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An ultracentrifugation analysis of two hundred fish genomes

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Abstract

The goal of this study was to provide a comprehensive view of the compositional characteristics of fish genomes. We therefore expanded the number of fish species that we had explored so far in their DNAs by analytical ultracentrifugation in CsCl density gradient from 122 to 201. This study included representatives from three out of nine orders of Elasmobranchs (sharks and rays), both orders of dipnoan lungfishes, and both orders of chondrosteans (sturgeons and bichirs). We also studied 19 out of 38 teleostean orders, which represent all but four (minor) superorders of the subdivision *Teleostei*, a group comprising about 23,600 species (96% of all extant fishes). This leaves for further studies two subclasses, *Holocephali* (chimaeras), and *Coelacanthimorpha* (gombessas). In spite of this substantial increase in the number of species and orders analysed, all average properties (the modal buoyant density, ρ_0, the average buoyant density, $\langle\rho\rangle$, the CsCl profile asymmetry, A, and the compositional heterogeneity, H), and all their ranges were unchanged compared to a previous study [J. Mol. Evol. 31 (1990) 265]. This suggests that, in all likelihood, the properties reported in the present paper can be considered as generally valid for all fish genomes.

Keywords: Analytical ultracentrifugation; Phylogeny; Genome; Base composition; Fish

1. Introduction

Previous investigations from our laboratory analysed the compositional patterns of fish genomes by studying the profiles obtained in analytical CsCl ultracentrifugation experiments. This was done in three steps involving a total of two (Thiery et al., 1976), 34 (Hudson et al., 1980), 39 (Pizon et al., 1983) and 122 (Bernardi and Bernardi, 1990a) species, respectively. Since the early studies (Thiery et al., 1976) the compositional properties of fish genomes were shown to be strikingly different from those of birds and mammals. While birds and mammals display genomes with vast heterogeneities, 10–15% of their genomes containing GC-rich sequences, fish genomes, in general, display DNAs characterized by a lower heterogeneity, GC-rich sequences being less GC-rich and less abundant that un warm-blooded vertebrates. In contrast, the distribution of genes is similar in all vertebrates, in that about half of the genes are located in the GC-rich sequences of the genomes, even if theses sequences are less GC-rich in fishes compared to mammals and birds (Bernardi and Bernardi, 1990b).

The transition in genome organization between cold- and warm-blooded vertebrates is therefore a crucial step in vertebrate DNA evolution. In order to fully understand this transition, it is essential to have a clear picture of the compositional properties of fish genomes. Since fish represent a huge variety of vertebrate forms (half of all vertebrate species), it is essential to study a phylogenetically representative sample of fish species. Thus we decided to expand the genomes explored so far to a total of 201 species.

The reasons for exploring such a large number of fish genomes was (i) as mentioned above, to obtain a phylogenetically representative sample of different orders as fishes include a vast array of distantly related vertebrates with about 25,000 species, corresponding to about half of the extant vertebrate species (Nelson, 1994); and (ii) to extend as much as possible comparisons of fish orders, families, and genera, in order to investigate the compositional differences that arose over evolutionary time (see Bernardi and Bernardi, 1990b).

Abbreviations: A, asymmetry; GC, molar fraction of guanine and cytosine in DNA; H, heterogeneity; , $\bar{H}$, mean heterogeneity (interspecific); kb, kilobase pair(s); MW, molecular weight; ρ, buoyant density; ρ_0, modal buoyant density; , $\langle\rho\rangle$, mean buoyant density; σ, standard deviation (interspecific)

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2. Materials and methods

2.1. Fish samples

Original fish samples were obtained for 73 species. Fish were collected by spear while free or scuba diving except *Gillichthys seta*, *Gillichthys mirabilis*, which were collected with minnow traps, and *Leuresthes sardina* which was collected using a beach seine. Sampling localities are given in Table 3. Fishes from Panama were collected by Kenneth Clifton (by spear), the sample from Venezuela was collected by Jason Podrabsky (by hand net).

2.2. DNA preparation

The samples of fish DNAs investigated here were obtained from either liver or, in the case of small fishes, from whole bodies, using the method of Kay et al. (1952).

2.3. Ultracentrifugation

Analytical ultracentrifugation was performed using a Beckman ultracentrifuge Optima XL-A.

The modal and mean buoyant densities, ρ_0 and $\langle\rho\rangle$, the asymmetry of the CsCl main band, $A = \langle\rho\rangle - \rho_0$, the intermolecular compositional heterogeneity, H, and the molar ratio, GC, of deoxyguanosine + deoxycytidine were calculated as described by Bernardi and Bernardi (1990a).

3. Results and discussion

Table 1 presents a classification of fishes derived from Nelson (1994) in order to indicate the taxonomic position of the species studied. This classification puts the fishes in an order that reflects their postulated evolutionary relationship, ranging from the most ancient splits in vertebrate evolution, *Chondrichthyes* (sharks, rays), *Sarcopterygii* (Lungfish,

Table 1
Classification of fishes[a]

Class	Subclass[b]	Infraclass	Division	Subdivision	Superorder	Order[c]
Chondrichthyes	*Elasmobranchi*			*Euselachii*	*Lamniformes*	
						Squaliformes
						Rajformes
	**Holocephali*					
Sarcopterygii	*Dipnoi*				*Ceratodontimorpha*	*Ceratodontiformes*
						Lepidosireniformes
Actinopterygii	*Chondrostei*					*Polypteriformes*
						Acipenseriformes
	Neopterygii		*Teleostei*	*Osteoglossomorpha*		*Osteoglossiformes*
				Elopomorpha		*Anguilliformes*
				Clupeomorpha		*Clupeiformes*
				Eutelostei	*Ostariophysi*	*Cypriniformes*
						Characiformes
						Siluriformes
					Protacanthopterygii	*Salmoniformes*
					**Stenopterygii*	
					Cyclosquamata	*Aulopiformes*
					**Scopelomorpha*	
					**Lampridiomorpha*	
					**Polymixiomorpha*	
					Paracanthopterygii	*Gadiformes*
						Ophidiiformes
						Batrachoidiformes
						Lophiiformes
					Acanthopterygii	*Cyprinodontiformes*
						Syngnathiformes
						Dactylopteriformes
						Scorpaeniformes
						Perciformes
						Pleuronectiformes
						Tetraodontiformes

[a] From Nelson, 1994.
[b] Asterisks indicate groups not investigated.
[c] Only orders listed in Table 2 are presented.

Table 2
Properties of DNAs from fishes[a]

Sample no.	Order	Family	*Species*	MW (kb)	ρ_0 (g/cm^3)	$\langle\rho\rangle$ (g/cm^3)	*A* (mg/cm^3)	*H* (%GC)	GC (%)	*c*
1	*Lamniformes*	*Scyliorhinidae*	*Scyliorhyinus stellaris*	29.55	1.7053					6.2
2		*Carcharinidae*	*Mustelus mosis*	34.55	1.7044	1.7047	0.3	2.9	45.6	
3			*Scoliodon terranovae*	13.98	1.7030	1.7035	0.5	2.9	44.4	3.6
4			*Carcharinus galapagensis*	15.24	1.7033	1.7037	0.4	2.3	44.7	
5		*Sphyrnidae*	*Sphyrna lewini*	1.57	1.7030	1.7042	1.2	4.6	45.1	3.5
6	*Squaliformes*	*Squalidae*	*Squalus acanthias*	48.50	1.7050	1.7059	0.9	4.3	46.8	7.2
7		*Squatinidae*	*Squatina dumerili*	29.55	1.7050	1.7072	2.2	5.0	48.2	
8	*Rajiformes*	*Rajidae*	*Raja erinacea*	33.25	1.7035	1.7041	0.6	3.7	45.0	3.5
9			*Raja stellulata**	117.00	1.7014					
10		*Dasyatidae*	*Gymnura altavela*	44.44	1.7015	1.7022	0.7	2.7	43.1	
11		*Myliobatidae*	*Myliobatis freminvillei*	33.25	1.7029	1.7039	1.0	4.4	44.8	4.9
12		*Torpedinidae*	*Torpedo marmorata*	45.36	1.7011					7.0
13			*Torpedo ocellata*	49.46	1.7012					7.5
14	*Ceratodontiformes*	*Ceratodontidae*	*Neoceratodus forsteri*		1.7031	1.7027	−0.4		43.5	
15	*Lepidosireniformes*	*Protopteridae*	*Protopterus sp.*	55.15	1.7005	1.7001	−0.4	2.5	40.9	50.0
16	*Polypteriformes*	*Polypteridae*	*Polypterus senegalus*	28.84	1.6995	1.7008	1.3	2.3	41.6	
17	*Acipenseriformes*	*Acipenseridae*	*Acipenser sturio*			1.7019			42.8	1.6
18	*Osteoglossiformes*	*Pantodontidae*	*Pantodon buchholzi*	5.53	1.7022	1.7023	0.1	2.0	43.2	0.8
19		*Notopteridae*	*Notopterus notopterus*	27.91	1.7015	1.7021	0.6	3.6	42.9	
20		*Mormyridae*	*Gnathonemus petersii*	24.58	1.7029	1.7033	0.4	2.5	44.2	1.2
21	*Anguilliformes*	*Anguillidae*	*Anguilla anguilla*	13.68	1.7018	1.7031	1.3	2.3	44.0	0.9
22			*Anguilla rostrata*	35.07	1.7011	1.7015	0.7	3.5	42.6	1.4
23	*Clupeiformes*	*Clupeidae*	*Brevoortia tyrannus*	20.52	1.7021	1.7024	0.3	2.5	43.4	0.8–1.4
24			*Sardinella anchovia*	20.14	1.7022	1.7022	0.0	3.3	43.1	
25	*Cypriniformes*	*Cyprinidae*	*Carassius auratus*	24.36	1.6970	1.6971	0.1	1.8	37.9	1.7
26			*Cyprinus carpio*	72.03	1.6963	1.6965	0.2	2.0	37.2	1.7
27			*Brachydanio rerio*	49.46	1.6959	1.6962	0.3	2.6	36.9	1.8
28			*Labeo bicolor*	18.65	1.6959	1.6965	0.7	2.0	37.2	1.3
29		*Cobitididae*	*Acanthophtalmus semicinctus*		1.6975	1.6978	0.3		38.6	
30	*Characiformes*	*Characidae*	*Astyanax mexicanus*		1.6995	1.6998	0.3		40.6	1.1–2.1
31	*Siluriformes*	*Callichthydae*	*Corydoras aeneus*		1.6979	1.6980	0.1		38.8	4.4
32	*Salmoniformes*	*Salmonidae*	*Salmo salar*	10.99	1.7028	1.7035	0.7		44.4	
33			*Salmo fario*	45.97	1.7030	1.7039	0.9	1.9	44.8	
34			*Onchorhynchus mykiss*	28.14	1.7024	1.7026	0.2	3.0	43.5	3.2
35			*Onchorhynchus kisutch*	31.24	1.7033	1.7036	0.3	3.0	44.5	3.0
36			*Onchorhynchus keta*	30.51	1.7038				44.7	
37			*Onchorhynchus nerka*	27.68	1.7035				44.4	
38			*Coregonus autunnalis migr.*	3.49	1.7034	1.7035	0.1	3.1	44.4	
39	*Aulopiformes*	*Synodontidae*	*Synodus foetens*	32.24	1.7025	1.7029	0.4	2.5	43.8	1.2
40			*Synodus intermedius*	1.31	1.7021	1.7027	0.6	5.6	43.6	
41			*Trachinocephalus myops*	31.00	1.7034	1.7045	0.9	3.5	45.4	

(*Continued overleaf*)

Table 2 (*continued*)

Sample no.	Order	Family	*Species*	MW (kb)	ρ_0 (g/cm^3)	$\langle\rho\rangle$ (g/cm^3)	*A* (mg/cm^3)	*H* (%GC)	GC (%)	*c*
42	*Gadiformes*	*Gadidae*	*Urophycis regius*	22.29	1.7065	1.7068	0.3	3.3	47.8	0.9
43			*Urophycis chuss*	5.86	1.7070	1.7063	−0.7	4.2	47.2	
44		*Merluccidae*	*Merluccius bilinearis*	52.43	1.7041	1.7042	0.1	2.5	45.1	0.9
45	*Ophidiiformes*	*Ophidiidae*	*Ophidion holbrooki*	6.43	1.7018	1.7027	0.9	4.2	43.6	0.7–0.8
46	*Batrachoidiformes*	*Batrachoidae*	*Opsanus tau*	23.73	1.6996	1.7001	0.5	2.4	40.9	2.8
47			*Porichthys notatus**	46.00	1.6979					2.2
48			*Porichthys porosissimus*	42.34	1.6998	1.7000	0.2	2.3	40.8	1.7
49	*Lophiiformes*	*Lophiidae*	*Lophius americanus*	13.08	1.6996	1.6998	0.2	3.5	40.6	1.0
50	*Cyprinodontiformes*	*Aplocheilidae*	*Aplocheilus dayi*		1.7002	1.7008	0.6		41.6	
51			*Aphyosemion elegans*	13.23	1.7008	1.7007	−0.1	1.8	41.5	
52			*Aphyosemion cameronense*	15.40	1.7009	1.7012	0.3	2.7	42.0	
53			*Aphyosemion punctatum*	6.43	1.7009	1.7011	0.2	1.7	41.9	0.6
54			*Aphyosemion schelii*	17.59	1.7011	1.7014	0.3	1.4	42.2	
55			*Aphyosemion hertzogii*	36.95	1.7013	1.7014	0.1	2.0	42.2	
56			*Aphyosemion amieti*	13.38	1.7016	1.7020	0.4	1.8	42.9	
57			*Aphyosemion marmoratum*	25.22	1.7018	1.7021	0.3	3.7	43.0	
58			*Aphyosemion spoorenbergi*	10.60	1.7032	1.7032	0.0	1.9	44.1	
59			*Aphyosemion striatum*	10.47	1.7053	1.7058	0.5	1.3	46.7	0.7
60			*Aphyosemion australe*	65.34	1.7066	1.7071	0.5	2.1	48.1	0.6
61			*Diapteron cyanosticum*	17.24	1.7012	1.7016	0.4	1.6	42.4	
62			*Diapteron cyanosticum + georgiae*	17.07	1.7009	1.7016	0.7	2.2	42.4	
63			*Epiplatys chaperi*	1.48	1.7010				41.9	
64			*Pachypanchax playfairii*		1.7046	1.7063	1.7		47.2	
65			*Rivulus holmiae*	18.12	1.6984	1.6987	0.3	1.8	39.5	
66			*Rivulus agilae*	13.83	1.7007	1.7015	0.8	1.3	42.3	
67		*Cyprinodontidae*	*Fundulus heteroclitus*	18.65	1.6993	1.6996	0.3	1.7	40.4	1.5
68			*Cyprinodon salinus*	10.21	1.6984	1.7000	1.6	4.4	40.8	
69			*Cyprinodon macularius califor.*	20.33	1.6989	1.6996	0.7	2.8	40.4	
70			*Cyprinodon n. nevadensis*	8.08	1.6983	1.6989	0.6	1.7	39.7	
71			*Cyprinodon variegatus*	7.54	1.6987	1.6998	1.1	3.1	40.6	1.6
72			*Austrofundulus limnaei**	102.19	1.6971	1.6973	0.2	1.9	38.0	
73			*Jordanella floridae*		1.6987	1.6987	0.0		39.5	
74		*Poecilidae*	*Poecilia sphenops mel.*	8.42	1.6987	1.6994	0.7	3.8	40.2	0.8
75			*Xiphophorus maculatus*	5.21	1.6985	1.6987	0.2	2.9	39.5	0.9
76	*Syngnathiformes*	*Syngnathidae*	*Hippocampus sp.*	25.66	1.7031	1.7038	0.7	3.0	44.7	0.7
77	*Gobiesociformes*	*Gobiesocidae*	*Gobiesox meandricus**	40.00	1.6998	1.7002	0.3	4.0	41.0	
78	*Dactylopteriformes*	*Dactylopteridae*	*Dactylopterus volitans*	9.84	1.7033	1.7040	0.7	3.9	44.9	
79	*Scorpaeniformes*	*Scorpaenidae*	*Scorpaena calarata*	29.31	1.6995	1.6999	0.4	3.5	40.7	
80			*Scorpaena brasiliensis*	33.25	1.6988	1.6990	0.2	4.2	39.8	1.4
81			*Scorpaena guttata**	27.20	1.6974	1.6978	0.4	3.8	38.6	
82			*Sebastes atrovirens**	22.53	1.6984					
83		*Triglidae*	*Prionotus carolinus*	3.04	1.7011	1.7021	1.0	3.5	42.9	1.0

Table 2 (*continued*)

Sample no.	Order	Family	*Species*	MW (kb)	ρ_0 (g/cm^3)	$\langle\rho\rangle$ (g/cm^3)	*A* (mg/cm^3)	*H* (%GC)	GC (%)	*c*
84		*Cottidae*	*Hemitripterus americanus*	4.62	1.7010	1.7013	0.3	2.9	42.1	0.9
85			*Leptocottus armatus**	58.90	1.7012	1.7020	0.8	2.5	42.9	
86			*Clinocottus analis**	63.00	1.7005	1.7004	−0.1	3.8	41.2	0.9
87		*Hexagrammidae*	*Ophiodon elongatus**	21.00	1.6997	1.7006	0.9	3.9	41.5	
88			*Pleurogrammus azonus**	11.56	1.6997	1.7004	0.7	3.4	41.2	
89	*Perciformes*	*Serranidae*	*Centropristis striatus*	8.53	1.6981	1.6985	0.4	3.0	39.3	1.2
90			*Epinephelus striatus*	18.29	1.7004	1.7006	0.2	1.5	41.4	1.3
91			*Epinephelus guttatus*	10.09	1.7015	1.7013	−0.2	1.7	42.1	1.2
92			*Paralabrax maculatofasciatus**	22.00	1.6979					
93			*Alphestes afer**	27.20	1.6988	1.6989	0.1	3.0	39.7	
94			*Paranthias colonus**	28.10	1.6997					
95			*Paralabrax clathratus**	23.60	1.6978					
96		*Acanthuridae*	*Acanthurus bahianus**	35.28	1.6990	1.6988	0.8	2.8	39.6	
97			*Acanthurus chirurgus**	43.00	1.6981	1.6985	0.3	2.5	39.2	
98			*Acanthurus coeruleus**	36.00	1.6979	1.6983	0.4	2.4	39.1	
99			*Acanthurus triostegus**	23.29	1.6989					
100		*Atherinidae*	*Leuresthes sardina**	46.60	1.6983					
101		*Cebidichthydae*	*Cebidichthys violaceus**	55.00	1.6997					
102		*Chaetodontidae*	*Johnrandallia nigrirostris**	24.30	1.6997					
103		*Echeneidae*	*Echeneis naucrates**	44.00	1.6993	1.69911	−0.2	2.8	40.1	0.7
104		*Embiotocidae*	*Amphisticus argenteum**	27.06	1.6984					
105			*Brachyistius frenatus**	22.00	1.6988					
106			*Cymatogaster aggregata**	45.30	1.6990					0.7
107			*Damalichthys vacca**	23.80	1.6985					
108			*Embiotoca lateralis**	28.10	1.6989	1.6993	0.4	2.8	40.1	
109			*Embiotoca jacksoni**	50.00	1.6990	1.6992	0.2	2.7	40.0	1.0
110			*Hyperprosopon argenteum**	64.00	1.6992					
111			*Hyperprosospon anale**	23.43	1.7002	1.7010	0.8	2.6	41.8	
112			*Micrometrus minimus**	29.00	1.6991					
113		*Gobidae*	*Coryphopterus nicholsii**	36.60	1.6985					
114			*Gillichthys seta**	33.00	1.6984	1.6997	1.3	3.6	40.6	
115			*Gillichthys mirabilis**	20.80	1.6976					
116		*Hexagrammidae*	*Oxylebius pictus**	30.21	1.7000	1.6997	−0.3	2.8	40.5	
117		*Pomacanthidae*	*Holocanthus passer**	37.00	1.6999					
118		*Kyphosidae*	*Hermosilla azurea**	20.08	1.6997	1.7001	0.4	3.9	40.9	

(*Continued overleaf*)

Table 2 (*continued*)

Sample no.	Order	Family	*Species*	MW (kb)	ρ_0 (g/cm^3)	$\langle\rho\rangle$ (g/cm^3)	*A* (mg/cm^3)	*H* (%GC)	GC (%)	*c*
119	*Perciformes* (cont'd)	*Labridae*	*Bodianus diplotaenia**	34.00	1.6997	1.6996	0.0	2.2	40.5	
120			*Bodianus rufus**	44.00	1.6990	1.6994	0.4	2.2	40.2	
121			*Clepticus parrae**	46.30	1.6990	1.6988	−0.1	2.7	39.6	
122			*Coris julis*	22.70	1.7006	1.7015	0.9	2.9	42.3	1.2
123			*Gomphosus varius**	36.00	1.6972	1.6977	0.5	2.5	38.4	
124			*Halichoeres garnoti**	60.00	1.6985	1.6987	0.2	2.6	39.5	
125			*Halichoeres nicholsi**	30.00	1.6977					
126			*Halichoeres pictus**	40.50	1.6979					
127			*Halichoeres poeyi**	51.00	1.6986	1.6988	0.2	2.6	39.6	
128			*Pseudodax moluccanus**	19.00	1.6995	1.7000	0.5	3.0	40.8	
129			*Semicossyphus pulcher**	28.16	1.6995					
130			*Symphodus cinereus*	24.79	1.7002	1.7016	1.4	3.2	42.4	1.2
131			*Symphodus mediterraneus*	17.94	1.7006	1.7015	0.9	3.1	42.3	0.6
132			*Symphodus ocellatus*	20.14	1.7004	1.7012	0.8	3.2	42.0	1.1
133			*Thalassoma amblycephalum**	31.80	1.6971					
134			*Thalassoma bifasciatum**	25.80	1.6982	1.6979	−0.2	3.5	38.7	1.0
135			*Thalassoma grammaticum**	29.80	1.6974	1.6979	0.5	3.8	38.7	
136			*Thalassoma hardwicke**	62.00	1.6975	1.6976	0.1	2.5	38.3	
137			*Thalassoma lucasanum**	25.00	1.6976					
138			*Thalassoma purpureum**	63.00	1.6976	1.6975	−0.2	2.3	38.2	
139			*Thalassoma trilobatum**	26.30	1.6971					
140			*Thalassoma hebraicum**	24.60	1.6970	1.6972	0.2	3.2	37.9	
141			*Xyrichthys novacula*	28.14	1.6998	1.7001	0.3	2.0	40.9	
142		*Labrisomidae*	*Dialommus fuscus**	53.00	1.7001	1.7007	0.5	2.7	41.5	
143		*Pomacentriade*	*Chromis atrovirens**	27.00	1.6986					
144			*Chromis chromis*	17.76	1.7005	1.6993	−1.2	4.4	40.1	1.2
145			*Chromis cyanea**	17.57	1.6982	1.6987	0.5	5.2	39.5	
146			*Chromis multilineata**	38.50	1.6983	1.6984	0.1	3.1	39.2	
147			*Dascyllus aruanus**	32.40	1.6991					
148			*Dascyllus flavicaudus**	29.90	1.6985					
149			*Microspathadon chrysurus**	30.00	1.6979	1.6984	0.5	3.4	39.2	
150			*Microspathodon dorsalis**	35.00	1.6985	1.6989	0.4	4.1	39.7	
151			*Stegastes dorsopunicans**	18.77	1.6990	1.6990	−0.1	3.8	39.8	
152			*Stegastes planifrons**	21.00	1.6991	1.6992	0.1	3.0	40.0	
153		*Paracirrhytidae*	*Paracirrhytices forsteri**	22.23	1.7006	1.7015	0.9	3.4	42.3	
154		*Scaridae*	*Calotomus carolinus**	56.00	1.6967					
155			*Hypposcarus harid**	39.00	1.6976	1.6980	0.4	2.6	38.8	
156			*Nicholsina denticulata**	62.00	1.6965	1.6964	−0.1	3.0	37.2	
157			*Scarus coelestinus**	78.00	1.6974	1.6976	0.2	2.5	38.4	
158			*Scarus ghobban**	77.00	1.6973	1.6982	0.9	2.9	39.0	
159			*Scarus gibbus*	12.79	1.6984	1.6988	0.4	1.6	39.6	1.9–2.1
160			*Scarus psitticus**	50.00	1.6973	1.6977	0.4	2.7	38.5	
161			*Scarus schlegeli**	24.20	1.6970	1.6964	−0.6	3.7	37.2	
162		*Zanclidae*	*Zanclus cornutus**	31.80	1.6975	1.6979	0.4	2.4	38.7	
163		*Lutjanidae*	*Lutjanus synagris*	2.62	1.7010	1.7022	1.2	4.2	43.1	0.9–1.4

Table 2 (*continued*)

Sample no.	Order	Family	Species	MW (kb)	ρ_0 (g/cm^3)	$\langle\rho\rangle$ (g/cm^3)	A (mg/cm^3)	H (%GC)	GC (%)	c
164		*Lethrinidae*	*Lethrinus nebulosus*	11.53	1.6999	1.7001	0.2	1.7	40.9	
165		*Cichlidae*	*Alcolapia alcalicus grahami*	8.64	1.7007	1.7012	0.5	3.6	42.0	
166			*Cichlasoma meeki*		1.7007	1.6994	− 1.3		40.2	1.4
167			*Orechromis spilurus*	6.15	1.7003	1.7006	0.3	2.2	41.4	0.9
168			*Oreochromis aureus*	9.11	1.7003	1.7008	0.5	2.3	41.6	1.2
169			*Oreochromis mossambicus*	9.71	1.7009	1.7010	0.1	3.0	41.8	1.0
170			*Oreochromis niloticus*	7.65	1.7002	1.7009	0.7	2.0	41.7	0.9
171			*Symphysodon discus*	24.36	1.7004	1.7005	0.1	2.5	41.3	1.5
172			*Tilapia buettikoferi*	25.88	1.7005	1.7009	0.4	2.5	40.8	
173		*Sphyraenidae*	*Sphyraena barracuda*	8.64	1.7015	1.7017	0.2	1.8	42.6	0.8–1.2
174			*Sphyraena ensis*	8.76	1.7024	1.7027	0.3	2.7	43.6	
175		*Nototheniidae*	*Pagothenia borchgrevinki*	15.56	1.7002	1.7010	0.8	2.5	41.8	
176			*Trematomus bernacchii*	4.25	1.7011	1.7020	0.9	3.1	42.9	1.9
177			*Trematomus centronotus*	1.82	1.7009	1.7017	0.8	4.1	42.5	2.0
178			*Trematomus nicolai*	4.71	1.7010	1.7017	0.7	3.7	42.5	
179			*Trematomus newnesi*	1.53	1.7005	1.7018	1.3	4.5	42.6	2.1
180			*Trematomus hansoni*	13.38	1.6999	1.7002	0.3	2.4	41.1	1.8
181			*Dissosticus mawsoni*	9.84	1.7003	1.7007	0.4	2.5	41.5	
182		*Bathydraconidae*	*Gymnodraco acuticeps*	17.24	1.7007	1.7012	0.5	3.5	42.1	1.9
183		*Blenniidae*	*Ophioblennius atlanticus*	7.33	1.7007	1.7011	0.4	2.8	41.9	0.8–1.0
184		*Callyonimidae*	*Synchiropus splendidus*	11.12	1.7073	1.7076	0.3	2.7	48.6	
185	*Pleuronectiformes*	*Pleuronectidae*	*Siacium papillosum*	2.62	1.7024	1.7025	0.1	4.7	43.4	0.7–1.0
186			*Limanda aspera*	6.62	1.6995	1.7002	0.7	3.5	41.0	0.6–1.0
187			*Limanda ferruginosa*	10.34	1.7012	1.7021	0.9	4.7	42.9	
188			*Psettichthys melanostictus*	80.00	1.7002	1.7006	0.3	2.7	41.4	
189			*Pleuronichthys californicus**	49.00	1.6996	1.6992	− 0.4	2.7	40.0	
190	*Tetraodontiformes*	*Balistidae*	*Melichthys vidua**	12.79	1.7014	1.7022	0.8	2.5	43.1	
191			*Balistes capriscus*	17.07	1.7023	1.7024	0.1	2.4	43.3	0.7
192			*Monacanthus tuckeri**	37.80	1.7042	1.7046	0.4	3.6	45.5	
193			*Rhinecanthus aculeatus*	8.19	1.7047	1.7048	0.1	1.5	45.7	
194			*Stephanolepis hispidus*	3.73	1.7037	1.7043	0.6	4.4	45.2	0.7
195			*Aluterus schoepfi*	1.44	1.7038	1.7039	0.1	5.4	44.8	0.6
196		*Ostraciidae*	*Acanthostracion quadricornis*	51.10	1.7001	1.7008	0.7	2.7	41.6	
197		*Tetraodontidae*	*Lagocephalus laevigatus*	9.35	1.7022	1.7026	0.4	3.5	43.5	0.4–0.5
198			*Arothron diadematus*	10.09	1.7025	1.7036	1.1	3.0	44.5	
199			*Arothron meleagris*	26.77	1.7034	1.7058	1.4	4.8	46.7	
200			*Sphoeroides annulatus*	18.47	1.7047	1.7053	0.6	3.6	46.2	
201		*Diodontidae*	*Diodon holocanthus*	6.24	1.7009	1.7012	0.3	2.1	42.0	0.9

[a] Asterisks indicate values from this work; all other values are from Bernardi and Bernardi (1990a); see this reference for additional information and for the sources of *c* values. The sources of samples are given in Bernardi and Bernardi (1990a) and in Table 3.

Table 3
Sampling localities of the fish species investigated

Species	Sampling locality
*Raja stellulata**	Panama, San Blas Islands
*Porichthys notatus**	Monterey, California
*Austrofundulus limnaei**	Venezuela
*Gobiesox meandricus**	Monterey, California
*Scorpaena guttata**	San Diego, California
*Sebastes atrovirens**	Monterey, California
*Leptocottus armatus**	Monterey, California
*Clinocottus analis**	Monterey, California
*Ophiodon elongatus**	Monterey, California
*Pleurogrammus azonus**	Alaska
*Paralabrax maculatofasciatus**	Sea of Cortez
*Alphestes afer**	Sea of Cortez
*Paranthias colonus**	Galapagos Islands
*Paralabrax clathratus**	Catalina Island, California
*Acanthurus bahianus**	Panama
*Acanthurus chirurgus**	Panama
*Acanthurus coeruleus**	Panama
*Acanthurus triostegus**	Moorea, French Polynesia
*Leuresthes sardina**	Sea of Cortez
*Cebidichthys violaceus**	Monterey, California
*Johnrandallia nigrirostris**	Galapagos Islands
*Echeneis naucrates**	California
*Amphisticus argenteum**	Monterey, California
*Brachyistius frenatus**	Monterey, California
*Cymatogaster aggregata**	Monterey, California
*Damalichthys vacca**	Monterey, California
*Embiotoca lateralis**	Monterey, California
*Embiotoca jacksoni**	Monterey, California
*Hyperprosopon argenteum**	Monterey, California
*Hyperprosospon anale**	Monterey, California
*Micrometrus minimus**	Monterey, California
*Coryphopterus nicholsii**	Monterey, California
*Gillichthys seta**	Sea of Cortez
*Gillichthys mirabilis**	Monterey, California
*Oxylebius pictus**	Monterey, California
*Holocanthus passer**	Sea of Cortez
*Hermosilla azurea**	Sea of Cortez
*Bodianus diplotaenia**	Galapagos Islands
*Bodianus rufus**	Panama, San Blas Islands
*Clepticus parrae**	Brazil
*Gomphosus varius**	Moorea, French Polynesia
*Halichoeres garnoti**	Panama, San Blas Islands
*Halichoeres nicholsi**	Sea of Cortez
*Halichoeres pictus**	Panama, San Blas Islands
*Halichoeres poeyi**	Panama, San Blas Islands
*Pseudodax moluccanus**	Rangiroa, Tuamotu, French Polynesia
*Semicossyphus pulcher**	Baja California, Mexico
*Thalassoma amblycephalum**	Moorea, French Polynesia
*Thalassoma bifasciatum**	Panama, San Blas Islands
*Thalassoma grammaticum**	Sea of Cortez
*Thalassoma hardwicke**	Moorea, French Polynesia
*Thalassoma lucasanum**	Moorea, French Polynesia
*Thalassoma purpureum**	Moorea, French Polynesia
*Thalassoma trilobatum**	Moorea, French Polynesia
*Thalassoma hebraicum**	Zanzibar
*Dialommus fuscus**	Galapagos Islands
*Chromis atrovirens**	Panama, San Blas Islands
*Chromis cyanea**	Panama, San Blas Islands
*Chromis multilineata**	Panama, San Blas Islands
*Dascyllus aruanus**	Moorea, French Polynesia
*Dascyllus flavicaudus**	Moorea, French Polynesia
*Microspathodon chrysurus**	Panama, San Blas Islands
*Microspathodon dorsalis**	Galapagos Islands
*Stegastes dorsopunicans**	Panama, San Blas Islands
*Stegastes planifrons**	Panama, San Blas Islands
*Paracirrhytices forsteri**	Moorea, French Polynesia
*Calotomus carolinus**	Zanzibar
*Hypposcarus harid**	Rangiroa, Tuamotu, French Polynesia
*Nicholsina denticulata**	Sea of Cortez
*Scarus coelestinus**	Panama, San Blas Islands
*Scarus ghobban**	Galapagos Islands
*Scarus psitticus**	Moorea, French Polynesia
*Scarus schlegeli**	Moorea, French Polynesia
*Zanclus cornutus**	Moorea, French Polynesia
*Pleuronichthys californicus**	Monterey, California
*Melichthys vidua**	Moorea, French Polynesia
*Monacanthus tuckeri**	Panama, San Blas Islands

Coelacanths and Tetrapods) and *Actinopterygii* (ray-finned fishes), to the most recent teleost orders, like *Tetraodontiformes* (puffers) and *Pleuronectiformes* (flounders). This study included representatives from three out of nine orders of *Elasmobranchs* (sharks and rays), both orders of dipnoan lungfishes, and both orders of chondrosteans (sturgeons and bichirs). We also studied 19 out of 38 teleostean orders, which represent all but four (minor) superorders of the subdivision *Teleostei*, a group comprising about 23,600 species (96% of all extant fishes). This leaves for further studies two subclasses, *Holocephali* (chimaeras), and *Coelacanthimorpha* (gombessas). Table 2 lists all species studied with their properties. Table 3 lists the sampling localities.

In a previous study of the analytical CsCl profiles of fish DNAs (Bernardi and Bernardi, 1990a), only three DNA

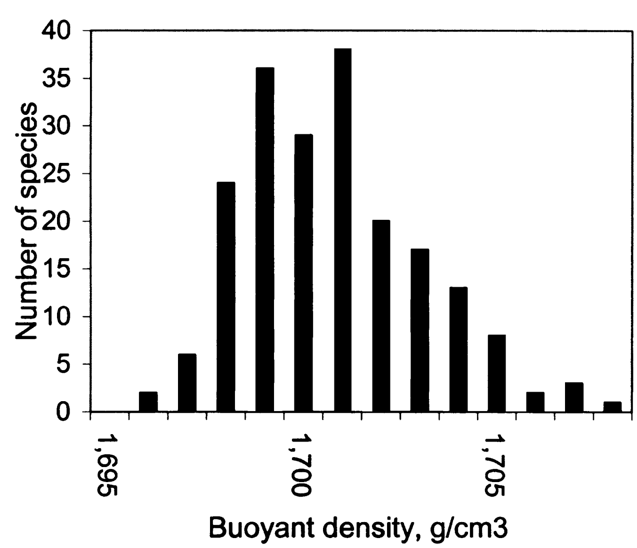

Fig. 1. The number of fish species is plotted against modal buoyant densities. Only species characterized by different modal buoyant densities were used within a genus.

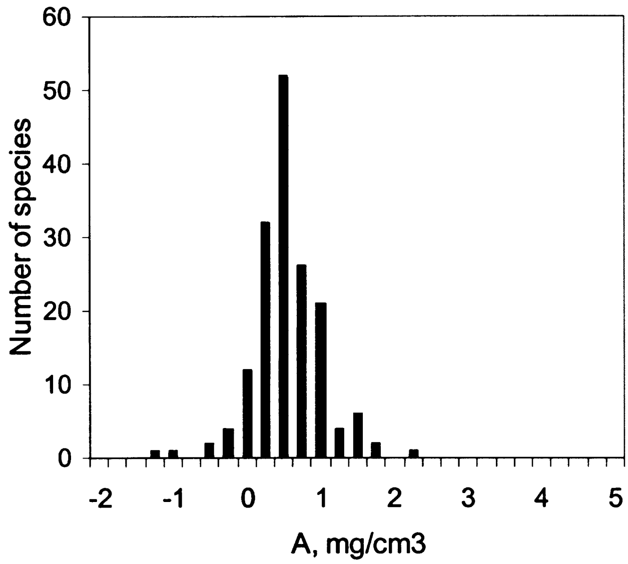

Fig. 2. The number of fish species is plotted against the CsCl band asymmetry.

samples (4, 11, 12; all of them from *Chondrichtyes*) showed sizable amounts of resolved satellite bands, which were on the heavy, GC-rich side of the main band. Minor satellite bands were found in other DNA samples, mainly on the heavy side and more rarely on the light side. Finally, some other samples showed poorly resolved satellite on the heavy side.

Fig. 1 displays the distribution of the modal buoyant densities of the fish DNAs studied. The modal buoyant density range covered was 1.696–1.708 g/cm^3. In the case of *Actinopterygii*, the average ρ_0 was 1.7014 g/cm^3 and the corresponding standard deviation, σ, was 2.2 mg/cm^3. Most values were between 1.699 and 1.704 g/cm^3. In the case of *Chondrichthyes*, the average ρ_0 was 1.7035 g/cm^3, and σ was 1.1 mg/cm^3. Most values were in the 1.702–1.704 g/cm^3 range.

Because the distribution of ρ_0 values could be biased by the overrepresentation of species from the same genera or families of fishes, a histogram was constructed in which only one DNA sample per genus was taken into account when the other species within the genus had the same buoyant density. This histogram was not, however, strikingly different from that including all species.

The distribution of DNA CsCl band asymmetries for all fish species investigated are presented in the histogram of Fig. 2. The majority of fishes showed values around 0.5 mg/cm^3 ($A = 0.65$ mg/cm^3; $\sigma = 0.54$ mg/cm^3 in the case of *Actinopterygii*).

Intermolecular compositional heterogeneities (*H*) basically reflect the spread of GC levels among different fragments in a given DNA preparation. Fig. 3 displays the distribution of H values for different fish species. Values ranged from 0.5% GC for *Aphyosemion striatum* (sample 59) to 4.7% GC for *Arothron meleagris* (sample 199; the higher value of 4.9% for *S. dumerili*, sample 7, was due to a shoulder on the heavy side of the main band, indicative of a satellite). The average heterogeneity, $\bar{H}$, of DNAs from *Osteichthyes* was equal to 2.6% GC ($\sigma = 1.1\%$ GC). Values higher than 3% GC were often due to poorly resolved satellites (see, for instance, sample 7). Artefactual reasons, like low molecular weight of DNA samples, may also account for some high values of *H*. A plot of CsCl band asymmetry versus intermolecular compositional heterogeneity (Fig. 4) shows a linear relationship. The slope of the line was significantly different from zero ($P < 0.001$).

The very small increase in the number of *c* values, relative to those reported in previous work (Bernardi and Bernardi, 1990a) made it unnecessary to replot the data against GC values, as previously done.

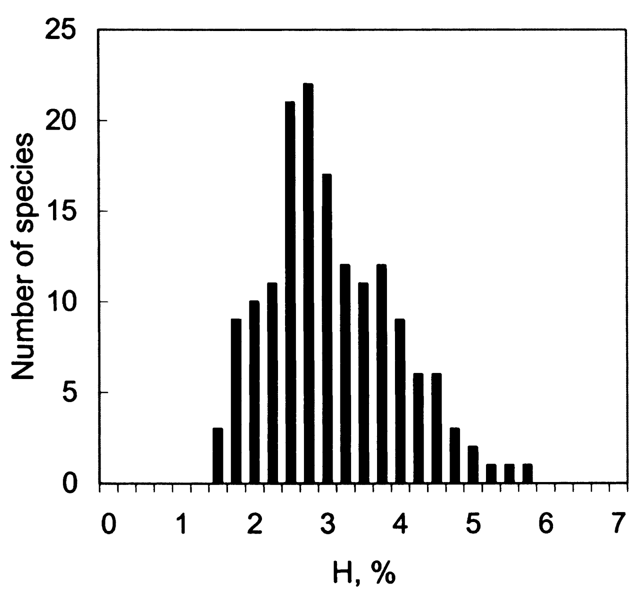

Fig. 3. The number of fish species is plotted against the intermolecular compositional heterogeneity of DNAs.

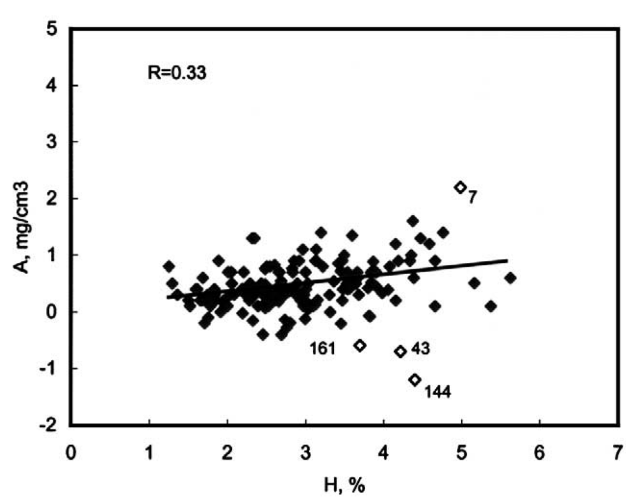

Fig. 4. Plot of CsCl band asymmetry against compositional heterogeneity of DNAs from fishes. Four outliers were not taken into consideration because the anomalous asymmetries were due to satellite DNAs.

In conclusion, an almost twofold increase in the fish DNAs investigated, including species from a number of orders and families not previously explored, did not lead to any significant differences in the range and average values of modal buoyant densities, asymmetry and heterogeneity compared to previous investigations (Bernardi and Bernardi, 1990a). This suggests that these values are unlikely to change with further increases in the fish samples explored.

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