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DNA BASE COMPOSITION AND NUCLEOTIDE DISTRIBUTION AMONG FIFTEEN SPECIES OF TELEOSTEAN FISHES

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Abstract—1. Genomic DNAs were isolated from 15 species of teleostean fishes and examined for % GC (guanine–cytosine base pair) values and nucleotide distributions using thermal denaturation.

2. The %GC values among the species ranged from 37.3 to 41.6; compositional heterogeneity values were relatively uniform and ranged from 8.8 to 13.5; asymmetry values were generally low.

3. Discrete melting components, indicative of blocks of repeated DNA sequences and identified by the presence of shoulders or minor peaks in differential melting rate profiles, were found in only two species.

4. Based on comparable data from other major vertebrate groups, it appears that fish genomes may be organized differently from other vertebrates and that genome evolution in fishes may have proceeded at a slower rate.

INTRODUCTION

DNA base compositions, nucleotide distributions, and differential melting rate and/or caesium chloride (CsCl) buoyant density gradient profiles have been used historically in microorganisms to study genome evolution, differentiate species, and identify taxonomic relationships (Schildkraut *et al.*, 1962). The approach provides data on DNA base composition and heterogeneity within genomes, and in higher organisms, including both animals and plants, often reveals discrete DNA components which cover a wide range of GC (guanine–cytosine) base pair contents (Huguet and Jouanin, 1972; Pivec *et al.*, 1974; Thiery *et al.*, 1976; Mayfield, 1977; Guttman *et al.*, 1977; Wada *et al.*, 1977; Olmo, 1981). The usual inference is that the discrete DNA components indicate the presence of repeated families of DNA sequences which differ in %GC base pairs from the average or mainband DNA. In many cases, these "minor" DNA components appear to be systematically or taxonomically informative, and frequently the DNA base compositions and/or DNA component profiles have been found to differ substantially even between closely related species (Guttman *et al.*, 1977; Olmo, 1981).

Relatively few comparable data are available on DNA base compositions and nucleotide distributions among genomes of teleostean fishes. This is surprising to the extent that teleosts comprise a vast array of nearly 20,000 species representing over half of the lining vertebrates (Nelson, 1976). Hudson *et al.* (1980) examined genomic DNAs from 29 teleostean species using buoyant density gradient centrifugation and found a striking degree of genomic homogeneity as compared to other vertebrate groups. In mammals, for example, several discrete DNA components usually exist, most of which typically have GC base pair contents greater than mainband DNA (Thiery *et al.*, 1976; Mayfield, 1977; Guttman *et al.*, 1977). Most of the teleostean fish species examined, however, have relatively low intermolecular compositional heterogeneity values and generally uniform DNA component profiles (Hudson *et al.*, 1980). Similar results were obtained for a few reptilian and amphibian species by Thiery *et al.* (1976), leading to the suggestion by Hudson *et al.* (1980) that genome organization in cold-blooded vertebrate species may differ considerably from that observed in warm-blooded species.

A potential problem with the studies of Thiery *et al.* (1976) and Hudson *et al.* (1980) is that the methodology employed to generate DNA component profiles was buoyant density gradient centrifugation. This procedure identifies minor DNA components differing in base compositions from mainband DNA, but is less sensitive than thermal denaturation analysis in terms of resolving minor genomic DNA components (Mayfield, 1977). Olmo (1981), for example, used thermal denaturation to study the genomes of 23 reptilian species including representatives from three families of turtles, four families of lizards, and two families of snakes. All but two of the species (a gekkonid lizard and a boid snake) were found to possess one or more well-defined DNA components in addition to the mainband peak, thereby falsifying the hypothesis that cold-blooded vertebrates differed from warm-blooded ones by the absence of numerous minor DNA components.

In this report, we document the results of thermal denaturation of genomic DNAs from 15 species of fish representing five different teleostean orders. The purpose of the study was to use this more sensitive approach and ask whether teleostean genomes do in fact differ from other vertebrate genomes by the conspicuous absence of various DNA thermal components. Previously (Karel and Gold, 1987), we had examined melting rate profiles from 20 species be-

longing to a single teleostean family, the Cyprinidae, and found a prominent and distinct minor DNA component in the genomes of only three species.

MATERIALS AND METHODS

Fish samples were obtained, with one exception, by seine or by angling from natural populations. Species (collection localities) were as follows: *Aphredoderus sayanaus* and *Hybognathus nuchalis* (Navasota R., Brazos Co., TX); *Notropis braytoni, Notropis jemezanus, Cyprinodon pecosensis, Fundulus grandis,* and *Fundulus zebrinus* (Pecos R., Val Verde Co., TX); *Pimephales notatus* (West Thompson Cr., West Feliciana Par., LA); *Fundulus chrysotus* (Little Pine Bayou, Hardin Co., TX); *Noturus nocturnus* (Little Brazos R., Brazos Co., TX); *Lepomis megalotis* and *Micropterus salmoides* (Stanmire L., Leon Co., TX); *Micropterus treculi* (Llano R., Llano Co., TX); and *Pomoxis annularis* (San Gabriel R., Milam Co., TX). The *F. grandis* population in the Pecos River is probably a transplant since the species does not normally occur this far west (Lee *et al.*, 1980). Specimens collected in the field were transported live to College Station, or were frozen immediately in liquid nitrogen. A sample of *Sciaenops ocellatus* was obtained from Dr W. H. Neill of Texas A & M and represents the descendants of Gulf of Mexico fish spawned at the John Wilson Marine Fish Hatchery at Flour Bluff, TX.

Genomic DNAs were isolated using the protocols of Zimmer *et al.* (1981); full details may be found in Karel and Gold (1987). DNA base composition data and differential melting rate profiles were generated via thermal denaturation of the DNA following Mandel and Marmur (1968) and Karel and Gold (1987). Three replicates per species were assayed with *Escherichia coli* DNA (Type VIII, Sigma) serving as the external standard. 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

DNA base compositions of the 15 species assayed are shown in Table 1 in the form of %GC, compositional heterogeneity, and asymmetry values. Compositional heterogeneity values reflect the degree to which GC and AT (adenine–thymine) base pairs are interspersed within the genome and essentially define the transition width of the melting curve (Mandel and Marmur, 1968). Asymmetry values reflect the skewness of the melting curve and represent the difference between the mean and modal values in %GC base pairs (Hudson *et al.*, 1980).

The %GC values among the 15 species ranged from 37.3 in *Cyprinodon pecosensis* to 41.6 in *Sciaenops ocellatus*. This range is slightly lower than that observed among other vertebrates (Thiery *et al.*, 1976; Mayfield, 1977; Guttman *et al.*, 1977; Hudson *et al.*, 1980; Olmo, 1981), but well within the extremes reported for vertebrates as a whole. The impression that %GC values are more similar within familial or generic groups (e.g., the four species of cyprinids and centrarchids, and the three species of *Fundulus*, cf. Table 1) is somewhat misleading since the range in %GC values among the 24 North American cyprinid species now examined is 36.1–41.3 (Karel and Gold, 1987; this paper).

Compositional heterogeneity values among the 15 species ranged from 8.8 in *Micropterus salmoides* and *Sciaenops ocellatus* to 13.5 in *Cyprinodon pecosensis*. Few comparative data on compositional heterogeneities are available for other vertebrates. The range of values among these 15 species, however, are generally the same as observed by Karel and Gold (1987) among 20 species of North American cy-

Table 1. Base compositions of genomic DNAs from 15 species of teleostean fishes. %GC and %CH (compositional heterogeneity) values were calculated following Mandel and Marmur (1968). Asymmetry values represent the difference between mean and modal %GC values (Hudson *et al.*, 1980)

Taxon*	%GC ± SE	%CH ± SE	Asymmetry
Paracanthopterygii			
Percopsiformes			
Aphredoderidae			
1. *Aphredoderus sayanus*	41.1 ± 0.1	13.1 ± 0.5	4.1
Acanthopterygii			
Cypriniformes			
Cyprinidae			
2. *Notropis braytoni*	38.5 ± 0.1	10.6 ± 0.4	0.2
3. *Notropis jemezanus*	39.9 ± 0.3	12.1 ± 0.2	2.6
4. *Hybognathus nuchalis*	38.3 ± 0.2	11.5 ± 0.5	1.5
5. *Pimephales notatus*	39.1 ± 0.1	12.5 ± 0.4	0.7
Siluriformes			
Ictaluridae			
6. *Noturus nocturnus*	40.3 ± 0.6	10.1 ± 0.9	1.7
Atheriniformes			
Cyprinodontidae			
7. *Cyprinodon pecosensis*	37.3 ± 0.1	13.5 ± 0.6	2.1
8. *Fundulus chrysotus*	39.2 ± 0.6	9.1 ± 0.7	2.8
9. *Fundulus grandis*	40.0 ± 0.1	11.5 ± 0.7	2.7
10. *Fundulus zebrinus*	40.8 ± 0.2	9.9 ± 0.7	2.5
Perciformes			
Centrarchidae			
11. *Lepomis megalotis*	39.8 ± 0.2	10.6 ± 0.3	2.5
12. *Micropterus salmoides*	41.5 ± 0.2	8.8 ± 0.2	2.2
13. *Micropterus treculi*	39.1 ± 0.1	10.9 ± 0.4	1.8
14. *Promoxis annularis*	40.4 ± 0.2	11.7 ± 0.2	1.6
Sciaenidae			
15. *Sciaenops ocellatus*	41.6 ± 0.1	8.8 ± 0.2	2.3

*Taxonomic placements follow the AFS (1980) checklist and Lauder and Liem (1983).

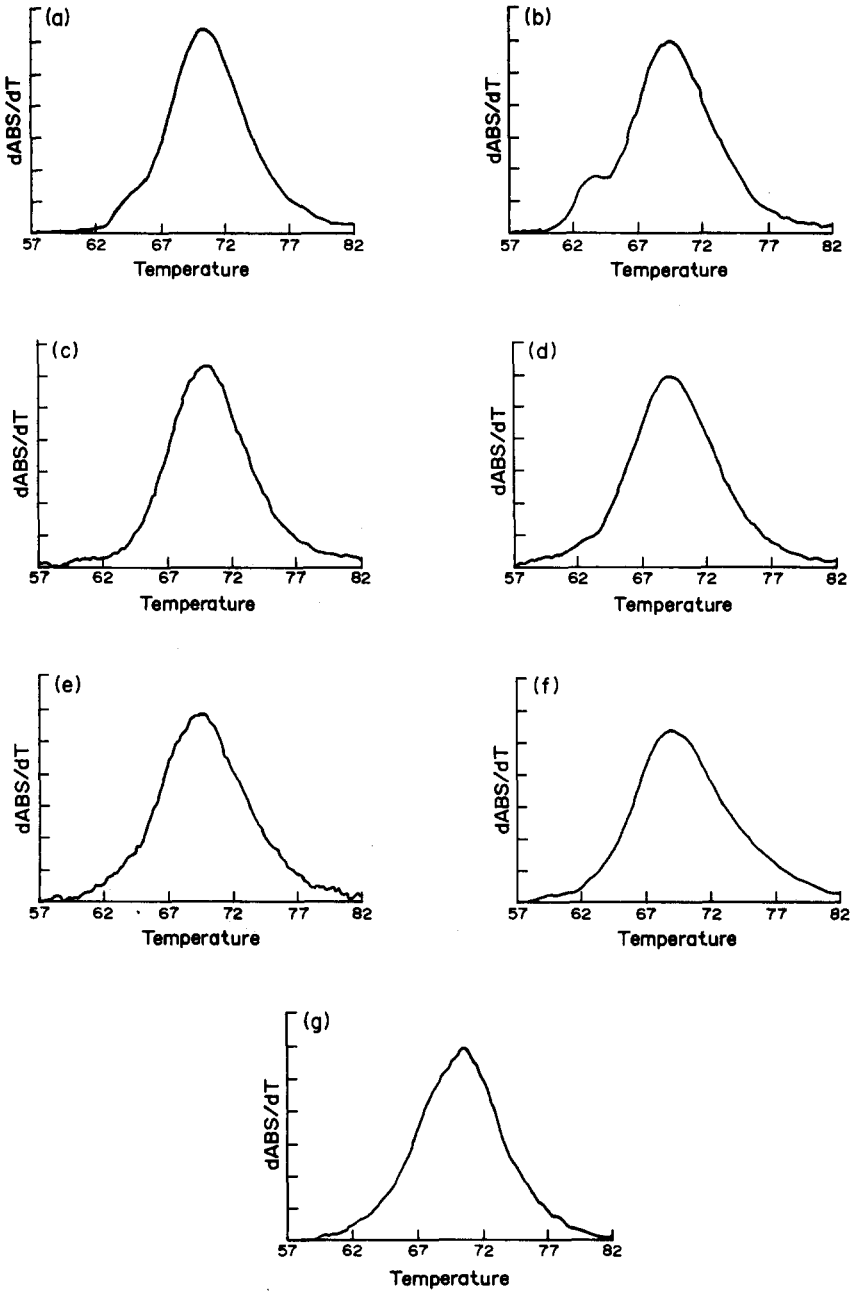

Fig. 1. Differential melting rate profiles. Abscissa: denaturation temperature; ordinate: increase in absorbance for each step increase (0.2°C) in temperature. The species shown are: A, *Sciaenops ocellatus*; B, *Lepomis megalotis*; C, *Micropterus salmoides*; D, *Micropterus treculi*, E, *Pomoxis annularis*; F, *Aphredoderus sayanus*; G, *Noturus nocturnus*.

prinids. This would appear to suggest that most of the genomes, both within and across families, have fairly uniform nucleotide distributions and that minor DNA components differing substantially in base pair composition from mainband DNA are either absent or present in low frequency.

Asymmetry values among the 15 species ranged from 0.2 in *Notropis braytoni* to 4.1 in *Aphredoderus sayanus*. Values for most species fell in the range 1.5–2.6, again suggesting that most of the genomes do not possess DNA components differing markedly in base composition. The one possible exception is the percopsiform species *A. sayanus*, where the comparatively high asymmetry value of 4.1 may indicate that a significant fraction of the genome of this species differs in base composition.

Differential melting rate profiles of the 15 species are shown in Figs 1 and 2. All of the profiles were asymmetrical to varying degrees as expected based on the calculated asymmetry values. Discrete DNA melting components, detectable as broad shoulders or peaks other than the mainband peak, were apparent in only two species: *S. ocellatus*, a sciaenid (Fig. 1a), and *Lepomis megalotis*, a centrarchid (Fig. 1b).

In both species, the minor DNA components were to the left or light side of the mainband peak indicating the presence of DNA sequences enriched in AT base pairs. The profiles of the remaining 13 species showed little or no evidence of discrete melting components.

DISCUSSION

The data obtained to date demonstrate that the genomes of teleostean fish by and large display considerable uniformity in nucleotide distributions and lack DNA components which differ significantly in base pair composition from mainband DNAs. This includes two studies using thermal denaturation of genomic DNAs (Karel and Gold, 1987; this paper) where only five of the 35 teleostean species examined possessed discrete, minor DNA components, and one study (Hudson *et al.*, 1980) which employed buoyant density gradient centrifugation. In the latter, only three of twenty-nine teleosts displayed a visible minor band, although five other species appeared to have very slight shoulders to the right or left of the mainband peak.

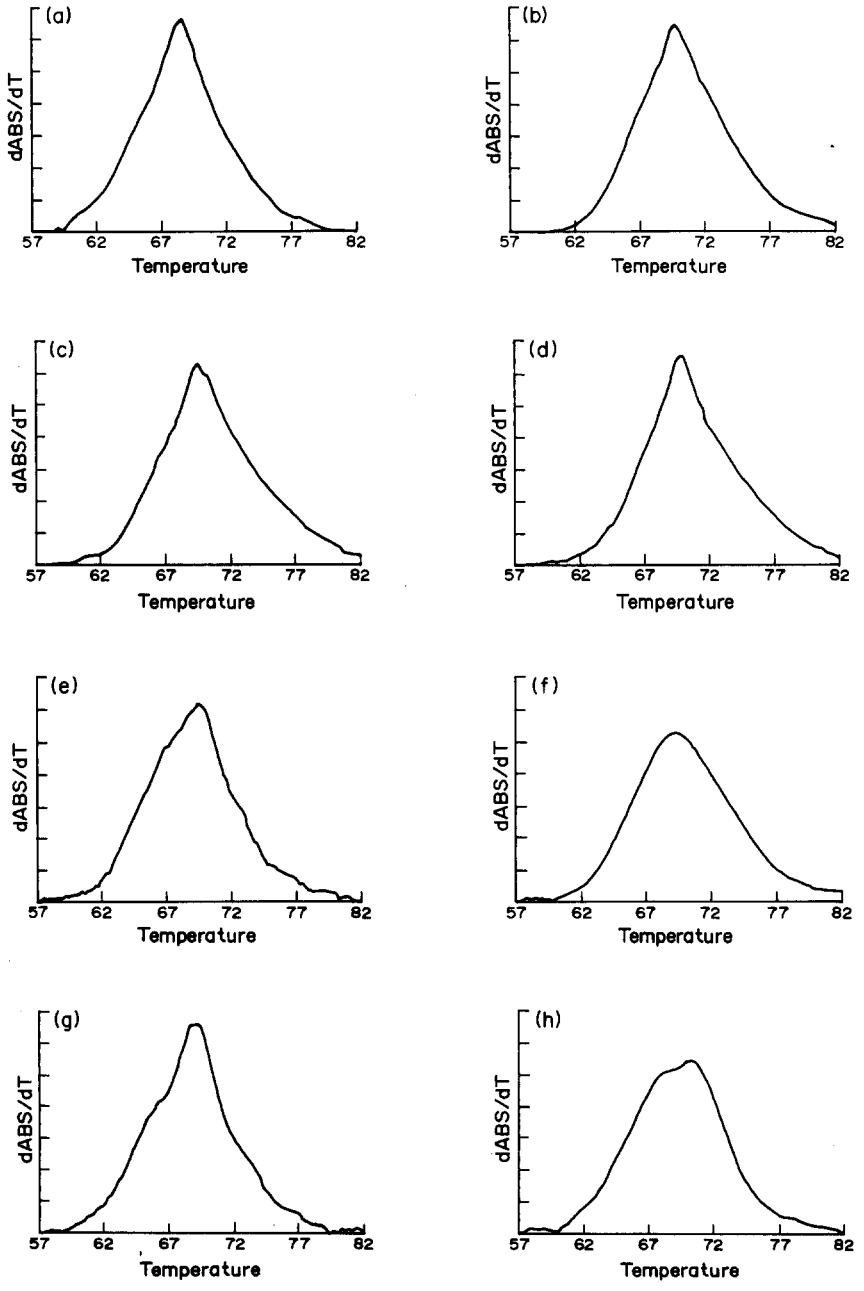

Fig. 2. Differential melting rate profiles. Abscissa: denaturation temperature; ordinate: increase in absorbance for each step increase (0.2°C) in temperature. The species shown are: A, *Cyprinodon pecosensis*; B, *Fundulus chrysotus*; C, *Fundulus grandis*; D, *Fundulus zebrinus*; E, *Notropis braytoni*; F, *Notropis jemezanus*; G, *Hybognathus nuchalis*; H, *Pimephales notatus*.

The apparent homogeneity of teleostean genomes is in sharp contrast to the genomes of species in other major vertebrate groups where the presence of numerous DNA components (in addition to the mainband DNA) appears to be the rule rather than the exception (Thiery *et al.*, 1976; Mayfield, 1977; Guttman *et al.*, 1977; Wada *et al.*, 1977; Olmo, 1981). Hudson *et al.* (1980) were the first to notice this discrepancy and suggested that genome organization as a whole in lower vertebrates might differ trenchantly from that in higher vertebrates. Their suggestion was predicated, in part, on the buoyant density gradient study of Thiery *et al.* (1976) which had shown similar homogeneity in the genomes of other lower vertebrates. Olmo (1981), however, employed the more sensitive thermal denaturation approach (Mayfield, 1977) and was able to demonstrate that the genomes of turtles, lizards, and snakes contained numerous minor DNA components in addition to the mainband DNA. On this basis, it would appear that the generalized property of genomic uniformity is a singular feature of teleostean fish genomes and not a difference between lower and higher vertebrates or between cold-blooded vs warm-blooded species.

The overall conservatism of teleostean fish genomes would appear to suggest that genome organization in fishes may differ from that in other vertebrates. Hudson *et al.* (1980) suggested that the difference between teleostean and other vertebrate genomes could be due to a particular frequency of short oligonucleotides, or to a short sequence design. With regard to the latter, the interspersion pattern of DNA sequences in fish genomes has not been formally studied. With regard to the former, reassociation kinetic analyses of a few fish genomes have shown that the complexities and quantities of repeated DNA sequences in fish are similar to those found in other vertebrates (Hanham and Smith, 1979; Schmidtke *et al.*, 1979). In addition, a highly repeated or satellite DNA family from one fish species has been characterized and found to have a repeat unit length typical of many eukaryotes (Moyer, 1986). These observations would appear to indicate that the primary difference between the genomes of teleostean fish and other vertebrates is in the absence of genomic components which differ in base composition and not in organization *sensu strictu*. Finally, the generalized uniformity and similarity of teleostean genomes as compared to other vertebrates may indicate that genome evolution in fishes has proceeded at a slower rate than in other vertebrate groups.

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