèces qu'entre les espèces elles-mêmes. En ce qui concerne les individus au sein de chaque population, les moyennes maximales se sont situées autour de 6% des génomes, ce qui correspond sensiblement à la quantité d'ADN théoriquement requise pour constituer la totalité des gènes structuraux des ciprinidés. Pour les 20 espèces, la distribution des variations en teneur d'ADN s'est située, chez la majorité des espèces, au dessus du niveau des individus à l'intérieur de ces populations. Des comparaisons portant sur des différences ou des distances des moyennes des dimensions de génomes entre les individus ont été établies à divers niveaux d'organisation taxonomique. Ces comparaisons ont indiqué qu'une divergence très considérable est survenue chez le genre *Notropis*, lequel est extrêmement spécieux, par comparaison aux autres genres ciprinidés de l'Amérique du Nord. Ceci permet de supposer que des changements de dimension des génomes ont été concentrés dans des épisodes de spéciation. Finalement, aucune association n'a été trouvée entre les variations interspécifiques des dimensions des génomes et cinq des caractères évolutifs. Il s'ensuit donc qu'une large part des variations de la dimension des génomes à l'intérieur et entre les 20 espèces étudiées peut n'avoir aucune importance au plan phénotypique.

Mots clés: dimension des génomes, poissons cyprinidés de l'Amérique du Nord, évolution. [Traduit par la revue]

Introduction

A historical problem in diploid eukaryotic evolution is the quantitative variation in nuclear DNA content or genome size (the C value). Extensive data demonstrate that variation in genome size among eukaryotes spans several orders of magnitude and that substantial differences in DNA quantity can be found even between closely related species (Bachmann *et al.* 1972; Sparrow *et al.* 1972; Rees and Jones 1972; Hinegardner and Rosen 1972; Price 1976). These data also have shown that no significant correlations exist between genome size and genetic or organismal complexity. This is referred to as the C-value paradox.

The underlying cause(s) of genome size variation are not well known. Most investigators have hypothesized that the variation in multicellular eukaryotes is strongly influenced by natural selection and has an adaptive basis. Direct data on the issue are primarily the long-standing correlations observed between genome size and biophysical parameters such as cell or nuclear size (or volume) and minimum meiotic or mitotic cycle times (Bennett 1971, 1972; Szarski 1974; Cavalier-Smith 1978, 1982, 1985*a*, 1985*b*). Bennett (1971, 1972) termed the sum of the biophysical parameters the "nucleotype" and hypothesized that C values evolve directly in response to selection acting on the nucleotype. Other "adaptive" theories have been proposed by Cavalier-Smith (1978, 1980, 1982, 1985*b*) and Szarski (1983). More indirect data favoring the "adaptive" hypotheses have come from studies where genome size was found to be correlated with organismal parameters that might be related to growth and developmental rates. These parameters have included body size, clinal or general habitat differences, and a few life-history characteristics (Miksche 1971; Ebeling *et al.* 1971; Bennett 1976; Mazin 1980; Shuter *et al.* 1983). A point to note is that these "adaptive" hypotheses are predicated on the assumptions that the variations in genome size are nonrandomly

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distributed, are affected by developmental, growth and (or) metabolic rates, and are reflected in organismal biology. The adaptive hypotheses also indirectly suggest that changes in genome size could follow phylogenetic trends and possibly be involved in speciation episodes.

The above considerations are confounded by the problem that almost all available data on genome sizes are from distinct species or higher taxa. Studies of DNA quantity variation at lower hierarchical levels are few, and historically, differences within species have been regarded as insignificant (Bennett and Smith 1976). In a few recent studies, however, it has clearly been shown that genome size variation within species can often exceed differences between species and, moreover, that the extent of the variation within species can often preclude meaningful consideration of the variation among species (Sherwood and Patton 1982; Price *et al.* 1983; Gold and Price 1985; Ragland and Gold 1986). As succinctly pointed out by Sherwood and Patton (1982), it will be very difficult to evaluate the meaning of genome size variation between species without knowledge on the extent of genome size variation within species.

In this paper, genome sizes from 200 individuals representing 20 species of North American cyprinid fishes are presented. Briefly, the cyprinids endemic to North America comprise a diverse array of some 250 species placed in 35–40 genera (Miller 1959; Lee *et al.* 1980). Most of these are placed in a single, essentially monophyletic subfamily which dates to the Miocene (Hubbs 1955; Miller 1965). The majority of North American cyprinid fossils, however, have been found in Pliocene and Pleistocene deposits (Miller 1965; Smith 1981), suggesting that many extant species are of recent origin. The apparent trend in North American Cyprinidae towards evolving a multitude of small, morphologically similar forms is best exemplified by the genus *Notropis*, which contains well over 100 living species (Miller 1959, 1965). Most of the comparative genetic work on North American cyprinids has focused either on structural gene (allozyme) divergence within and between species or on gross karyotypic differences (Avis and Ayala 1975; Avise 1977; Gold *et al.* 1978; Gold 1980). These studies have demonstrated that despite the rapid speciation and morphological evolution exhibited by the group, structural gene and chromosomal evolution appear decelerated, especially in the genus *Notropis*.

The problems investigated in this study are the dynamics of genome size variation within and between North American cyprinid species and the distribution of the variation in relation to life-history and habitat differences among the species. In our initial paper (Gold and Price 1985), it was inferred from a survey of five species that the focus of genome size variation in North American cyprinids was between individuals within populations and that genome size differences within and between species were the result of small accumulations of both gains and losses of DNA. The data presented here augment the latter, but not the former, in that considerable genome size differentiation may be occurring during cyprinid speciation episodes. The genome size variation among species, however, appears to be independent of several biological variables that differentiate the species.

Materials and methods

The current classifications and collection locales of the 20 species examined in this study are given in Table 1. Five of the species are from the previous study (Gold and Price 1985) and were included to enlarge the data set. Except for *Notemigonus crysoleucas*, all fish were collected by seining from natural populations. Individuals sampled outside Texas were processed in the field or at facilities in Oklahoma (see Acknowledgments); individuals sampled in Texas were returned live to College Station. All specimens ultimately will be deposited in the Texas Cooperative Wildlife Collection at Texas A&M University.

Relative genome sizes of individual fish (10 individuals/species) were determined microspectrophotometrically using Feulgen-stained erythrocyte nuclei. Details of slide preparation, staining, and microdensitometry are given in Gold and Price (1985). Control experiments to ensure that preparation and fixation of fish nuclei in the field would not adversely affect Feulgen staining were carried out and demonstrated that fish nuclei could be fixed on slides and held under dessicated conditions at 4°C for up to 2 weeks without significantly altering absorbancy values. Fifteen nuclei were measured from each of two slides per fish (=30 nuclei/individual) and standardized as a percent of the mean absorbancy of chicken erythrocyte nuclei on the same slide. The chicken blood served as an internal standard for each slide and was obtained from full sibs of a very highly inbred line (SPAFAS) available from the Texas A&M College of Veterinary Medicine. The decision to measure 30 nuclei per individual was based on experiments described in Gold and Price (1985), which showed an average coefficient of variation (per slide and per individual) of 3–4%. This means that measuring 30 nuclei per fish should differentiate a 2–3% difference in mean genome size at α and β probability levels of 0.05 (Gold *et al.* 1975).

Standardized absorbancy values of fish nuclei were loaded onto minidiscs using a small laboratory computer, coded for convenience by multiplying the percent chicken standard (for each fish nucleus) by 20, and then transferred to the University mainframe computer. For conversion to picograms of DNA, the standardized, decoded data were multiplied by 2.5, the generally accepted DNA value of diploid chicken erythrocyte nuclei (Rasch *et al.* 1971). Statistical analyses of the data as described below were carried out using either SAS programs or statistical packages available at the University computer center.

Results

The coded absorbancy data were organized into a number of different sampling distributions and each was tested for normality using the g_1 and g_2 indices (Sokal and Rohlf 1969). The distributions tested included (*i*) all measurements (nuclei) over all 20 species ($n = 6000$); (*ii*) all DNA values of individuals over all species ($n = 200$); (*iii*) all measurements (nuclei) within each species (20 sampling distributions, $n = 200$ each); and (*iv*) a rankit distribution reflecting the distribution of DNA values of individuals within populations of species (summed over all species, $n = 200$). The last was generated from rankit scores of DNA values of individuals according to Eq. 1:

$$[1] \quad \text{Rankit}_{jk} = \bar{X}_j - \bar{X}_k/\text{SD}_k$$

where Rankit_{jk} is the rankit score of the jth individual in the kth species, $\bar{X}_j$ is the mean DNA content of the jth individual based on all measurements (nuclei) from that individual ($n = 30$), $\bar{X}_k$ is the mean DNA content of the kth species based on all individuals assayed from that species ($n = 10$), and SD_k is the standard deviation of $\bar{X}_k$. The purposes of the transformation to rankit values were to remove scaling effects owing to individuals being drawn from different species and to increase the sample size in the test of normality for the distribution of DNA values of individuals within populations of species. As expected (Sokal and Rohlf 1969), the rankit distribution had a zero mean and near unit variance.

The results of the distribution normality tests are shown in Table 2. The distributions of all measurements and of all DNA values of individuals across species were significantly nonnormal; the deviations from normality in both cases, however, do

TABLE 1. Classification and collection locales of species

Taxon	Collection site	Drainage	County and State
Family Cyprinidae			
Subfamily Abramidinae			
*Notemigonus crysoleucas**†	—	—	—
Subfamily Leuciscinae			
Genus *Notropis*			
Subgenus *Alburnops*			
N. girardi	S. Canadian R.	Arkansas R.	McClain, OK
N. stramineus	Caddo Cr.	Washita R.	Carter, OK
Subgenus *Cyprinella*			
N. lutrensis†	Little Brazos R.	Brazos R.	Brazos, TX
N. venustus†	Bull Cr.	Colorado R.	Travis, TX
N. whipplei	Clear Cr.	Arkansas R.	Crawford, AR
Subgenus *Hydrophlox*			
N. nubilus	White R.	White R.	Washington, AR
N. rubellus	Blue R.	Red. R.	Johnston, OK
Subgenus *Luxilus*			
N. chrysocephalus	Blue R.	Red R.	Johnston, OK
N. pilsbryi	White R.	White R.	Washington, AR
Subgenus *Lythrurus*			
N. umbratilis	Blue R.	Red R.	Johnston, OK
Subgenus *Notropis*			
N. shumardi	Arkansas R.	Arkansas R.	Crawford, AR
Unknown affinity			
N. boops	Blue R.	Red R.	Johnston, OK
Other genera			
Campostoma anomalum†	Boardhouse Cr.	Blanco R.	Blanco, TX
Campostoma oligolepis	White R.	White R.	Washington, AR
Pimephales notatus	Blue R.	Red R.	Johnston, OK
Pimephales promelas	Briar Cr.	Red R.	Marshall, OK
Pimephales vigilax†	Little Brazos R.	Brazos R.	Brazos, TX
Phoxinus erythrogaster	Bob Jordan Spr.	Illinois R.	Washington, AR
Semotilus atromaculatus	Osage Cr.	Illinois R.	Benton, AR

NOTE: Classification is based on Hubbs (1955), Gibbs (1957), Gilbert (1964), Snelson (1968, 1972), and Swift (1970).
*Obtained from a local bait shop in Bryan, TX.
†Taxa studied by Gold and Price (1985).

not appear large (Fig. 1), and both distributions are continuous and overlapping. Seven of the 20 distributions of measurements within species were significantly nonnormal. However, there was no tendency for these nonnormal distributions to be either skewed or kurtotic, and the deviations from normality in each case were only slight (e.g., Fig. 2 in Gold and Price 1985). The rankit distribution, reflecting DNA values of individuals within populations of species, was normal. These findings indicate that genome size variation in cyprinids is essentially normally distributed within populations of species but not across them. The distributions of DNA contents across species, however, are both continuous and overlapping.

Descriptive statistics from the distribution of DNA values of individuals within species are shown in Table 3. The variation over all individuals over all species ranged from 2.12 (one individual of *N. boops*) to 2.81 pg DNA (one individual of *N. shumardi*) or 32.5%. The differences between species means ranged from 0% (*Semotilus atromaculatus* vs. *N. whipplei* and *N. nubilus* vs. *N. rubellus*) to 24.2% (*N. boops* vs. *N. shumardi*) and averaged 7.86% (190 pairwise comparisons). The maximum variation between individuals within populations of species ranged from 2.56% in *N. rubellus* to 13.49% in *N. crysoleucas* and averaged 5.95%. The comparison of DNA quantity variation within and between the 20 species indicates that the average maximum difference in genome size between individuals within populations of cyprinid species is nearly as great as the average difference in genome size between species. Moreover, the average maximum genome size variation between individuals within populations (5.95% or roughly 1.3×10^8 base pairs of DNA) is nearly as large as the quantity of DNA theoretically needed for the entire cyprinid structural gene component. The latter is based on assuming a liberal figure of 50 000 structural genes in the cyprinid genome and 1 500 coding DNA base pairs per gene (total = 1.5×10^8 base pairs of DNA).

Single classification analysis of variance (ANOVA) was used to test for significant heterogeneity in genome size variation among species and among individuals within populations of species. For reasons outlined in Gold and Price (1985), only the distribution of DNA values of individuals was used in the test of heterogeneity among species. Significant heterogeneity of mean DNA values at $\alpha = 0.05$ was found among species and the results of a Duncan's multiple range test are shown in Table 4. All species fell into a continuous series of groupings, with considerable overlap of mean DNA values among the species with smaller genome sizes. Given the range of DNA values of individuals within populations of species (Table 3), it is clear that the distribution of DNA values over all species is continuous and overlapping and that no single species can be unequivocally differentiated solely on the basis of genome size.

Separate single classification ANOVA's were used to test for

TABLE 2. Distribution normality statistics

Sampling distribution	n	Skewness (g_1)	Kurtosis (g_2)
Measurements (nuclei) among species	6000	0.471*	−0.266*
Individuals among species	200	0.501*	−0.527
Measurements (nuclei) within species			
Notropis shumardi	300	−0.078	−0.288
Notropis umbratilis	300	0.263	−0.147
Phoxinus erythrogaster	300	0.090	−0.679*
Notropis stramineus	300	0.223	−0.320
Semotilus atromaculatus	300	0.042	−0.212
Notropis whipplei	300	0.236	−0.237
Notropis pilsbryi	300	0.128	−0.407
Notropis venustus†	300	0.086	−0.452
Notropis nubilus	300	−0.100	−0.711*
Notropis rubellus	300	−0.041	−0.251
Notropis lutrensis†	300	−0.028	−0.289
Notropis girardi	300	0.117	−0.283
Notropis chrysocephalus	300	0.291*	0.244
Campostoma anomalum†	300	0.155	−0.214
Notemigonus crysoleucas†	300	0.376*	−0.091
Campostoma oligolepis	300	0.284*	−0.643*
Pimephales notatus	300	−0.314*	−0.093
Pimephales promelas	300	0.028	−0.247
Pimephales vigilax†	300	0.018	−0.343
Notropis boops	300	−0.069	−0.720*
Individuals within species‡	200	0.048	−0.314

*Significant at $\alpha = 0.05$. Positive g_1 values indicate skewness toward higher values; negative g_1 values indicate skewness toward lower values; negative g_2 values indicate platykurtosis.

†Data from Gold and Price (1985).

‡Rankit distribution of DNA values of individuals (cf. text).

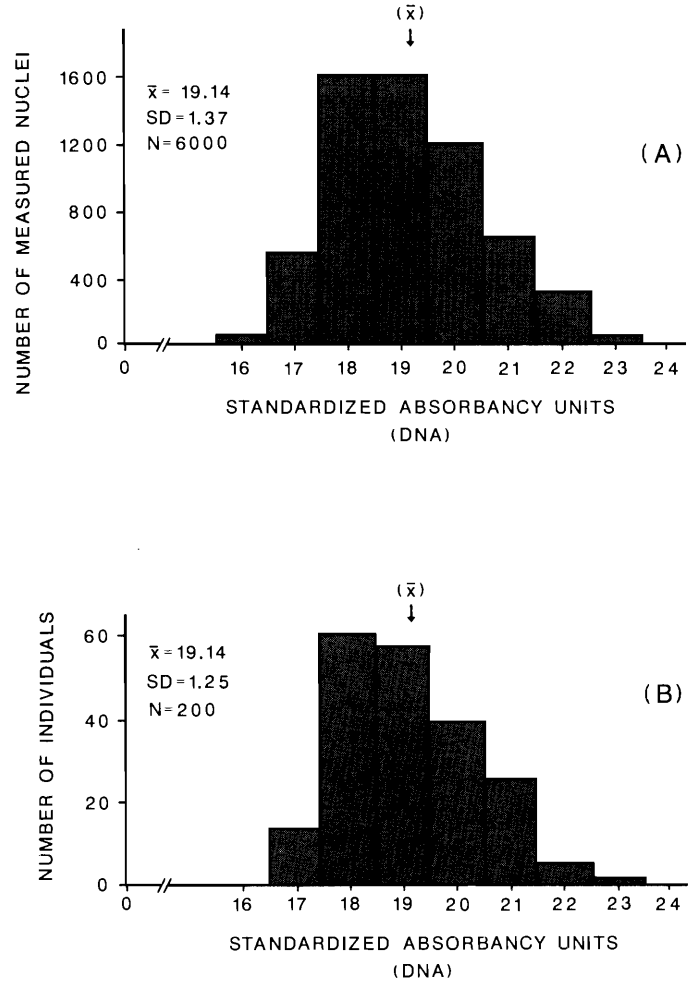

FIG. 1. Frequency distribution (coded data) of DNA measurements (nuclei) over all 20 cyprinid species (A) and DNA values of individuals over all 20 cyprinid species (B).

heterogeneity of DNA values of individuals within each species using the distribution of measurements (nuclei) of that species. All F-tests were significant at $\alpha = 0.05$. A synopsis of the results of Duncan's multiple range tests on each species is shown in Table 5. On the average, between four and five (range = two to six) significantly different groupings of individuals in terms of DNA content are found in populations of the 20 species. In all cases, the heterogeneity of DNA values among individuals within populations was distributed continuously, with modal DNA values being approximately the same as species mean DNA values. These results indicate that significant differences in genome size occur among individuals within populations of cyprinid species.

The foregoing indicated that variation in genome size appears to be continuously distributed within and across the 20 cyprinid species and that significant heterogeneity in mean genome size occurs both among species and among individuals within populations of species. In addition, the average maximum difference in genome size between individuals within populations approaches the average difference in genome size between species. A nested analysis of variance, however, revealed that although significant heterogeneity in genome size existed at each experimental level from between slides within individuals to between species, the majority (77%) of the variation in genome size occurs among species (Table 6). To examine this further, the magnitude of genome size differences at ascending taxonomic levels was evaluated by calculating the average genome size difference (or distance) between individuals drawn at random from different levels. This was accomplished using Eqs. 2 and 3.

$$[2]\quad \mathrm{GSD}_{kl} = \frac{\sum_{i=1}^{i=m}\sum_{j=1}^{j=n}\left|a_{ki} - a_{lj}\right|}{mn}$$

where GSD_{kl} is the minimum genome size difference (distance) between species k and species l, m is the number of individuals in species k, n is the number of individuals in species l, a_{ki} is the DNA value of the ith individual in species k, and a_{lj} is the DNA value of the jth individual in species l.

$$[3]\quad \mathrm{GSD}_k = \frac{\sum_{i=1}^{i=n_{k-1}}\sum_{j=i+1}^{j=n_k}\left|a_{ki} - a_{kj}\right|}{n_k(n_{k-1})/2}$$

where GSD_k is the average genome size difference (or distance) between all individuals in species k, n_k is the total number of individuals in species k, a_{ki} is the DNA value of the ith individual in species k, and a_{kj} is the DNA value of the jth individual in species k. The GSD values generated from Eq. 2 represent the average of all possible pairwise differences in genome size between all individuals of any two species. Since 10 individuals were examined from each species, there are 100 possible comparisons in estimating the GSD value between any two species. The 19 × 20 GSD distance matrix generated from these calculations is not shown but may be obtained upon request from the first author. The GSD values generated from

TABLE 3. Descriptive statistics* of genome size variation within and between 20 species of North American Cyprinidae

Taxon	2C DNA content (pg)		Difference from *Notropis shumardi*	
	Mean ± SE	Range (%)	pg	%
Notropis shumardi	2.72±0.02	2.65–2.81(6.04)	—	—
Notropis umbratilis	2.65±0.01	2.57–2.68(4.28)	0.07	2.64
Phoxinus erythrogaster	2.63±0.01	2.57–2.71(5.45)	0.09	3.42
Notropis stramineus	2.51±0.01	2.48–2.58(4.03)	0.21	8.37
Semotilus atromaculatus	2.50±0.01	2.43–2.57(5.76)	0.22	8.80
Notropis whipplei	2.50±0.01	2.44–2.56(4.92)	0.22	8.80
Notropis pilsbryi	2.48±0.01	2.41–2.56(6.22)	0.24	9.68
Notropis venustus	2.42±0.01	2.36–2.47(4.66)	0.30	12.40
Notropis nubilus	2.38±0.01	2.32–2.42(4.31)	0.34	14.29
Notropis rubellus	2.38±0.01	2.34–2.40(2.56)	0.34	14.29
Notropis lutrensis	2.37±0.01	2.31–2.44(5.63)	0.35	14.77
Notropis girardi	2.34±0.01	2.26–2.41(6.64)	0.38	16.24
Notropis chrysocephalus	2.30±0.01	2.24–2.37(5.80)	0.42	18.26
Campostoma anomalum	2.29±0.02	2.21–2.38(7.69)	0.43	18.78
Notemigonus crysoleucas	2.28±0.03	2.15–2.44(13.49)	0.44	19.30
Campostoma oligolepis	2.26±0.02	2.18–2.36(8.26)	0.46	20.35
Pimephales notatus	2.24±0.01	2.18–2.31(5.96)	0.48	21.43
Pimephales promelas	2.22±0.01	2.17–2.28(5.07)	0.50	22.52
Pimephales vigilax	2.21±0.01	2.14–2.26(5.61)	0.51	23.08
Notropis boops	2.19±0.01	2.12–2.26(6.60)	0.53	24.20

*From the distribution of DNA values of individuals where $n = 10$ for each taxon.

TABLE 4. Results of Duncan's multiple range test on the distribution of DNA values of individuals

Species	$\bar{X}$ DNA values (pg)
Notropis shumardi	2.72*a*
Notropis umbratilis	2.65*b*
Phoxinus erythrogaster	2.63*b*
Notropis stramineus	2.51*c*
Semotilus atromaculatus	2.50*c*
Notropis whipplei	2.50*c*
Notropis pilsbryi	2.48*c*
Notropis venustus	2.42*d*
Notropis nubilus	2.38*e*
Notropis rubellus	2.38*e*
Notropis lutrensis	2.37*e*
Notropis girardi	2.34*e*
Notropis chrysocephalus	2.30*f*
Campostoma anomalum	2.29*gf*
Notemigonus crysoleucas	2.28*hgf*
Campostoma oligolepis	2.26*hg*
Pimephales notatus	2.24*hi*
Pimephales promelas	2.22*ji*
Pimephales vigilax	2.21*ji*
Notropis boops	2.19*j*

NOTE: Mean DNA values of species with the same letter (Duncan's test grouping) are not significantly different at $\alpha = 0.05$.

Eq. 3 represent the average of all possible pairwise comparisons between all individuals of any one species. Since 10 individuals were examined from each population of a species, there are 45 possible comparisons in estimating the GSD value between individuals within a population of a species. These GSD values for all 20 species were then averaged to obtain an estimate of the average genome size difference between individuals within populations of species. Both GSD values are minimum linear difference or distance estimates and as such may underestimate the true difference if reversed or reticulated patterns of change occur (Sneath and Sokal 1973). Alternatively, such linear estimates are the only metrics suitable for nonrooted quantitative data such as genome sizes.

In Table 7, the average genome size differences between individuals drawn from successive levels of evolutionary divergence are shown. To obtain estimates of genome size differences between species in subgenera of *Notropis* and between species in *Notropis* and in other genera, subsets of GSD values were extracted from the 19 × 20 GSD distance matrix. As an example, the mean genome size difference between species in subgenera of *Notropis* involved first computing an average GSD value for each subgenus based on all pairwise comparisons between species in that subgenus. The average GSD values for the subgenera were then averaged to obtain the value shown in Table 7. The same approach was used to estimate the average GSD value between species in genera other than *Notropis*. The estimate for species in *Notropis* is simply the average of all pairwise comparisons among the 12 *Notropis* species examined (cf. Table 1).

Examination of the data in Table 7 reveals that, on the average, individuals drawn at random from two different cyprinid species will differ by 1.47 GSD units (approximately 0.184 pg DNA), whereas any two individuals drawn at random from a population of the same species will differ by only 0.381 GSD units (approximately 0.048 pg DNA). This indicates that the majority of genome size divergence in cyprinids occurs above the level of individuals within populations of species and corroborates the results obtained from the nested analysis of variance. What is more interesting, however, is the degree of divergence in genome size that has apparently occurred in the cyprinid genus *Notropis* as compared with other cyprinid genera. The average difference in genome size between

TABLE 5. Results of single classification ANOVA's and Duncan's multiple range tests for heterogeneity of DNA values of individuals within populations of species

Species	*F* value*	No. of significantly different groups†
Notropis shumardi	14.53	3
Notropis umbratilis	6.96	3
Phoxinus erythrogaster	7.05	4
Notropis stramineus	7.48	3
Semotilus atromaculatus	7.57	3
Notropis whipplei	4.94	4
Notropis pilsbryi	9.88	5
Notropis venustus	8.68	5
Notropis nubilus	8.32	5
Notropis rubellus	7.32	2
Notropis lutrensis	13.36	6
Notropis girardi	9.78	6
Notropis chrysocephalus	7.04	5
Campostoma anomalum	17.90	5
Notemigonus crysoleucas	46.73	6
Campostoma oligolepis	15.40	6
Pimephales notatus	12.03	5
Pimephales promelas	9.09	3
Pimephales vigilax	16.52	4
Notropis boops	8.91	6
$\bar{X}$		4.45

*All *F* values are significant at $\alpha = 0.05$.

†Refers to groupings of significantly different means using Duncan's multiple range tests at $\alpha = 0.05$.

TABLE 6. Nested analysis of variance of the distribution of measurements (nuclei) over all species

Variance source	df	MS	*F*	Variance component	%
Total	5999	1.88	—	1.958	100
Species	19	458.71	128.8*	1.517	77.50
Individuals	180	3.56	1.6*	0.045	2.28
Slides	200	2.22	8.2*	0.130	6.65
Error	5600	0.27	—	0.266	13.57

*Significant at $\alpha = 0.05$.

TABLE 7. Average genome size difference (distance) between individuals from successive levels of evolutionary divergence

Level	Mean genome size difference ± SE*	No. of pairwise comparisons
Individuals within populations of species	0.381±0.028	900
Species in subgenera (of *Notropis*)	0.919±0.284†	6
Species in genera		
Notropis	1.379±0.115	66
Other genera	0.434±0.076‡	4
Species in family	1.471±0.071	190

*In coded DNA values. Picograms are estimated by dividing coded values by eight.

†Average of mean genome size distances between species in four subgenera of *Notropis* (cf. Table 1).

‡Average of mean genome size distances between species in *Campostoma* and *Pimephales*.

TABLE 8. Assignment of species to a life-history characteristic

Taxon	Peak annual spawning time*	Range breadth†	Water volume preference‡	Dietary habit§
Notropis shumardi	2	3	3	2
Notropis umbratilis	2	2	1	3
Phoxinus erythrogaster	1	2	1	1
Notropis stramineus	2	1	2	2
Semotilus atromaculatus	1	1	1	3
Notropis whipplei	2	2	2	3
Notropis pilsbryi	1	3	1	3
Notropis venustus	2	2	1	3
Notropis nubilus	2	3	2	1
Notropis rubellus	1	1	2	2
Notropis lutrensis	2	1	2	3
Notropis girardi	2	3	3	3
Notropis chrysocephalus	1	1	1	3
Campostoma anomalum	1	1	2	1
Notemigonus crysoleucas	2	1	—	2
Campostoma oligolepis	1	3	2	1
Pimephales notatus	1	1	2	2
Pimephales promelas	2	1	1	2
Pimephales vigilax	2	2	2	3
Notropis boops	3	2	1	3

*Peak annual spawning times: 1, early (April–May); 2, intermediate (June–July); 3, late (July–August).

†Range breadth: 1, widespread distribution; 2, moderate distribution; 3, limited distribution.

‡Water volume preference: 1, small- to medium-sized creeks or streams; 2, medium- to large-sized streams or small rivers; 3, medium-sized streams to large rivers.

§Dietary habit: 1, herbivorous; 2, omnivorous; 3, carnivorous.

individuals in different species of *Campostoma* and *Pimephales* is only 0.434 GSD units, whereas the average difference between individuals in different *Notropis* species (1.379 GSD units) is nearly the same as the average difference between individuals drawn from any two species in the family. Moreover, much of the divergence in genome size within *Notropis* appears to have occurred at the subgeneric rather than generic level. Although additional species from other cyprinid genera need to be examined, the tentative implication of these data is that genome size divergence in *Notropis* has been far greater than that in other North American cyprinid genera.

The final set of analyses carried out with the cyprinid genome size data set were tests of association between interspecific variation in genome size and variation in several life-history parameters. These tests were in response to studies in other organisms (see Introduction), where significant associations between genome size and life-history characteristics were taken as evidence that variation in genome size has an adaptive basis. The parameters used here included body size (=length), peak annual spawning time, range breadth, water volume preference, and dietary habit and were chosen in large part because at least 2 of the 20 cyprinid species studied could generally be assigned into two or more categories within each of the life-history parameters. The assignments were made on the basis of the primary literature and are shown in Table 8. The tests for association between interspecific genome size and body size involved computation and significance testing of both parametric (Pearson's product moment) and nonparametric (Spearman's rank) measures of correlation. Three separate measures of body size were used, viz., mean body lengths for each of the populations (species) sampled in the study and estimated

directly from the specimens examined for genome size, and average and maximum body lengths for each species taken from the primary literature. No significant correlations at $\alpha = 0.05$ were found between genome size and any of the three body size estimates. The tests for association between variation in genome size and variation in the four life-history characteristics were carried out using the nonparametric Kruskal–Wallis one-way analysis of variance to determine whether independent samples (species DNA values) were drawn from different sampling distributions as shown in Table 8. All computed Kruskal–Wallis H statistics (Siegel 1956) were nonsignificant at $\alpha = 0.05$, indicating that species DNA values are randomly distributed within the variation that occurs in these life-history characteristics.

Discussion

The normality (or near normality) of DNA content distributions within populations of cyprinid species suggests that changes in DNA quantity at this level are small in amount, involve both gains and losses of DNA, and are cumulative and independent in effect. This is based primarily on the assumption that genome size variation within populations of species follows the premises of the normal probability density function (Sokal and Rohlf 1969). Similar conclusions were reached by Gold and Price (1985) from a much smaller cyprinid data set. The distribution of DNA contents across cyprinid species, although nonnormal, is essentially continuous and overlapping suggesting that differences between species also result from the steady accumulations of relatively small changes in DNA quantity. Johnson and Utter (1986) and Ragland and Gold (1986) found almost identical patterns in their studies of genome size variation among salmonid and centrarchid fish, respectively. This pattern of continuous genome size change appears to typify many animal groups (Bachmann *et al.* 1972, 1985) and differs markedly from the "quantized" (Cavalier-Smith 1985*b*) change in genome size that appears to occur in many plant groups. In the latter, species DNA values are often discontinuously distributed and species groups may be distinguished on the basis of quantum differences in genome size (Narayan 1983; Raina *et al.* 1986). This may reflect either a difference between animals and plants in the mechanism(s) by which DNA sequences are gained or lost, a difference in the rates of DNA quantity change, or perhaps a difference in the intensity of selection on individuals with intermediate DNA values (Cavalier-Smith 1985*b*). There are, however, plant groups where interspecific DNA contents are continuously distributed (Kenton 1983). Regardless, in cyprinids at least, genome size differences between individuals within populations and among species appear to result from small changes in DNA quantity. Since all but one of the 20 cyprinids have $2n = 50$ very similar chromosomes (Gold *et al.* 1980; C. T. Amemiya and J. R. Gold, unpublished), most or all of the genome size changes are not due to chromosomal aneuploidy.

The size of the DNA fraction that is apparently free to vary quantitatively within cyprinid genomes is minimally about 6% (the average maximum difference between individuals within populations of species) and could be as high as 13.5% (the range of variation in *N. crysoleucas*). Comparable data in other organisms are rare since most of the work on genome size variation has focused on differences between fully differentiated species and higher level taxa. Sherwood and Patton (1982) and Price *et al.* (1983), however, have reported intraspecific differences in genome sizes of 35 and 20% in the rodent genus *Thomomys* and the composite genus *Microseris*, respectively, which along with the cyprinid data clearly indicate that intraspecific genome size variation is neither insignificant nor unimportant. The estimate of 6% in cyprinids represents about 1.3×10^8 base pairs of DNA, which is nearly as large as the quantity of DNA theoretically needed to code for the entire cyprinid structural gene component. In all likelihood, however, the DNAs gained or lost in cyprinid genomes are probably not coding sequences *per se* since this would be expected to significantly interfere with normal cellular processes. In other organisms, the sequences that are known to vary quantitatively between species include repeated DNA classes (Flavell *et al.* 1974; Hutchinson *et al.* 1980); the same is very likely true for cyprinids. Recent work in our laboratory (Moyer 1986; Karel and Gold[1]; Gold *et al.* 1986; J. R. Gold and L. S. Pletscher, unpublished) has shown that cyprinids contain appreciable quantities of chromosomal heterochromatin and at least two different, highly repeated satellite DNAs. Experiments are currently underway to determine whether differences in the relative quantities of chromosomal heterochromatin and of the satellite DNAs are correlated with differences in genome size.

The majority of the genome size variation among all 20 cyprinids examined appears to be distributed above the level of individuals within populations of species. This was demonstrated both by the nested analysis of variance and by the estimated genome size difference (or distance) values between individuals at successively higher levels of taxonomic organization. The surprising finding was that the degree of genome size divergence was far greater between species in subgenera of the genus *Notropis* than between species in other cyprinid genera. In the past, the North American Cyprinidae, and especially the genus *Notropis*, have been used as a model system in which to search for those genetic or genomic changes whose evolutionary rate or degree of divergence correspond to the relatively rapid rate of speciation and organismal evolution exhibited by these fishes. The finding that average differences in genome size between *Notropis* species and between species in various *Notropis* subgenera are substantially greater than differences between species in other cyprinid genera is strong suggestive evidence that genome size changes in cyprinids may be concentrated in speciation episodes. Gold (1980) estimated that the net speciation rate within *Notropis* was nearly twice that of other confamilial genera, meaning that if genome size change is correlated with speciation the average difference in genome size between *Notropis* species should be substantially greater than the average difference between species in other cyprinid genera. The theoretical grounds for this expectation may be found in Avise and Ayala (1975). Exactly how genome size change might engender a speciation event is unknown and would be impossible to demonstrate empirically. In addition, since the evidence is correlative, it would be difficult to determine if the correlation was one of cause and effect or simply one of association. Further studies are in progress to determine whether these observations hold over a broader sampling range both within and among species.

The last question of interest is whether the interspecific differences in genome size in cyprinids are biologically meaningful with respect to organismal parameters. As noted in the Introduction, most investigators have hypothesized that genome

[1]Karel, W. J., and Gold, J. R. A thermal denaturation study of genomic DNA from North American minnows (Cyprinidae: Teleostei). Submitted.

size variation in multicellular eukaryotes has an adaptive basis and is strongly influenced by natural selection. The data in support of the adaptive hypotheses are the positive correlations between genome size and a variety of quantitative characters of potential adaptive or functional significance. Some examples of the latter include the volume and mass of metaphase chromosomes, nuclear and cell volumes or sizes, and the length of cell cycles and (or) the duration of meiosis (Cavalier-Smith 1985*a*). In flowering plants, at least, there also are correlations between genome size and climate (Bennett 1976; Rayburn *et al.* 1985) and between genome size and leaf cell size (Grime 1983, cited by Cavalier-Smith 1985*b*). In general, however, the adaptive hypotheses have been confounded by two problems. The first is that with increasing organismal complexity, it is very difficult to correlate cellular features such as nuclear or cell volume and mitotic or meiotic cycle times with organismal phenotypes that can be empirically evaluated. In addition, it is often difficult, if not impossible, to obtain direct and unbiased estimates of growth and developmental rates from the majority of higher eukaryotes. The second problem is that most of the data on this issue has been obtained from distinct species or higher taxa, and variation within species has been essentially ignored.

The tests for association between cyprinid species DNA values and five life-history characteristics, including body size, were all nonsignificant. This would appear to preclude any direct relationship between genome size variation and these organismal parameters. This should not, however, be taken as evidence for the absence of selection acting upon the genome size variation but rather as a demonstration of the difficulty in detecting correlations should they exist (Bachmann *et al.* 1985). Moreover, the findings that significant differences in genome size occur within populations of cyprinid species and that species genome size distributions overlap considerably confounds the problem of identifying significant correlations even if they exist. As noted by Bachmann *et al.* (1985), the question of whether selection for genome size results in changes in organismal phenotypes will ultimately depend on experimental evidence obtained in the laboratory and under defined conditions.

A final point to note is that thus far the cyprinid DNA quantity data are not inconsistent with the "selfish DNA" hypothesis that much of the variation in eukaryotic genome size reflects gains or losses of phenotypically inconsequential DNA (Doolittle and Sapienza 1980; Orgel and Crick 1980). This is based on the observations that (*i*) considerable quantitative DNA variation appears to occur within cyprinid species and has no noticeably adverse phenotypic effects, and (*ii*) species DNA values in cyprinids appear to be more or less randomly distributed within the variation which occurs. Empirical evidence confirming a direct relationship between genome size and selfish DNA proliferation, however, will be exceedingly difficult to obtain.

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