Genetica 74: 181–187, (1987)
© Dr W. Junk Publishers, Dordrecht – Printed in the Netherlands

A thermal denaturation study of genomic DNAs from North American minnows (Cyprinidae: Teleostei)

W. J. Karel & J. R. Gold*
Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843, USA

Received 16.7.1986 Accepted in revised form 27.5.1987

Abstract

Base compositions and differential melting rate profiles of genomic DNAs from twenty species of North American cyprinid fishes were generated via thermal denaturation. Base pair composition expressed as % GC values ranged among the twenty species from 36.1–41.3%. This range is considerably broader than that observed at comparable taxonomic levels in other vertebrate groups. Both the range and average difference in base pair composition between species in the diverse and rapidly evolving genus *Notropis* were considerably greater than those between species in other North American cyprinid genera. This may indicate that genomic changes at the level of base pair composition are frequent and possibly important events in cyprinid evolution. Compositional heterogeneity and asymmetry values among the twenty species were uniform and low, respectively, suggesting that most of the species lacked DNA components in their genomes which differed substantially from their main-band DNAs in base pair composition. The melting rate profiles revealed a prominent and distinct heavy or GC-rich DNA component in the genomes of three species belonging to the subgenus *Cyprinella* of *Notropis*. These and other data suggest that the heavy melting component may reflect a large, comparatively GC-rich family of highly repeated or satellite DNA sequences common to all three genomes.

Introduction

Thermal denaturation analysis of genomic DNAs has long been used to study genomic change, differentiate taxa, and identify species relationships among microorganisms (Schildkraut *et al.*, 1962). The approach provides data on DNA base pair composition and heterogeneity within genomes, and in higher organisms can often reveal discrete DNA melting components which indicate the presence of repeated families of DNA sequences (Pivec *et al.*, 1974; Guttman *et al.*, 1977; Mayfield, 1977). Comparative DNA thermal denaturation studies in higher organisms, however, are relatively rare. There is evidence which indicates that DNA base compositions and melting rate profiles often reveal specific details of differing DNA sequences within eukaryotes, and that such data are, in some cases, systematically or taxonomically informative (Arrighi *et al.*, 1970; Huguet & Jouanin, 1972; Pivec *et al.*, 1974; Mayfield, 1977; Guttman *et al.*, 1977; Wada *et al.*, 1980; Olmo, 1981). A few of these studies have shown that DNA base compositions or melting rate profiles can differ substantially between closely related species (Guttman *et al.*, 1977; Olmo, 1981).

In this report, we present the data from thermal denaturation of genomic DNAs from twenty species of North American cyprinid fishes. Twelve of the species examined were from the diverse cyprinid genus *Notropis* which contains more than 100 living representatives. The remaining eight species examined were from different genera among the 35–40 cyprinid genera extant in North America. The experiments were carried out primarily to examine the

* To whom reprint requests should be sent

distribution of DNA base compositions and melting rate profiles among these fishes as part of a long term study on genome evolution and systematics in the North American Cyprinidae (Gold *et al.*, 1978; Gold, 1980; Gold & Price, 1985). Of principle interest was the question of whether changes in whole genome composition might be associated with the rapid evolutionary divergence which characterizes these fishes.

Material and methods

Fishes were collected from natural populations in the southern United States. Specimens were transported live to College Station, Texas, or were frozen immediately in liquid nitrogen. Genomic DNA was isolated following the differential centrifugation protocol of Zimmer *et al.* (1981). Whole adult specimens (usually two to three per assay) were homogenized in 10 mM Tris-HCl, 5 mM EDTA, and 2 mM $MgCl_2$ (pH 7.2) on a Sorvall Omnimixer and a Brinkman Polytron. The nuclei were isolated by low speed centrifugation (400 × *g*) and were lysed in 20 mM Tris-HCl, 5 mM EDTA, and 10% Sarcosyl. Cesium chloride (1.1 g/ml) and ethidium bromide (50 μg/ml) were added to the lysate, and the DNA separated from other cellular constituents by preparative ultracentrifugation for 48 hours at 135000 × *g*. The DNA was withdrawn by side-puncture with a 20 gauge hypodermic needle and the ethidium bromide extracted via several washes with CsCl saturated isopropanol. The DNA was further purified by a series of ethanol/salt precipitations. Purity and concentration of the DNA samples were determined spectrophotometrically via readings at 260, 280, and 320 nm wavelengths.

Base composition data and differential melting rate profiles were generated via thermal denaturation of the DNAs (Mandel & Marmur, 1968). Isolated DNAs (approximately 60–80 μg per assay) were dissolved and exhaustively dialyzed in 0.1 × SSC (standard saline citrate) and then melted and monitored on a Gilford Model 2600 spectrophotometer with attached thermal programmer. The temperature was increased linearly at the rate of one degree C per minute and thermal denaturation readings were taken at 0.2 degree increments. Three replicates per species with *Escherichia coli* DNA (Type VIII, Sigma) as an external standard were generated. Data were transferred to a separate microprocessor and corrected for volume expansion prior to calculations of base composition values and plotting of melting rate profiles.

Results

Base compositions of the twenty North American cyprinid species examined are shown in Table 1 in the form of % GC (guanine-cytosine base pair) values, compositional heterogeneity values, and asymmetry values. Compositional heterogeneity values reflect the degree to which the GC and AT base pairs are interspersed within the genome and essentially define the transition width of the melting curve (Mandel & Marmur, 1968). Asymmetry values reflect the skewness of the melting curve and show the difference between the mean and modal values in % GC. Comparative data on compositional heterogeneity and asymmetry are available for some bacteria and protozoa (Gebers *et al.*, 1985; Schildkraut *et al.*, 1962), but few corresponding values are available for vertebrates.

The % GC values among the twenty species ranged from 36.1 to 41.3%. This range is considerably broader than that observed at comparable taxonomic levels in other vertebrate groups (Sueoka, 1961; Arrighi *et al.*, 1970; Hudson *et al.*, 1980; Olmo, 1981). Compositional heterogeneity values were relatively uniform, suggesting that most of the genomes lack DNA components which differ substantially in base pair composition from main-band DNA. The low asymmetry values (Table 1) also appeared to indicate the absence of DNA components differing substantially in base pair composition, although the generally positive asymmetry values did suggest a greater degree of base pair heterogeneity within the GC-rich fractions in most species. The slightly higher asymmetry values of the non-*Notropis* species may indicate that these species are less homogeneous in base composition than the *Notropis* species. Asymmetry values for the three species in the subgenus *Cyprinella* (*Notropis*) were not cal-

Table 1. Base compositions of genomic DNAs from twenty North American cyprinid species. % GC and CH (compositional heterogeneity) values were calculated according to Mandel & Marmur (1968). Asymmetry values represented the difference between mean and modal % GC values (Hudson *et al.*, 1980). Taxonomic placements of *Notropis* species follow Lee *et al.*, (1980).

Taxon	Mean % GC ± SE	CH ± SE	Asymmetry
Genus *Notropis*			
Subgenus *Notropis*			
N. amabilis	36.1 ± 0.1	14.6 ± 0.4	2.4
N. shumardi	37.1 ± 0.1	11.9 ± 0.5	0.5
Subgenus *Alburnops*			
N. girardi	37.7 ± 0.1	11.8 ± 0.5	2.5
N. texanus	38.2 ± 0.1	13.4 ± 0.3	2.1
N. sabinae	38.5 ± 0.1	12.8 ± 0.4	2.4
N. stramineus	38.5 ± 0.1	11.2 ± 0.6	2.4
Subgenus *Lythrurus*			
N. bellus	37.5 ± 0.1	15.2 ± 0.8	1.7
N. roseipinnis	39.0 ± 0.1	13.0 ± 0.4	1.7
N. ardens	39.3 ± 0.1	11.8 ± 0.3	2.9
Subgenus *Cyprinella*			
N. venustus	40.0 ± 0.1	12.1 ± 0.1	-
N. lutrensis	41.1 ± 0.2	14.3 ± 0.3	-
N. lepidus	41.3 ± 0.3	11.9 ± 0.6	-
Other genera			
Pimephales vigilax	37.1 ± 0.2	14.3 ± 0.4	−0.5
Hybognathus placitus	37.4 ± 0.2	12.1 ± 0.6	2.3
Hybopsis aestivalis	37.9 ± 0.1	12.9 ± 0.2	4.0
Phenacobius mirabilis	38.2 ± 0.1	12.1 ± 0.7	3.9
Phoxinus erythrogaster	38.2 ± 0.1	12.0 ± 0.4	3.0
Campostoma anomalum	38.3 ± 0.1	14.4 ± 0.4	3.0
Rhinichthys atratulus	38.3 ± 0.1	15.1 ± 0.5	2.6
Dionda episcopa	38.7 ± 0.1	11.4 ± 0.1	3.7

culated since the DNA melting rate profiles of all three were distinctly bimodal and asymmetry values are valid only for unimodal distributions (Hudson *et al.*, 1980).

Of particular interest was the over three-fold greater range in % GC values found among the *Notropis* species as compared to that among the species of the other eight cyprinid genera (Table 1). To examine this further, we calculated the average percent difference in % GC values between any two *Notropis* species and any two non-*Notropis* species by summing the percent differences in all pair-wise comparisons of species and dividing by the number of pair-wise comparisons made. The results (Ta-

Table 2. Ranges and average % differences between species pairs of % GC values for all *Notropis* and non-*Notropis* species examined. Average % difference is the mean % difference between any two species based upon all possible pair-wise comparisons.

Comparison	Range (in % GC)	Average % difference between species (± SE)	Number of pair-wise comparisons
Among *Notropis* species	36.1 − 41.3 (5.2)	1.83 ± 0.15	66
Among non-*Notropis* species	37.1 − 38.7 (1.6)	0.60 ± 0.08	28

ble 2) indicated that on the average any two *Notropis* species will differ considerably more (> threefold) in % GC value than any two species drawn at random from different North American cyprinid genera.

Melting rate profiles of genomic DNAs from the twenty species are shown in Figures 1 and 2. As expected based on the calculated asymmetry values (Table 1), almost all of the profiles were asymmetrical. However, most showed little evidence of discrete DNA melting components (i.e., broad shoulders or discrete peaks), again suggesting the absence of major DNA components with base compositions differing significantly from main-band DNA. A

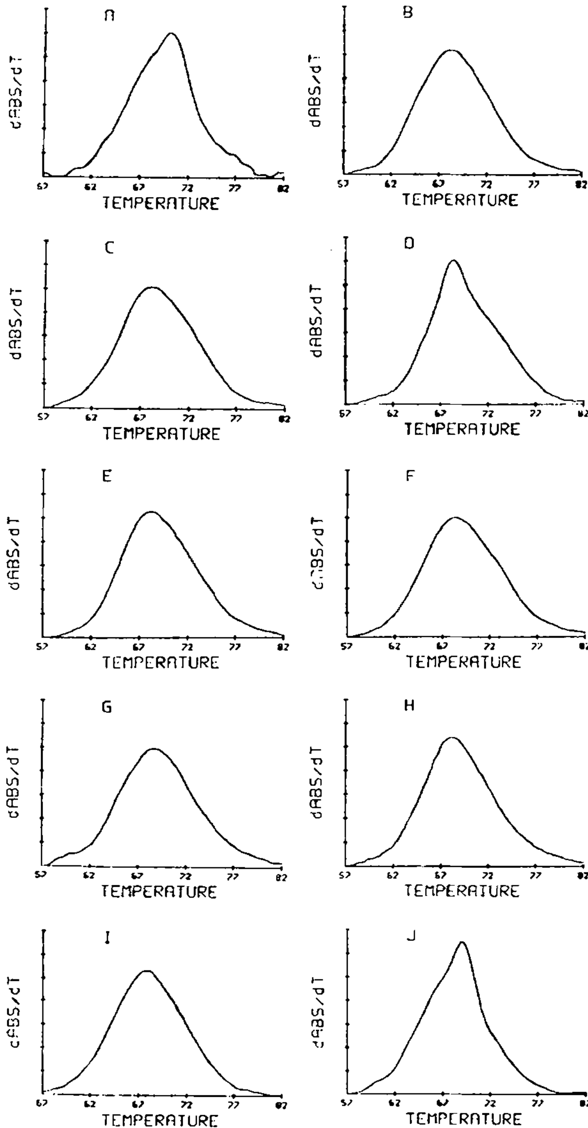

Fig. 1. Differential melting rate profiles. Abscissa: denaturation temperature; ordinate: increase in absorbance for each step increase (0.2 °C) in temperature. The species shown are: A, *Pimephales vigilax*; B, *Hybognathus placitus*; C, *Hybopsis aestivalis*; D, *Phenacobius mirabilis*; E, *Phoxinus erythrogaster*; F, *Campostoma anomalum*; G, *Rhinichthys atratulus*; H, *Dionda episcopa*; I, *Notropis amabilis*; J, *N. shumardi*.

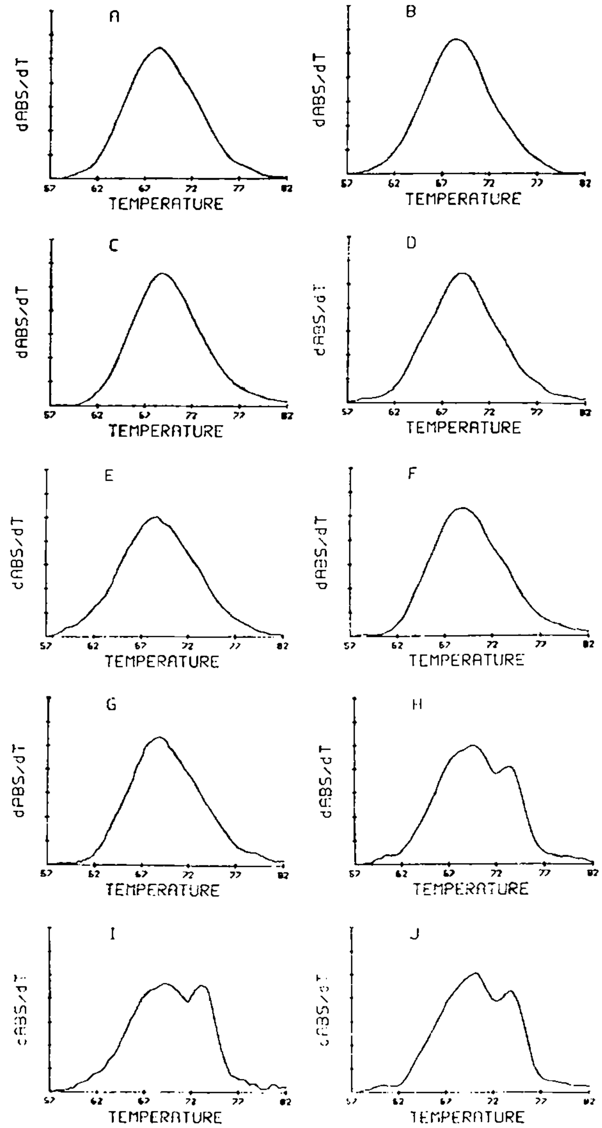

Fig. 2. Differential melting rate profiles. Abscissa: denaturation temperature; ordinate: increase in absorbance for each step increase (0.2 °C) in temperature. The species shown are: A, *Notropis texanus*; B, *N. girardi*; C, *N. sabinae*; D, *N. stramineus*; E, *N. bellus*; F, *N. roseipinnis*; G, *N. ardens*; H, *N. venustus*; I, *N. lutrensis*; J, *N. lepidus*.

Table 3. Guanine-cytosine content values of mainband DNA and the heavy DNA component of the three *Cyprinella* (*Notropis*) species. % GC values were calculated according to Mandel & Marmur (1968).

Taxon	% GC + SE	
	Mainband DNA	Heavy DNA Component
N. venustus	38.1 ± 0.6	47.6 ± 0.5
N. lutrensis	39.0 ± 0.4	47.9 ± 0.4
N. lepidus	39.5 ± 0.0	48.7 ± 0.5

prominent and distinct heavy or GC-rich DNA component was found in the genomes of the three species from the subgenus *cyprinella* (*Notropis*) (Fig. 2h – j). The % GC values of the heavy DNA component in all three species were nearly identical and differed considerably from the % GC values of the main-band DNAs in each of the three species (Table 3).

Discussion

The initial purpose of these experiments was to continue our search for genetic or genomic changes whose evolutionary rate or degree corresponds to the relatively rapid rate of speciation exhibited by North American cyprinid fishes. Briefly, the cyprinids endemic to North America comprise a diverse, essentially monophyletic assemblage of more than 200 extant species currently placed in some 35 – 40 genera (Miller, 1959; Lee *et al.*, 1980). All but one endemic species are placed in a single subfamily which is thought to have originated from one or a few ancestors that migrated to North America from Eurasia not prior to the Miocene (Hubbs, 1955; Miller, 1959). The majority of cyprinid fossils found in North America, however, are from Pliocene or Pleistocene deposits (Miller, 1965; Smith, 1981), suggesting that much of the group may be of relatively recent origin. Gold *et al.* (1978) estimated the rate of speciation within living North American cyprinid genera to be approximately 0.7 new species per lineage (genus) per million years. This rate is considerably higher than speciation rates estimated for many lower vertebrate taxa (Bush *et al.*, 1977). The apparent trend in North American Cyprinidae towards evolving a multitude of small, morphologically similar forms over short periods of time is best exemplified by the large genus *Notropis* which contains well over 100 living species (Miller, 1959, 1965).

Thus far, most of the evolutionary genetics work on North American cyprinids has focused either on structural gene (allozyme) divergence within and between species or on the variation between species in gross chromosomal karyotypes (Avise *et al.*, 1975; Avise, 1977; Gold *et al.*, 1978; Gold, 1980). In sum, these studies have demonstrated that despite the relatively rapid speciation and morphological evolution exhibited by North American cyprinids, structural gene and gross chromosomal evolution appear, if anything, to be decelerated (Avise, 1977; Gold, 1980). This is particularly well emphasized in the genus *Notropis*.

The salient finding of this survey was the broad range in % GC values among the twelve species of the genus *Notropis* where the extremes (36.1% in *N. amabilis vs.* 41.3% in *N. lepidus*) represent a difference of 14%. Large-scale surveys of base compositions of vertebrates are not extensive, but to date, the range in % GC values among the *Notropis* species appears to have no vertebrate analogue at a comparable taxonomic level, and in fact is comparable to or exceeds the range found in some vertebrate orders (Arrighi *et al.*, 1970; Hudson *et al.*, 1980; Olmo, 1981). This would appear to suggest first, that rather extensive genomic change has occurred during the evolution of North American Cyprinidae; and secondly, that the most dramatic changes have taken place within the genus *Notropis*. The latter is exemplified by the over three-fold difference in % GC value observed between the average *Notropis* species and the average species sampled independently from other North American cyprinid genera.

Relatively uniform DNA compositional heterogeneity and asymmetry values were found for all but three of the twenty cyprinid species examined. This indicates that the dispersion of DNA sequences in cyprinid genomes is generally homogeneous and that major DNA components differing substantially in base pair composition from main-band DNA are either absent, or present in low frequency. The

predominantly positive asymmetry values suggest either a greater degree of heterogeneity among GC-rich fractions in most genomes or the presence of minor DNA components comparatively rich in GC base pairs. The generalized absence of distinct heavy or GC-rich DNA components in cyprinid DNAs appears to typify cold-blooded vertebrate genomes as opposed to warm-blooded ones (Hudson *et al.*, 1980), although reptiles may prove to be a notable exception (Olmo, 1981).

The discrete, heavy DNA component found in the genomes of the three *Notropis* species belonging to the subgenus *Cyprinella* had nearly the same % GC value in all three species and differed considerably from the % GC values of the respective main-band DNAs. The displacement from the main-band DNA and the identity of % GC values suggest that the heavy melting components reflect a large, comparatively GC-rich family of highly repeated or satellite DNA sequences common in the genomes of all three species. The presence of satellite DNAs in *N. lutrensis* and *N. venustus* had been inferred previously based on the large amounts of C-band positive material on the chromosomes of both species (Gold *et al.*, 1986). More recently, we have obtained direct evidence of a discrete family of satellite DNA sequences in the *N. lutrensis* genome which conforms to expectations based on the *N. lutrensis* DNA melting rate profile. This family, referred to as the *Mbo*I satellite, minimally comprises about 8% of the *N. lutrensis* genome, is organized into tandem arrays of a 174 base pair repeat unit, and contains approximately 47% GC base pairs as estimated by nucleotide sequencing of one of the repeat units (Moyer, 1986). Experiments are now underway to determine if the *Mbo*I satellite is the heavy melting component in all three genomes. One other point to note is that the heavy DNA component found in these three species may also provide a systematically useful character. Thus far, the presence of the heavy DNA component in detectable frequency is unique to the three *Cyprinella* species, and on the basis of the commonality principle (Watrous & Wheeler, 1981) may represent a synapomorphic character state (*sensu* Hennig, 1966). If true, this would unite these three *Cyprinella* species into a derived, monophyletic species group or clade within the genus *Notropis*, and perhaps within the subgenus *Cyprinella*.

The finding of substantial differences in % GC values among the twelve *Notropis* species examined is strong suggestive evidence that genomic changes at the level of DNA base pair composition are frequent, possibly important events in cyprinid evolution. Gold (1980) estimated that the net speciation rate in *Notropis* was nearly twice that of other consubfamilial genera. This means that if base compositional change is associated with speciation events, the mean difference in base composition among *Notropis* species should be considerably greater than the mean difference among species in other cyprinid genera. The theoretical basis for this expectation is outlined in Avise & Ayala (1975) and Avise (1977). Exactly how changes in base composition might precipitate a speciation episode or a morphological alteration is unknown and would be difficult to detect. Moreover, since the evidence is correlative, it would be impossible at present to determine whether the relationship observed between the degree of base pair change and rapid evolution is one of cause and effect or simply one of association. What is clear is that separate levels of genome organization in North American cyprinids (i.e., protein coding genes, chromosomes, and DNA base composition) appear to be following different evolutionary paths. A more extensive survey of base pair compositions and melting rate profiles among North American Cyprinidae is now in progress to determine whether these observations hold over a broader sampling range.

Acknowledgements

We thank Dr. Ed Funkhauser and Tim Sherman, Texas A&M University, for use of the spectrophotometer and associated computer. We also thank Chris Amemiya and Chara Ragland (Texas A&M University), Dr. William Matthews (University of Oklahoma), and Dr. W. Mike Howell (Samford University) for assistance in collecting specimens, and Chris Amemiya and Chara Ragland for their constructive comments on the manuscript. The research was supported in part by NSF grant BSR-8415428 and in part by the Texas Agricultural Experiment Station under Projects H-6187 and H-6703.

References

Arrighi, F. E., Mandel, M., Bergendahl, J. & Hsu, T. C., 1970. Buoyant densities of DNA of mammals. Biochem. Genet. 4: 367–376.

Avise, J. C., 1977. Is evolution gradual or rectangular? Evidence from living fishes. Proc. natn. Acad. Sci. U.S.A. 74: 5083–5087.

Avise, J. C. & Ayala, F. J., 1975. Genetic change and rates of cladogenesis. Genetics 81: 757–773.

Avise, J. C., Smith, J. J. & Ayala, F. J., 1975. Adaptive differentiation with little genic change between two native California minnows. Evolution 29: 411–426.

Bush, G. L., Case, S. M., Wilson, A. C. & Patton, J. L., 1977. Rapid speciation and chromosomal evolution in mammals. Proc. natn. Acad. Sci. U.S.A. 74: 3942–3946.

Gebers, R., Wehmeyer, U., Roggentin, T., Schlesner, H., Kolbel-Boelke, J. & Hirsch, P., 1985. Deoxyribonucleic acid base compositions and nucleotide distributions of 65 strains of budding bacteria. Int. J. syst. Bacteriol. 35: 260–269.

Gold, J. R., 1980. Chromosomal change and rectangular evolution in North American cyprinid fishes. Genet. Res. 35: 157–164.

Gold, J. R., Womac, W. D., Deal, F. H. & Barlow, J. A., 1978. Gross karyotypic change in North American cyprinid fishes. Genet. Res. 32: 37–46.

Gold, J. R. & Price, H. J., 1985. Genome size variation among North American minnows (Cyprinidae). I. Distribution of the variation in five species. Heredity 54: 297–305.

Gold, J. R., Amemiya, C. T. & Ellison, J. R., 1986. Chromosomal heterochromatin differentiation in North American cyprinid fish. Cytologia 51: 557–566.

Guttman, T., Vitek, A. & Pivec, L., 1977. High resolution thermal denaturation of mammalian DNAs. Nucleic Acids Res. 4: 285–297.

Hennig, W., 1966. Phylogenetic systematics. Univ. Illinois Press, Urbana, Ill.

Hubbs, C. L., 1955. Hybridization between fish species in nature. Syst. Zool. 4: 1–20.

Hudson, A. P., Cuny, G., Cortadas, J., Haschemeyer, A. E. V. & Bernardi, G., 1980. An analysis of fish genomes by density gradient centrifugation. Eur. J. Bioch. 112: 203–210.

Huguet, T. & Jouanin, L., 1972. The heterogeneity of wheat nuclear DNA. Biochim. biophys. Acta. 262: 431–440.

Lee, D. S., Gilbert, C. R., Hocutt, C. H., Jenkins, R. E., McCallister, D. E. & Stauffer, J. R., Jr., 1980. Atlas of North American freshwater fishes. North Carolina Biological Survey, Pub. no. 1980-12, 867 pp.

Mandel, M. & Marmur, J., 1968. Use of ultraviolet absorbence-temperature profile for determining the guanine plus cytosine content of DNA. Meth. Enzymol. 12B: 195–206.

Mayfield, J. E., 1977. A comparison of the differential melting profiles with the CsCl density profiles of DNA from *Escherichia coli*, cow, mouse, rat, and chicken. Biochim. biophys. Acta 477: 97–101.

Miller, R. R., 1959. Origin and affinities of the freshwater fish fauna of western North America. Zoogeography. Amer. Assoc. Adv. Sci. Pub. 51: 187–222.

Miller, R. R., 1965. Quaternary freshwater fishes of North America. In: Wright, H. E., Jr. & Frey, D. G. (eds), The Quaternary of the United States, Princeton Univ. Press, Princeton, New Jersey, pp. 569–581.

Moyer, S. P., 1986. Isolation and characterization of a highly repeated satellite DNA sequence from the cyprinid fish *Notropis lutrensis*. M.S. Thesis, Texas A&M University, College Station, Texas.

Olmo, E., 1981. Evolution of genome size and DNA base composition in reptiles. Genetica 57: 39–50.

Pivec, L., Horska, H., Vitek, A. & Doskocil, J., 1974. Plurimodal distribution of base composition in DNA of some higher plants. Biochim. biophys. Acta 340: 199–206.

Schildkraut, C. L., Mandel, M., Levisohn, S., Smith-Sonneborn, J. E. & Marmur, J., 1962. Deoxyribonucleic acid base composition and taxonomy of some protozoa. Nature 196: 795–796.

Smith, G. R., 1981. Late cenozoic freshwater fishes of North America. Ann. Rev. Ecol. Syst. 12: 163–193.

Sueoka, N., 1961. Variation and heterogeneity of base composition of deoxyribonucleic acids: a compilation of old and new data. J. mol. Biol. 3: 31–40.

Wada, A., Yabuki, S. & Husimi, Y., 1980. Structure in the thermal denaturation of DNA: high temperature resolution spectrophotometric studies. CRC Crit. Rev. Bioch. 9: 87–144.

Watrous, L. E. & Wheeler, Q. D., 1981. The outgroup comparison method of character analysis. Syst. Zool. 30: 1–11.

Zimmer, E. A., Riven, C. J. & Walbot, V., 1981. Isolation of DNA and DNA recombinants from maize. Plant mol. Biol. Newsletter 2: 93–96.