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An Analysis of Fish Genomes by Density Gradient Centrifugation

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DNA was prepared from 33 species of fishes representing 12 of the 31 orders of Teleostei and one order of Chondrichthyes. DNA samples were investigated to determine modal and mean buoyant densities in CsCl (ϱ_0 and $\langle\varrho\rangle$), main-band asymmetry, intermolecular compositional heterogeneity and base composition. Modal buoyant densities ranged over 1.697–1.704 g/cm^3, generally with identical or close values for DNAs from species belonging to the same order. Main bands were practically symmetrical in most cases; when present, asymmetries were on the heavy side of the band and rather modest. In most cases, compositional heterogeneities were equal to, or lower than, those of bacterial DNAs. Both the CsCl band symmetry and the low compositional heterogeneity strikingly distinguish fish DNAs from the DNAs of warm-blooded vertebrates and indicate a major discontinuity in genome organization in the vertebrates. The overall properties of the main bands of fish DNAs are very similar to those exhibited by the light components of DNAs from mammals and birds.

Fractionation of native DNA fragments by density gradients centrifugation in the presence of DNA ligands like Ag^+ or BAMD, i.e. bis(aceto-mercurimethyl) dioxane [1–4], has shown [5, 6] that (neglecting satellite and minor components) DNAs from unicellular eukaryotes and invertebrates exhibit essentially symmetrical CsCl band profiles, as do prokaryotic DNAs; DNAs from cold-blooded vertebrates show either no asymmetry or a slight asymmetry on the heavy side of their CsCl bands; in contrast, DNAs from warm-blooded vertebrates are characterized by a strong asymmetry on the heavy side of their CsCl bands. The latter feature was demonstrated [5, 6] to be due to the fact that mammalian and avian genomes contain two heavy DNA components, exhibiting buoyant densities equal to 1.704 and 1.708 g/cm^3 and representing about 25% and 10% of the genome, respectively, in addition to one or two (according to the species) light component(s); the latter represent about 60% of the genome and have buoyant density(ies) in the range 1.697–1.703 g/cm^3, which is the same range as main-band DNA from cold-blooded vertebrates. It should be understood that what we call 'DNA components' are families of fragments deriving [6, 7] from very long DNA stretches (> 150000 bases or $> M_r = 10^8$) of rather homogeneous base composition, called isochores (Cuny, Soriano, Macaya, and Bernardi, unpublished results).

Abbreviation. BAMD, bis(acetato-mercurimethyl) dioxane.

The present investigations were carried out with a double aim. The first one was to put on a wider basis the large differences found at the DNA level between the genomes of warm-blooded and cold-blooded vertebrates. Previous work [5], in fact, had concerned DNAs from eleven mammalian and two avian species, but from only six cold-blooded vertebrates (two reptiles, two amphibians and two fishes). The second aim was to look for possible phylogenetic differences among the DNAs of the fish species analyzed; this point is of particular interest in the case of fishes, which include a vast array of distantly related vertebrates with 20000 species, representing half of the living vertebrate species [9].

MATERIALS AND METHODS

Sample Collection and Identification

Fish were collected during an expedition of the research vessel Alpha Helix in the eastern Pacific near the Galapagos Islands (samples 3, 4, 20) and Perlas Islands (sample 33) and in the western Caribbean near the San Blas Islands, Panama (samples 2, 17, 19, 30).

Antarctic species (samples 22–29) were collected in McMurdo Sound, Antarctica (77°51′ S, 166°37′ E). Other species were obtained at Woods Hole, Massachusetts (samples 6, 7, 11–15), in the Gulf of Alaska (samples 9, 31), in the Red Sea at Elat (samples 1, 18, 21) and Sharm-el-Sheik (sample 32), in the Bahamas (sample 16), in central Africa (sample 5), and from an aquarium dealer (sample 10). Salmon DNA (sample 8) was a commercial preparation investigated in a previous study [5].

Collection and identification of species were made possible through the collaboration of C. Lavett Smith (American Museum of Natural History), Ron McConnaughey and John Graves (Scripps Institute of Oceanography), Fred Nichy (National Marine Fisheries Service in Woods Hole), Randy Rice (University of Alaska), Warren Zeiler (Miami Seaquarium), Avi Baranes (The Hebrew University, Marine Biological Laboratory), and Robert Thompson (Hunter College of the City University of New York).

DNA Preparation

DNAs were generally prepared from the liver with the exception of sample 5 which was prepared from brain, and samples 9, 13, and 15 which were prepared from testes; all preparations were done using the method of Kay et al. [10] and were further purified by chromatography on hydroxyapatite in the presence of 3 M KCl [11].

Centrifugations and Other Methods

Sedimentation velocity determinations were performed as previously described [12], and all molecular weights of the various DNAs were calculated from the $s_{20,w}$ values using the relationship of Eigner and Doty [13].

Preparative density gradient centrifugation has already been described [2–8]. All analytical density gradient centrifugations were carried out as previously described [5], and the mean and modal buoyant densities, $\langle \varrho \rangle$ and ϱ_0, have been defined elsewhere [5, 6]. The asymmetry of the main-band DNAs is expressed as $\langle \varrho \rangle - \varrho_0$ [5] dG+dC content (the molar ratios of deoxyguanosine plus deoxycytidine) of the various DNAs analyzed were estimated from $\langle \varrho \rangle$ values using the relationship of Schildkraut et al. [14] for bacterial DNAs. In addition, dG+dC contents were determined by chemical analysis of nucleosides [15] for several samples. Intermolecular compositional heterogeneity (H) was estimated using the method of Schmid and Hearst [16].

Restriction enzymes were obtained from New England Biolabs or Bethesda Research Labs; acrylamide and bisacrylamide were obtained from Eastman Chemicals or from BioRad. Restriction enzyme digestion and acrylamide gel electrophoresis were performed as previously described [17].

RESULTS

Table 1 presents a classification of fishes derived from Nelson [9], in order to indicate the taxonomical position of the species studied here. This classification puts the fishes in an order that reflects their postulated evolutionary relationship, ranging from the ancient cartilaginous fishes (sharks, rays) to the most recent teleost orders, like Tetraodontiformes (puffers etc.). The present study includes species from all divisions and all superorders (except for Scopelomorpha) of the infraclass Teleostei, a group comprising about 18 000 species in all. The study includes species from 12 out of 31 teleost orders and one order of Chondrichthyes (cartilaginous fishes). This leaves for further studies four subclasses: Holocephali (ratfishes, chimaeras), Dipneusti (lungfishes), Crossopterygii (coelacanths), Brachioptergygii (bichirs), and two infraclasses: Chondrostei (sturgeons, paddlefishes) and Holostei (gars, bowfins). Although comprising only a very few species, these groups are important from an evolutionary point of view. The far more ancient superclass of Agnatha (lampreys, hagfishes), not included in Table 1 (which is only concerned with superclass Gnathostomata), would also be of interest.

The CsCl band profiles from analytical density gradient centrifugations for all species of fishes studied are presented in Fig. 1. Table 2 summarizes the physical properties of these DNAs, including modal and mean buoyant densities in CsCl (ϱ_0 and $\langle \varrho \rangle$, respectively), the asymmetry of the main-band DNA (given as $\langle \varrho \rangle - \varrho_0$), and the intermolecular compositional heterogeneity (H). Sedimentation coefficients for each DNA are also given. The dG+dC contents listed in Table 2 were obtained from $\langle \varrho \rangle$ and from nucleoside analysis (values in parentheses). DNA contents per haploid cell (c value) were obtained from Hinegardner and Rosen [18] for each species where possible, or for the family, based on other representatives.

The modal buoyant densities of the main-band DNAs, from Fig. 1 and Table 2, include a rather wide range of values (1.697–1.704 g/cm^3). All representatives of ancient fish groups up through and including the Salmoniformes (samples 1–9) show high main-band buoyant density values, in the range of 1.702–1.704 g/cm^3; interestingly, one barracuda (sample 20) and one puffer (sample 32) also fall in this range. For other species, the main-band buoyant density is centered in the range 1.699–1.701 g/cm^3, with the exception of the goldfish (*Carassius auratus*, sample 10) which shows a value of $\varrho_0 = 1.697$ g/cm^3. With the exception of one order which is discussed separately below, identical or very close values for ϱ_0 were found

Table. 1. *Classification of fishes*
The two classes of extant fishes, Chondrichthyes and Osteichthyes, belong to the superclass Gnathostomata (jawed fishes). Classes of fishes containing only extinct species are not listed. The list of orders is incomplete; it contains only those orders which comprise species studied here. Classification is from Nelson [9]

Classes	Subclasses and infraclasses	Divisions	Superorders	Orders
Chondrichthyes	Elasmobranchii		Selachimorpha	Lamniformes
	Holocephali			
Osteichthyes	Dipneusti			
	Crossopterygii			
	Brachiopterygii			
	Actinopterygii			
	Chondrostei			
	Holostei			
	Teleostei	Archaeophylaces		Mormyriformes
		Taenopaedia	Clupeomorpha	Clupeiformes
			Elopomorpha	Anguilliformes
		Euteleostei	Protacanthopterygii	Salmoniformes
			Ostariophysi	Cypriniformes
			Scopelomorpha	
			Paracanthopterygii	Batrachoidiformes
				Lophiiformes
			Acanthopterygii	Atheriniformes
				Scorpaeniformes
				Perciformes
				Pleuronectiformes
				Tetraodontiformes

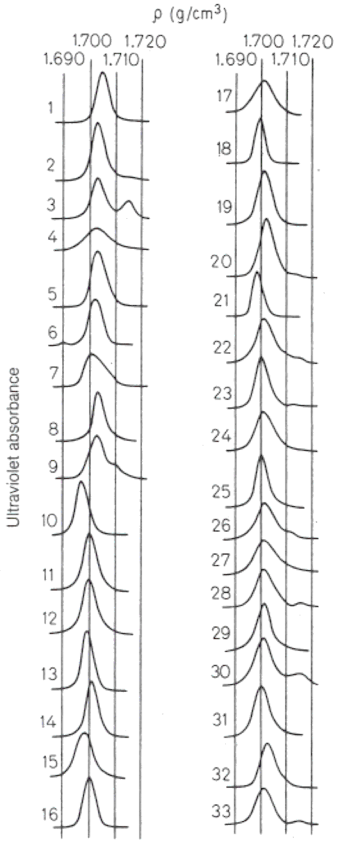

Fig. 1. *Density gradient profiles in CsCl of DNAs from 33 species of fishes.* Table 2 contains the numbering of samples

in the DNAs of fishes belonging to the same order. For example, the DNAs from three sharks examined (*Carcharinus galapagensis, Scoliodon terra-novae,* and *Sphyrna lewini,* samples 2–4) all had the same main-band buoyant density ϱ_0 value of 1.703 g/cm^3, while the fourth shark had a slightly higher value of 1.704 g/cm^3 (*Mustelus mosis,* sample 1). This was also the case for the DNAs of the two Salmonids (*Oncorhynchus kisutch* and *Salmo salar,* $\varrho_0 = 1.703$ g/cm^3, samples 8 and 9), and for the two species from related orders of Paracanthopterygii (*Opsanus tau* and *Lophius americanus,* $\varrho_0 = 1.6996$ g/cm^3, samples 11 and 12). The six Antarctic fishes of the family Nototheniidae and all of the same genus, *Trematomus,* also show very small variations, ranging from a low value of $\varrho_0 = 1.6999$ g/cm^3 in *Trematomus hansoni* (sample 25) to a high values of $\varrho_0 = 1.7010$ g/cm^3 in *Trematomus nicolai* (sample 26).

This uniformity was not the case, however, within the order Perciformes. This is the most diversified of all fish orders, and the vertebrate order containing the largest number of species [9]. Within this order, several differences were found in ϱ_0 values in very closely-related species, notably between the two barracudas of the same genus (*Sphyraena barracuda,* $\varrho_0 = 1.7015$ g/cm^3 and *Sphyraena ensis,* $\varrho_0 = 1.7024$ g/cm^3, samples 19 and 20), and the two serranids (*Centropristis striatus,* $\varrho_0 = 1.6981$ g/cm^3 and *Epinephelus striatus,* $\varrho_0 = 1.7004$ g/cm^3 samples 15 and 16).

Table 2. *Properties of fish DNAs*
Values of dG + dC content in parentheses were obtained from nucleoside analysis. c = DNA content/haploid cell (ranges refer to families)

Order	Family	Sample number	Genus and species	$s_{20,w}$	ϱ_0	$\langle\varrho\rangle$	$\langle\varrho\rangle-\varrho_0$	H	dG + dC	c
				S	g/cm³		mg/cm³	g/cm³	%	pg
Lamniformes	Carcharinidae (sharks)	1.	*Mustelus mosis*	32.6	1.7044	1.7047	0.3	2.8	45.6	2.8–9.8
		2.	*Scoliodon terra-novae*	22.6	1.7030	1.7035	0.5	2.7	44.4 (43.6)	
		3.	*Carcharinus galapagensis*	23.4	1.7033	1.7037	0.4	2.1	44.7	
	Sphyrnidae (hammerhead sharks)	4.	*Sphyrna lewini*	10.5	1.7030	1.7042	1.2	3.4	45.1	
Mormyriformes	Mormyridae (elephant fishes)	5.	*Gnathonemus petersii*	28.4	1.7029	1.7033	0.4	2.4	44.2	1.2
Clupeiformes	Clupeidae (herrings)	6.	*Brevoortia tyrannus*	26.4	1.7021	1.7024	0.3	2.3	43.4 (43.0)	0.8–1.4
Anguilliformes	Anguillidae (freshwater eels)	7.	*Anguilla rostrata*	32.8	1.7011	1.7015	0.7	3.4	42.6 (42.9)	1.4
Salmoniformes	Salmonidae (salmonids)	8.	*Salmo salar*	20.5	1.7028	1.7035	0.7		44.4	
		9.	*Oncorhynchus kisutch*	31.3	1.7033	1.7036	0.3	2.9	44.5 (43.0)	3.0
Cypriniformes	Cyprinidae (carps, minnows)	10.	*Carassius auratus*	28.3	1.6970	1.6971	0.1	1.6	37.9 (37.3)	2.0
Batrachoidiformes	Batrachoididae (toadfishes)	11.	*Opsanus tau*	28.0	1.6996	1.7001	0.5	2.3	40.9 (37.3)	2.8
Lophiiformes	Lophiidae (goosefishes)	12.	*Lophius americanus*	22.0	1.6996	1.6998	0.2	3.3	40.6 (40.1)	1.0
Atheriniformes	Cyprinodontidae (killifishes)	13.	*Fundulus heteroclitus*	30.0	1.6993	1.6996	0.3	1.5	40.4 (40.1)	1.5
Scorpaeniformes	Cottidae (sculpins)	14.	*Hemitripterus americanus*	14.9	1.7010	1.7013	0.3	2.3	42.1	0.94
Perciformes	Serranidae (sea basses)	15.	*Centropristis striatus*	18.8	1.6981	1.6985	0.4	2.7	39.3 (38.5)	1.2
		16.	*Epinephelus striatus*	25.2	1.7004	1.7006	0.2	1.2	41.4 (40.5)	1.3
	Lutjanidae (snappers)	17.	*Lutjanus synagris*	12.4	1.7010	1.7022	1.2	3.4	43.1	1.3–1.4
	Lethrinidae (scavengers)	18.	*Lethrinus nebulosus*	20.9	1.6999	1.7001	0.2	1.2	40.9	
	Sphyraenidae (barracudas)	19.	*Sphyraena barracuda*	18.6	1.7015	1.7017	0.2	1.3	42.6	0.8–1.2
		20.	*Sphyraena ensis*	18.7	1.7024	1.7027	0.3	2.4	43.6	
	Scaridae (parrot fishes)	21.	*Scarus gibbus*	21.8	1.6984	1.6988	0.4	1.2	39.6	1.9–2.1
	Nototheniidae	22.	*Trematomus bernacchii*	14.5	1.7011	1.7020	0.9	2.5	42.9	
		23.	*Trematomus borchgrevinki*	23.6	1.7002	1.7010	0.8	2.3	41.8 (41.2)	
		24.	*Trematomus centronotus*	11.0	1.7009	1.7017	0.8	2.9	42.5	
		25.	*Trematomus hansoni*	22.2	1.6999	1.7002	0.3	2.1	41.1 (39.4)	
		26.	*Trematomus nicolai*	15.0	1.7010	1.7017	0.7	3.2	42.5 (40.7)	
		27.	*Trematomus newnesi*	10.4	1.7005	1.7018	1.3	3.2	42.6	
		28.	*Dissostichus mawsoni*	19.6	1.7003	1.7007	0.4	2.2	41.5 (40.8)	
	Bathydraconidae	29.	*Gymnodraco acuticeps*	24.6	1.7007	1.7012	0.5	3.4	42.1 (40.8)	
	Bleniidae (blennies)	30.	*Ophioblennius atlanticus*	17.4	1.7007	1.7011	0.4	2.4	41.9	0.8–1.0
Pleuronectiformes	Pleuronectidae (right-eye flounders)	31.	*Limanda aspera*	16.7	1.6995	1.7002	0.7	3.2	41.0	0.7–1.0
Tetraodontiformes	Tetraodontidae (puffers)	32.	*Arothron diadematus*	19.8	1.7025	1.7036	1.1	2.7	44.5	0.4–0.5
	Diodontidae (porcupine fishes)	33.	*Diodon holocanthus*	16.3	1.7009	1.7012	0.3	1.4	42.0 (42.2)	0.9

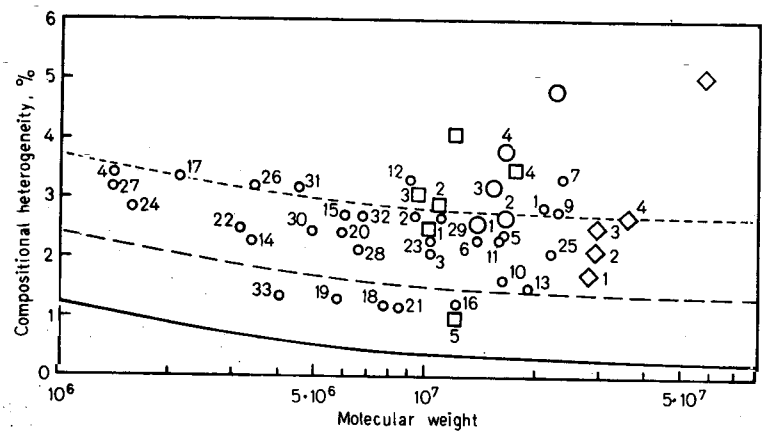

Fig. 2. *Intermolecular compositional heterogeneity (H) plotted as a function of molecular weight for DNAs of 33 fish species.* Numbering of species is from Table 2. The solid line represents the dependence of H on fragment size for a DNA of random sequence. The broken lines show this dependence for two bacterial DNAs: *E. coli* (51% dG+dC, upper dashed line) and *H. influenzae* (38% dG+dC, lower dashed line). Data for human (○), mouse (□), and chicken (◇) DNAs and their various components [1–4] are also given. Data for random DNA, bacterial, mammalian, and avian DNAs are from unpublished results of Cuny, Soriano, Macaya and Bernardi

As shown in Fig. 1, several fish DNAs showed satellite bands. The clupeoid *Brevoortia tyrannus* (sample 6) showed a light satellite ($\varrho_0 = 1.69$ g/cm^3); all other satellite bands were on the heavy side of the main band and frequently at a buoyant density close to 1.715 g/cm^3. One particularly prominent satellite band was found in the DNA of the shark *Carcharinus galapagensis* (sample 3) and this band composed some 30% of the total genome. Restriction enzyme digestion of this DNA with *Hae*III showed several heavy bands when analyzed on acrylamide gels, thus confirming that a substantial proportion of the genome in this shark is made up of highly repeated DNA sequences (data not shown).

The very strong asymmetry in the DNA of the moray eel *Anguilla rostrata* (sample 7) is also due to the presence of a heavy satellite which is evident on expanded-scale scans of this peak (data not shown).

The dG+dC content of the various fish genomes, as calculated from mean buoyant density, ranged from 38% to 45%. Values obtained from nucleoside analyses were identical to the calculated values or were, at most, 1% lower; this discrepancy is smaller than that found (2%) in the main-band components of higher vertebrates.

The main-band asymmetries of the fish DNAs, expressed as $\langle\varrho\rangle - \varrho_0$, ranged from a low value of 0.1 mg/cm^3 (sample 10) to a high value of 1.3 mg/cm^3 (sample 27), with the majority of species showing values less than 0.5 mg/cm^3. When high asymmetry values associated with low-molecular-weight samples are ignored, the fish DNAs average 0.4 mg/cm^3, compared with an average value of 3 mg/cm^3 in mammalian and avian DNAs [5].

Fig. 2 presents data on the intermolecular compositional heterogeneity (H) of the DNA samples as a function of molecular weight for the 33 fishes examined. This parameter reflects variation in dG+dC content among different fragments of the given molecular weight. The normal dependence of H on fragment size is illustrated by the solid line, calculated for a DNA of random sequence; the dependence of H on dG+dC content is indicated for two bacterial DNAs: *Escherichia coli* with a dG+dC content of 51% (upper broken line), and *Haemophilus influenzae* with a dG+dC content of 38% (lower broken line). Data points for human, mouse, and chicken DNAs and their components are also shown. A few fish DNAs exhibited a heterogeneity level slightly higher than that of bacterial DNAs having the same base composition and molecular weight; the majority of fish DNAs, however, showed a heterogeneity equal to or smaller than the bacterial DNAs (see also Fig. 3). Compared with the total genomic mammalian and avian DNAs, fish DNAs show a strikingly lower heterogeneity. In fact, the heterogeneities of the fish DNAs are comparable to those of the isolated major components of homeotherms shown in Fig. 2 (see also Fig. 3).

Fig. 3 presents the heterogeneity ratio for each of the 33 fish DNAs, as well as the ratios for the total genomic DNAs and their various components from mouse, human, and chicken. The heterogeneity ratio is the result of dividing the H value for each fish DNA (Table 2) by the corresponding value of a bacterial DNA having the same dG+dC content and the same molecular weight; this normalization of the H values facilitates comparison among all the species. The fish species in Fig. 3 are arranged in taxonomic order as in Table 2, with the more taxonomically 'primitive' fishes at the left and progressing toward the taxonomically 'advanced' fishes at the right of the figure. It is clear from these data that no general trend of increasing or decreasing level of heterogeneity exists as one moves from primitive to advanced fishes.

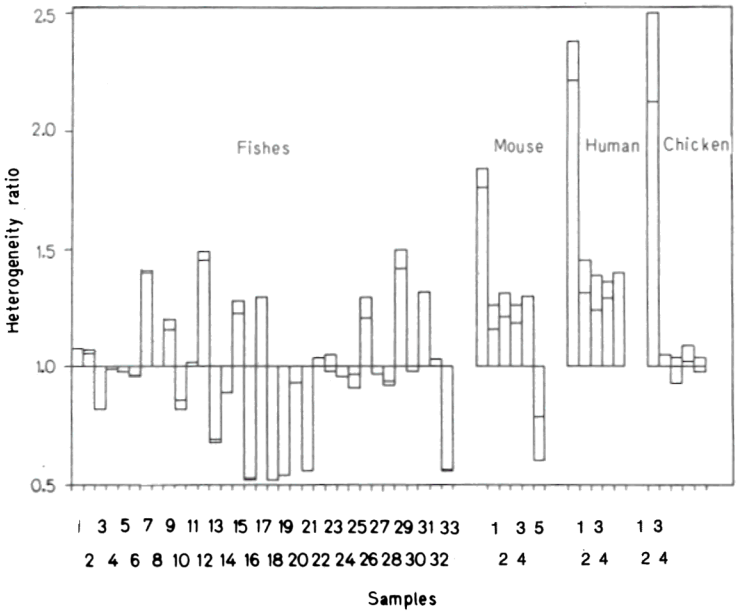

Fig. 3. *Comparison of heterogeneity ratios of DNAs from 33 species of fishes.* Ratios were obtained by dividing intermolecular compositional heterogeneity values from each species of fish by the corresponding value of bacterial DNA having the same dG+dC content and the same molecular weight (standard error ± 0.2). Numbering of samples is from Table 2. dGrdC contents for fish DNAs were calculated from $\langle \varrho \rangle$ values given in Table 2; values given by nucleoside analysis were also when available to calculate ratios; in this case ratios were always higher than those calculated from $\langle \varrho \rangle$ and are also shown in the figure. Results for mouse main-band DNA, total human and chicken DNAs, and their components [1–4], including mouse satellite [5], are also shown (unpublished results of Cuny, Soriano, Macaya and Bernardi)

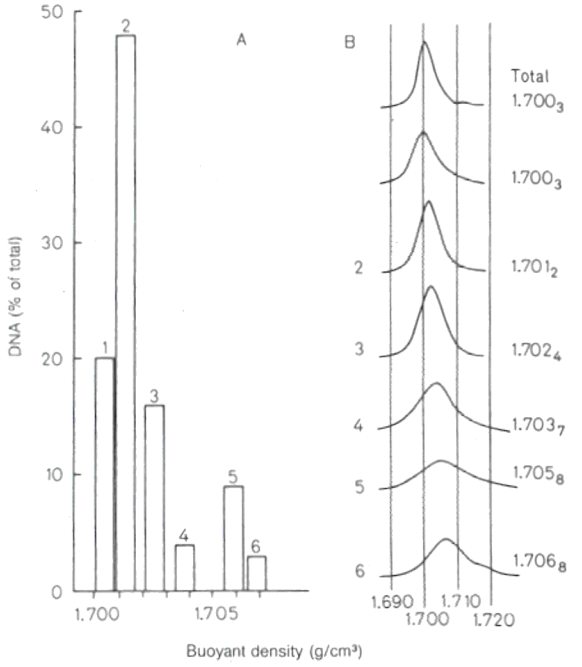

Fig. 4. *Histogram (A) illustrating relative amounts of six DNA fractions from* Trematomus borchgrevinki *(sample 23), as obtained from Cs_2SO_4/BAMD preparative density gradient centrifugation, as a function of their respective buoyant densities in CsCl.* See text for experimental details. Fraction 1 corresponds to DNA which was pelleted at the r_f used (r_f is the ligand/nucleotide ratio). (B) Density gradient profiles in CsCl of the six fractions from A

In order to investigate further the component structure and compositional heterogeneity of fish DNAs, several selected DNAs were fractionated in preparative Cs_2SO_4/BAMD density gradients, and each resulting fraction was run in a CsCl analytical density gradient. The results of such an analysis for the DNA of *Trematomus borchgrevinki* (sample 23) are shown in Fig. 4. This sample is characterized by a relatively high asymmetry coefficient, $\langle \varrho \rangle - \varrho_0 = 0.8$ mg/cm^3 (Table 2). The CsCl bands for the six fractions obtained show 85% of the DNA in the range 1.700–1.702 g/cm^3 (fractions 1–3). Higher density components (1.704–1.707 g/cm^3) account for 15% of the DNA. The most abundant of these latter fractions (fraction 5) showed three strong bands upon gel electrophoresis after *Hae*III digestion (data not shown), indicating the presence of a satellite component. This was also suggested for fraction 6 by the bimodal distribution of the CsCl profile. These results indicate that the two heaviest fractions, accounting together for some 12% of the genome, are formed at least to a large extent by satellite and minor components.

DISCUSSION

The present investigation confirms and very considerably extends the conclusions of previous work

work [5], which has suggested that genome organization in lower vertebrates differs from that of higher vertebrates in several important aspects. The fish DNAs investigated here generally exhibit much more symmetrical band profiles in CsCl analytical gradients than do DNAs from homeotherms. The average asymmetry of the fish DNAs is about 0.4 mg/cm^3, if one ignores some higher values which result from low molecular weights. This is in clear contrast to data [5] from mammalian and avian genomes, where CsCl band asymmetries were usually close to 3 mg/cm^3. Even in the fish DNAs with asymmetries higher than 0.4 – 0.5 mg/cm^3, the highest value is only 1.3 mg/cm^3. The fish asymmetry values are similar to those found for the isolated and purified major components of homeoterm DNAs, and for bacterial DNAs [5].

Data on intermolecular compositional heterogeneity in these fish DNAs leads to a similar comparison. Although a few fish DNAs showed heterogeneity levels slightly higher than values for bacterial genomes having similar dG + dC content, in general the fish genomes exhibited values close to those of bacterial genomes of similar dG+dC content; in several species, heterogeneity was actually lower than in comparable bacterial genomes. This is of some interest in that genome size in fishes is larger than that of bacteria by two to three orders of magnitude. Intermolecular heterogeneity in fish genomes, like their asymmetries, are very close to values found for the major components of warm-blooded vertebrates [5].

In the DNAs of higher vertebrates, the large asymmetry values observed are a result of the presence of several discrete DNA components covering a range of dG+dC contents [5]. The lack of comparable asymmetries, as well as the relatively low levels of compositional heterogeneity, in the DNAs of the fishes investigated in this study appear to be due to the lack of heavy components. This point was investigated in detail in the case of *Trematomus borchgrevinki,* an Antarctic fish of family Nototheniidae. This DNA was characterized by both a relatively high asymmetry coefficient and a high level of compositional heterogeneity. However, no evidence was found in this species for the existence of discrete components comparable to the heavy components of homeotherms. The main-band DNAs of fishes are quite similar to the main-band DNAs of other poikilotherms [5]. On the basis of buoyant density, compositional heterogeneity and asymmetry, the main bands of fish DNAs appear to correspond to the light component(s) found in the DNAs of homeotherms [5].

Moreover, fish DNAs appear to differ from the DNAs of warm-blooded vertebrates in that fishes show either no discrepancy or only a very small discrepancy between the dG+dC content as determined by chemical analysis and as calculated from $\langle \varrho \rangle$ values. Methylation cannot be responsible for this effect, since it would tend to cause a discrepancy in a direction opposite to that found [19]. The main reason for the discrepancy seems to be a particular frequency of short oligonucleotides, or a particular short-sequence 'design'. If this suggestion is correct, one would have to conclude that such a design is different in fish genomes than in the genomes of homeotherms. An analysis of short-sequence frequency carried out on DNAs from both cold-blooded and warm-blooded vertebrates [20] appears to support this conclusion.

Three other points which deserve mention are the following. The first is that, even if the number of species examined here is still limited, evidence has been found for similar or identical ϱ_0 values in species which are phylogenetically close. A more extensive survey might therefore provide information useful in solving taxonomic problems. The second point is that, in contrast with mammalian and avian genomes where c values are very highly clustered, teleost genomes exhibit a tenfold spread of c values (neglecting some exceptional cases, like lung fishes where the c value is as high as 200 pg). This affords an opportunity to study the relationships, if any, between c value and asymmetry and/or compositional heterogeneity. Finally, it will be useful to compare the genome properties collected here with renaturation kinetics studies for examination of sequence interspersion patterns, a subject where the available information is very scanty [20 – 24].

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