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Compositional Patterns in the Nuclear Genome of Cold-Blooded Vertebrates

Giacomo Bernardi and Giorgio Bernardi

Laboratoire de Génétique Moléculaire, Institut Jacques Monod, Tour 43, 2 Place Jussieu, 75005 Paris, France

Summary. DNA preparations obtained from 122 species of fishes, 5 species of amphibians, and 13 species of reptiles were investigated in their compositional properties by analytical equilibrium centrifugation in CsCl density gradients. These species represented 21 orders of Osteichthyes, 3 orders of Chondrichthyes, 2 orders of amphibians, and 3 orders of reptiles. Modal buoyant densities of fish DNAs ranged from 1.696 to 1.707 g/cm^3, the vast majority of values falling, however, between 1.699 and 1.704 g/cm^3, which is the range covered by the DNAs of amphibians and reptiles. In all cases, DNA bands in CsCl were only weakly asymmetrical and only very rarely were accompanied by separate satellite bands (mostly on the GC-rich side). Intermolecular compositional heterogeneities were low in the vast majority of cases, and, like CsCl band asymmetries, at least partially due to cryptic or poorly resolved satellites. The present findings indicate, therefore, that DNAs from cold-blooded vertebrates are characterized by a number of common properties, namely a very wide spectrum of modal buoyant densities, low intermolecular compositional heterogeneities, low CsCl band asymmetries, and, in most cases, small amounts of satellite DNAs. In the case of fish DNAs a negative correlation was found between the GC level and the haploid size (*c* value) of the genome. If polyploidization is neglected, this phenomenon appears to be mainly due to the fact that increases and decreases in GC are associated with contraction and expansion phenomena, respectively, of intergenic noncoding sequences, which are GC poor relative to coding sequences.

Offprint requests to: Giorgio Bernardi

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Introduction

Previous investigations from our laboratory showed (1) that analytical ultracentrifugation to equilibrium in a CsCl density gradient of large (30–100-kb), native DNA fragments is a powerful tool to investigate the compositional properties of vertebrate genomes; and (2) that preparative density gradient centrifugation of such fragments in the presence of sequence-specific DNA ligands, like Ag^+ or BAMD, bis(acetato-mercurimethyl)dioxane (Corneo et al. 1968; Cortadas et al. 1977), can provide an even finer analysis of their compositional distribution.

1) The analytical approach demonstrated (Macaya et al. 1976; Thiery et al. 1976) that, neglecting satellite and minor components (like ribosomal DNA), DNAs from warm-blooded vertebrates exhibit a strong asymmetry on the heavy side of their CsCl bands, whereas DNAs from cold-blooded vertebrates show only a slight asymmetry. It should be pointed out that DNAs from the invertebrates, unicellular eukaryotes, and prokaryotes tested also exhibit essentially symmetrical CsCl band profiles (Thiery et al. 1976).

2) The preparative approach (Filipski et al. 1973; Macaya et al. 1976; Thiery et al. 1976; Cortadas et al. 1979; Cuny et al. 1981; Olofsson and Bernardi 1983; Bernardi et al. 1985; Salinas et al. 1986; Zerial et al. 1986) showed, in addition, that the compositional features of DNAs from warm-blooded DNAs are due to the presence of GC-rich major DNA components, so designated to distinguish them from satellite and minor components (see above). These GC-

rich components exhibit buoyant densities in the 1.704–1.712-g/cm³ range, represent about one-third of the genome, and accompany GC-poor components, which correspond to the remaining two-thirds of the genome and have buoyant densities in the range 1.697–1.703 g/cm³. This is the range covered by main-band DNA from most cold-blooded vertebrates. It should be understood that the major DNA components just mentioned are families of fragments having similar although not identical base compositions. These fragments derive from much longer (over 200–300-kb) DNA segments, called isochores (Cuny et al. 1981; Bernardi et al. 1985) for identical regions. Isochores are characterized by a remarkably homogeneous base composition (above segment sizes of at least 3 kb).

Another point shown by our previous investigations is that the compositional patterns of vertebrate genomes, namely the compositional distributions of large DNA fragments and of coding sequences, can lead to new insights into genome evolution (see Bernardi et al. 1985, 1988; Bernardi and Bernardi 1985, 1986; Mouchiroud et al. 1987, 1988; Perrin and Bernardi 1987; Bernardi 1989).

The present investigations were carried out with the aim of providing a larger group of examples of the compositional properties of the genomes of cold-blooded vertebrates and of providing a better comparison with the genomes of warm-blooded vertebrates. In fact, early work (Thiery et al. 1976) had dealt only with DNAs from six cold-blooded vertebrates including two fish species. These results were later expanded by investigations concerning DNAs from 32 additional fishes (Hudson et al. 1980; Pizon et al. 1984). We have now further extended the analysis of genomes from cold-blooded vertebrates to a total of 122 fishes, 5 amphibians, and 13 reptiles.

The large number of fish genomes investigated was (1) to obtain a representative sample of different orders; fishes include a vast array of distantly related vertebrates with about 20,000 species, corresponding to about half of the extant vertebrate species (Nelson 1984); and (2) 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 1990).

On the other hand, the expansion of the sample of DNA from amphibians and reptiles was required for deciding whether the different genome organizations recognized by Thiery et al. (1976) differentiated cold-blooded and warm-blooded vertebrates, as claimed by those authors, or anamniotes and amniotes, as suggested by Birstein (1982) and Olmo et al. (1989). In the latter case, the genomes of reptiles should be similar to those of warm-blooded and not to those of cold-blooded vertebrates. This issue is not a trivial one, as the two hypotheses suggest completely different reasons for the differences that exist in the genome organizations of vertebrates. Preliminary accounts of some of the data reported here were presented elsewhere (Pizon 1983; Bernardi et al. 1986).

Materials and Methods

Specimen Collection and Identification. Fish specimens were collected and identified at the locations and by the persons mentioned in Table 1. Identification of some species was made possible through the collaboration of Avi Baranes (Marine Biological Laboratory, Elat, Israel), Mikhail Grachev (Limnology Institute, Listvenitchnoye, Baikal Lake, USSR), John Graves and Ron McConnaughey (Scripps Institute of Oceanography, La Jolla, CA, USA), Fred Nichy (National Marine Fisheries Service, Woods Hole, MA, USA), Randy Rice (University of Alaska, Fairbanks, AL, USA), C. Lavett Smith (American Museum of Natural History, New York, NY, USA), Robert Thompson (Hunter College of the City University, New York, NY, USA), Jean Vaugelas (Marine Science Station, Aqaba, Jordan), Warren Zeiler (Miami Seaquarium, Miami, FL, USA), the Fisheries Department of Nairobi (Kenya), and the Freshwater Fish Institute of Mariental (Namibia).

Table 2 presents a classification of fishes derived from Nelson (1984) in order to indicate the taxonomic 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) and Pleuronectiformes (flounders). The present study includes species from all infradivisions and all superorders (except for Stenopterygii) of the subdivision Teleostei, a group comprising about 18,000 species. The study includes species from 19 out of 35 teleost orders, 1 order each from the subclasses Dipneusti and Brachiopterygii, and 3 orders out of 5 from the subclass Elasmobranchii. Table 3 lists all species studied. This leaves for further studies two subclasses, Holocephali (chimaeras) and Crossopterygii (coelacanths); one infraclass, Chondrostei (sturgeons; sturgeon DNA was studied, however, by Felix et al. 1956); one division, Ginglymodi; and one subdivision, Halecomorphi (see Table 2).

Specimens of amphibians and reptiles were collected and identified by: Drs. Maria Luiza and Willy Beçak, Instituto Butantan, Sao Paulo, Brazil (specimens 15, 16, 18); Dr. Ahmad Disi, Yarmuk University, Amman, Jordan (specimen 17); Dr. Chris Foggins, Veterinary Research Laboratory, Harare, Zimbabwe (specimen 6); Dr. D. Robinson, School of Biology, University of Costa Rica, San José, Costa Rica (specimen 9); Dr. Denise Peccinini-Seale, Universidade de Sao Paulo, Sao Paulo, Brazil (specimen 12); Dr. Rashika El Ridi, Department of Zoology, University of Cairo, Cairo, Egypt (specimens 11, 13). Specimens 1, 2, 3, 7, and 8 were obtained from animal dealers in Paris, France; specimens 4, 10, and 14 were obtained by Dr. Jordi Cortadas in Spain; specimen 5 was obtained from Professor J. C. Lacroix (Université Paris VI, Paris, France). Two orders of amphibians (Anura and Caudata) and three orders of reptiles (Crocodylia, Chelonia, and Squamata) were investigated, leaving one amphibian order (Gymnophiona) and one reptilian order (Rhyncocephalia) for further studies. Table 4 shows the taxonomic position of the species investigated.

DNA Preparation. Fish DNAs were prepared from the liver, with the exception of sample 17 prepared from brain; of samples

Table 1 Geographical origin, collection, and identification of the fish specimens used

Geographical origin		Collection and identification[a]	Specimen number (see Table 3)
Africa			
Central Africa:	East coast		
	Machakos (Kenya)	(a)	83
	Lake Magadi (Kenya)	(a)	82
	Mombasa (Kenya)	(a)	84, 85
	Zanzibar (Tanzania)	(b)	60
	West coast	(b)	13–17, 50, 52–54, 59, 87
	Libreville (Gabon)	(c)	55, 56
	Makokou (Gabon)	(c)	47–49, 51, 57, 58
South Africa:	Mariental (Namibia)	(a)	86
America			
North America:	Alaska	(d)	32, 103
	Death Valley (CA)	(a)	64, 66, 67
	Miami (FL)	(b, a)	68, 78
	Salton Sea (CA)	(a)	65
	Southeast coast	(d)	5–10, 36–39, 41, 42, 44, 72–75, 107, 109, 113, 116–118, 121
	Woods Hole (MA)	(d)	19, 20, 40, 43, 45, 63, 76, 77
	Hawaii	(b)	112
Central America:	Mexico	(b)	27, 69, 70, 88
	Pacific Coast	(d)	4, 21, 81, 92, 110, 111, 115
	Panama (freshwater)	(b)	28
	(east coast)	(d)	3, 91
	(Perlas Islands)	(d)	122
South America:	Brasil	(e)	89
	French Guyana	(c)	61, 62
Antarctica:	McMurdo Sound	(d)	99–106
Asia			
Near East:	Elat (Israel)	(a)	2, 80, 98
	Red Sea	(b)	71, 79, 108, 114, 120
	Sharem-el-Sheik (Egypt)	(a)	119
Central:	Lake Baikal (USSR)	(a)	35
Far East:	Kamchatka (USSR)	(f)	33, 34
South East:	India	(b)	24
	Indonesia	(b)	25, 26
	Sri Lanka	(b)	46
Europe:	Naples (Italy)	(g)	1, 11, 12, 90, 93–97
	Paris (France)	(b)	18, 22, 23, 30, 31
	Commercial prep.		29

[a] (a) Giacomo Bernardi and/or Giorgio Bernardi; (b) Fish dealers; (c) André Brosset (Muséum d'Histoire Naturelle, Brunoy, France); (d) Audrey Haschemeyer (Hunter College of the City University, New York, NY, USA); (e) Lurdes Foresti de Almeida Toledo (Sao Paulo University, Sao Paulo, Brazil); (f) Vadim Kavsan and Alla Rynditch (Institute of Molecular Biology and Genetics, Ukrainian Academy of Sciences, Kiev, USSR); (g) Flegra Bentivegna (Stazione Zoologica, Naples, Italy)

32, 36, 40, 63, and 77 prepared from testes; and of samples 26–28, 46–62, and 64–70 prepared from the whole body. All preparations were obtained using the method of Kay et al. (1952). They were further purified by treatment with RNase followed by dialysis and alcohol precipitation, or by chromatography on hydroxyapatite in the presence of 3 M KCl (Bernardi 1971), or by preparative CsCl centrifugation.

DNAs from amphibians and reptiles were prepared (on the location, up to the sodium dodecyl sulfate treatment) from liver (samples 2, 3, 4, 8, 10–12), blood (samples 1, 6, 7, 9, 13–18), or testes (sample 5).

Ultracentrifugation and Other Methods. Sedimentation velocities of DNAs were determined as described by Prunell and Bernardi (1973), and molecular weights were calculated from the sedimentation coefficient values, s20,w, using the relationship of Eigner and Doty (1965)

$$s20,w = 0.034M^{0.405} \quad (1)$$

Preparative and analytical density gradient centrifugations were performed as already described (Thiery et al. 1976; Cortadas et al. 1979). In order to calculate the buoyant density, ρ, at any point of abscissa r from the rotation axis, the relationship

$$\rho = \rho_{r_\kappa} - \frac{\omega^2}{2\beta_0}(r^2_\kappa - r^2) \quad (2)$$

was used, where the subscript κ refers to the density marker, ω is the angular velocity in radians$\cdot s^{-1}$, and β was taken as equal to 1.19×10^9 cm$^5\cdot$g$^{-1}\cdot$s^{-2} (Ifft et al. 1961). Under such conditions, using phage 2*c* DNA ($\rho = 1.742$ g/cm^3; Szybalski 1968) as

Table 2. Classification of fishes (Nelson 1984)

Class	Subclass, infraclass, division, subdivision[a]	Infradivision	Superorder	Order[b]
Chondrichthyes	Elasmobranchi		Selachimorpha	Lamniformes
				Squaliformes
			Batidoidimorpha	Rajiformes
	*Holocephali			
Osteichthyes	Dipneusti		Ceratodontimorpha	Lepidosireniformes
	*Crossopterygii			
	Brachiopterygii			Polypteriformes
	Actinopterygii			
	*Chondrostei			
	Neopterygii			
	*Ginglymodi			
	Halecostomi			
	*Halecomorphi			
	Teleostei	Osteoglossomorpha		Osteoglossiformes
		Elopomorpha		Anguilliformes
		Clupeomorpha		Clupeiformes
		Euteleostei	Ostariophysi	Cypriniformes
				Characiformes
				Siluriformes
			Protacanthopterygii	Salmoniformes
			*Stenopterygii	
			Scopelomorpha	Aulopiformes
			Paracanthopterygii	Gadiformes
				Ophidiiformes
				Batrachoidiformes
				Lophiiformes
			Acanthopterygii	Cyprinodontiformes
				Syngnathiformes
				Dactylopteriformes
				Scorpaeniformes
				Perciformes
				Pleuronectiformes
				Tetraodontiformes

[a] Asterisks indicate groups not investigated
[b] Only orders studied in the present work are listed

a density marker, a reproducible modal buoyant density, ρ_0 (density at the peak maximum, located at a distance r_0 from the rotation axis), of 1.7103 g/cm^3 was obtained for *Escherichia coli* DNA. The mean buoyant density, $\langle\rho\rangle$, of the main band (satellites were disregarded when present) was calculated from the first moment $\langle r\rangle$ of the band profile about the center of rotation

$$\langle\zeta\rangle = \frac{\int c_\zeta \, d\zeta}{d\zeta} \qquad (3)$$

and from Eq. (2), c_ζ being the DNA concentration at point of abscissa r. The variance of the profile, $\langle\delta^2\rangle$, is equal to the second moment about the mean

$$\langle\delta^2\rangle = \frac{\int c\delta \, d\delta}{\text{[illegible]}} \qquad (4)$$

with $\delta = \langle r\rangle - r_0$.

Integrals of Eqs. (3) and (4) were calculated using Simpson's rule, with radial steps corresponding to 0.04 or 0.08 mm in cell dimensions.

The asymmetry, A, of CsCl main bands was determined as $A = \langle\rho\rangle - \rho_0$. The intermolecular compositional heterogeneity, H, of CsCl main bands was calculated according to Schmid and Hearst (1972)

$$H = 100\left[\left(\frac{G r_0^2 \omega^4 \langle\delta^2\rangle}{RT\rho_0} - \frac{1}{M_3}\right)\left(\frac{RT\rho_0}{\beta_0^2 a^2 G}\right)\right]^{1/2} \qquad (5)$$

where G is the buoyancy factor, M_3 is the molecular weight of the dry Cs salt of DNA, R is the universal gas constant, T is the absolute temperature, and a is the slope, 0.098, of the relationship of Schildkraut et al. (1962).

$$\rho = 1.66 + a\ \%GC \qquad (6)$$

The molar ratios of deoxyguanosine plus deoxycytidine, GC, of the various DNAs analyzed were estimated from $\langle\rho\rangle$ values by using Eq. (6) (that was established for bacterial DNAs), and, in some cases, by direct nucleotide analysis (Hudson et al. 1980).

Results

The Buoyant Densities of DNAs from Fishes

The modal, ρ_0, and mean, $\langle\rho\rangle$, buoyant densities, the sedimentation coefficients, s20,w, and the GC levels of the DNAs investigated are reported in Ta-

Table 3. Properties of fish DNAs. Continued on pages 270–271

Order	Family	Sample number[a]	Species	s20,w s	ρ_0 g/cm³	$\langle\rho\rangle$ g/cm³	A mg/cm³	H %GC	%GC	c
Lamniformes	Scyliorhinidae	1	*Scyliorhinus stellaris*	30.6	1.7053					6.2 (a)
	Carcharinidae	2*	*Mustelus mosis*	32.6	1.7044	1.7047	0.3	[illegible] 8	45.6	
		3*	*Scoliodon terranovae*	22.6	1.7030	1.7035	0.5	[illegible] 7	44.4	3.6 (b)
		4*	*Carcharinus galapagensis*	23.4	1.7033	1.7037	0.4	[illegible] 1	44.7	
	Sphyrnidae	(5)*	*Sphyrna lewini*	10.5	1.7030	1.7042	1.2	3.4	45.1	3.5 (c)
Squaliformes	Squalidae	6†	*Squalus acanthias*	37.4	1.7050	1.7059	0.9	4.3	46.8	7.2 (d)
	Squatinidae	7†	*Squatina dumerili*	30.6	1.7050	1.7072	2.2	4.9	48.2	
Rajiformes	Rajidae	8†	*Raja erinacea*	32.1	1.7035	1.7041	0.6	3.6	45.0	3.5 (c)
	Dasyatidae	9†	*Gymnura altavela*	36.1	1.7015	1.7022	0.7	2.6	43.1	
	Myliobatidae	10†	*Myliobatis freminvillei*	32.1	1.7029	1.7039	1.0	4.3	44.8	4.9 (c)
	Torpedinidae	11	*Torpedo marmorata*	36.4	1.7011					7.0 (a)
		12	*Torpedo ocellata*	37.7	1.7012					7.5 (a)
Lepidosireniformes	Protopteridae	13	*Protopterus* sp.	39.4	1.7005	1.7001	−0.4	2.4	40.9	50 (e)
Polypteriformes	Polypteridae	14	*Polypterus senegalus*	30.3	.6995	1.7008	1.3	2.2	41.6	
Acipenseriformes	Acipenseridae		*Acipenser sturio*			(1.7019)			42.8	1.6
Osteoglossiformes	Pantodontidae	15	*Pantodon buchholzi*	15.8	1.7022	1.7023	0.1	1.1	43.2	0.77 (f)
	Notopteridae	16	*Notopterus notopterus*	29.9	1.7015	1.7021	0.6	3.5	42.9	
	Mormyridae	17*	*Gnathonemus petersii*	28.4	1.7029	1.7033	0.4	2.4	44.2	1.2 (f)
Anguilliformes	Anguillidae	18	*Anguilla anguilla*	22.4	1.7018	1.7031	1.3	2.1	44.0	0.9 (e)
		19*	*Anguilla rostrata*	32.8	1.7011	1.7015	0.7	3.4	42.6	1.4 (f)
Clupeiformes	Clupeidae	20*	*Brevoortia tyrannus*	26.4	1.7021	1.7024	0.3	2.3	43.4	(0.77–1.4)
		21	*Sardinella anchovia*	26.2	1.7022	1.7022	0.0	3.2	43.1	
Cypriniformes	Cyprinidae	22*	*Carassius auratus*	28.3	1.6970	1.6971	0.1	1.6	37.9	1.7 (g)
		23	*Cyprinus carpio*	43.9	1.6963	1.6965	0.2	1.9	37.2	1.7 (f)
		24	*Brachydanio rerio*	37.7	1.6959	1.6962	0.3	2.5	36.9	1.8 (f)
		25	*Labeo bicolor*	25.4	1.6959	1.6965	0.7	1.8	37.2	1.3 (f)
	Cobitididae	26	*Acanthophthalmus semicinctus*		1.6975	1.6978	0.3		38.6	
Characiformes	Characidae	27	*Astyanax mexicanus*		1.6995	1.6998	0.3		40.6	(1.1–2.1)
Siluriformes	Callichthyidae	28	*Corydoras aeneus*		1.6979	1.6980	0.1		38.8	4.4 (f)
Salmoniformes	Salmonidae	29*	*Salmo salar*	20.5	1.7028	1.7035	0.7		44.4	
		30	*Salmo fario*	36.6	1.7030	1.7039	0.9	1.8	44.8	
		31	*Salmo gairdneri*	30.0	1.7024	1.7026	0.2	2.9	43.5	3.2 (g)
		32*	*Oncorhynchus kisutch*	31.3	1.7033	1.7036	0.3	2.9	44.5	3.0 (f)
		33	*Oncorhynchus keta*	31.0	1.7038				44.7	
		34	*Oncorhynchus nerka*	29.8	1.7035				44.4	
		35	*Coregonus autunnalis migr.*	13.6	1.7034	1.7035	0.1	2.3	44.4	
Aulopiformes	Synodontidae	36†	*Synodus foetens*	31.7	1.7025	1.7029	0.4	2.4	43.8	1.2 (f)
		(37)†	*Synodus intermedius*	9.9	1.7021	1.7027	0.6	4.5	43.6	
		38†	*Trachinocephalus myops*	31.2	1.7034	1.7045	0.9	3.4	45.4	
Gadiformes	Gadidae	39†	*Urophycis regius*	27.3	1.7065	1.7068	0.3	3.2	47.8	0.90
		40	*Urophycis chuss*	16.1	1.7070	1.7063	−0.7	3.9	47.2	
	Merlucciidae	41†	*Merluccius bilinearis*	38.6	1.7041	1.7042	0.1	2.4	45.1	0.93 (f)
Ophidiiformes	Ophidiidae	42†	*Ophidion holbrooki*	16.5	1.7018	1.7027	0.9	3.9	43.6	(0.68–0.84)
Batrachoidiformes	Batrachoidae	43*	*Opsanus tau*	28.0	1.6996	1.7001	0.5	2.3	40.9	2.8 (f)
		44†	*Parichthys porosissimus*	35.4	1.6998	1.7000	0.2	2.2	40.8	1.7 (f)

Table 3. Continued

Order	Family	Sample number[a]	Species	s20,w s	ρ_0 g/cm³	$\langle\rho\rangle$ g/cm³	A mg/cm³	H %GC	%GC	c
Lophiiformes	Lophiidae	45*	*Lophius americanus*	22.0	1.6996	1.6998	0.2	3.3	40.6	1.0 (f)
Cyprinodontiformes	Aplocheilidae	46	*Aplocheilus dayi*		1.7002	1.7008	0.6		41.6	
		47	*Aphyosemion elegans*	22.1	1.7008	1.7007	−0.1	1.4	41.5	
		48	*Aphyosemion cameronense*	23.5	1.7009	1.7012	0.3	2.5	42.0	
		49	*Aphyosemion punctatum*	16.5	1.7009	1.7011	0.2	0.7	41.9	0.6 (h)
		50	*Aphyosemion sheeli*	24.8	1.7011	1.7014	0.3	1.0	42.2	
		51	*Aphyosemion hertzogi*	33.5	1.7013	1.7014	0.1	1.9	42.2	
		52	*Aphyosemion amieti*	22.2	1.7016	1.7020	0.4	1.5	42.9	
		53	*Aphyosemion marmoratum*	28.7	1.7018	1.7021	0.3	3.6	43.0	
		54	*Aphyosemion spoorenbergi*	20.2	1.7032	1.7032	0.0	1.5	44.1	
		55	*Aphyosemion striatum*	20.1	1.7053	1.7058	0.5	0.5	46.7	0.73 (h)
		56	*Aphyosemion australe*	42.2	1.7066	1.7071	0.5	2.0	48.1	0.65 (h)
		57	*Diapteron cyanosticum*	24.6	1.7012	1.7016	0.4	1.3	42.4	
		58	*D. cyanosticum* + *Diapteron georgiae*	24.5	1.7009	1.7016	0.7	2.0	42.4	
		(59)	*Epiplatys chaperi*	10.3	1.7010				41.9	
		60	*Pachypanchax playfairii*		1.7046	1.7063	1.7		47.2	
		61	*Rivulus holmiae*	25.1	1.6984	1.6987	0.3	1.6	39.5	
		62	*Rivulus agilae*	22.5	1.7007	1.7015	0.8	0.7	42.3	
	Cyprinodontidae	63*	*Fundulus heteroclitus*	25.4	1.6993	1.6996	0.3	1.5	40.4	1.5 (f)
		64	*Cyprinodon salinus*	19.9	1.6984	1.7000	1.6	4.2	40.8	
		65	*Cyprinodon macularius calif.*	26.3	1.6989	1.6996	0.7	2.7	40.4	
		66	*Cyprinodon n. nevadensis*	18.1	1.6983	1.6989	0.6	1.0	39.7	
		67	*Cyprinodon variegatus*	17.6	1.6987	1.6998	1.1	2.8	40.6	1.6 (f)
		68	*Jordanella floridae*		1.6987	1.6987	0.0		39.5	
	Poecilidae	69	*Poecilia sphenops mel.*	18.4	1.6987	1.6994	0.7	3.6	40.2	0.85 (i)
		70	*Xyphophorus maculatus*	15.5	1.6985	1.6987	0.2	2.4	39.5	0.95 (f)
Syngnathiformes	Syngnathidae	71	*Hippocampus* sp.	28.9	1.7031	1.7038	0.7	2.9	44.7	0.66 (f)
Dactylopteriformes	Dactylopteridae	72†	*Dactylopterus volitans*	19.6	1.7033	1.7040	0.7	3.7	44.9	
Scorpaeniformes	Scorpaenidae	73	*Scorpaena calarata*	30.5	1.6995	1.6999	0.4	3.4	40.7	
		74	*Scorpaena brasiliensis*	32.1	1.6988	1.6990	0.2	4.1	39.8	1.4 (f)
	Triglidae	75	*Prionotus carolinus*	13.0	1.7011	1.7021	1.0	2.7	42.9	0.99 (f)
	Cottidae	76*	*Hemitripterus americanus*	14.9	1.7010	1.7013	0.3	2.3	42.1	0.94 (f)
Perciformes	Serranidae	77*	*Centropristis striatus*	18.8	1.6981	1.6985	0.4	2.7	39.3	1.2 (f)
		78*	*Epinephelus striatus*	25.2	1.7004	1.7006	0.2	1.2	41.4	1.3 (f)
		79	*Epinephelus guttatus*	19.8	1.7015	1.7013	−0.2	1.2	42.1	1.2 (f)
	Lutjanidae	80*	*Lutjanus synagris*	12.4	1.7010	1.7022	1.2	3.4	43.1	(0.95–1.4)
	Lethrinidae	81*	*Lethrinus nebulosus*	20.9	1.6999	1.7001	0.2	1.2	40.9	
	Cichlidae	82	*Oreochromis a. grahami*	18.6	1.7007	1.7012	0.5	3.4	42.0	
		83	*Oreochromis aureus*	19.0	1.7003	1.7008	0.5	1.9	41.6	1.21 (i)
		84	*Oreochromis spilurus*	16.2	1.7003	1.7006	0.3	1.6	41.4	0.95 (j)

Table 3. Continued

Order	Family	Sample number[a]	Species	s20,w s	ρ_0 g/cm³	$\langle\rho\rangle$ g/cm³	A mg/cm³	H %GC	%GC	c
		85	*Oreochromis niloticus*				0.7	1.5	41.7	0.95 (i)
		86	*Oreochromis mossambicus*	19.5	1.7009	1.7010	0.1	2.7	41.8	1.0 (j)
		87	*Tilapia butticoferi*	29.0	1.7005	1.7009	0.4	2.4	40.8	
		88	*Cichlasoma meeki*		1.7007	1.6994	−1.3		40.2	1.4 (f)
		89	*Symphysodon discus*	28.3	1.7004	1.7005	0.1	2.4	41.3	1.5 (n)
	Pomacentridae	90	*Chromis chromis*	24.9	1.7005	1.6993	−1.2	4.3	40.1	1.21 (k)
	Sphyraenidae	91*	*Sphyraena barracuda*	18.6	1.7015	1.7017	0.2	1.3	42.6	(0.83–1.2)
		92*	*Sphyraena ensis*	18.7	1.7024	1.7027	0.3	2.4	43.6	
	Labridae	93	*Xyrichthys novacula*	30.0	1.6998	1.7001	0.3	1.9	40.9	
		94	*Coris julis*	27.5	1.7006	1.7015	0.9	2.8	42.3	1.21 (l)
		95	*Symphodus mediterraneus*	25.0	1.7006	1.7015	0.9	3.0	42.3	0.57 (m)
		96	*Symphodus cinereus*	28.5	1.7002	1.7016	1.4	3.1	42.4	1.25 (l)
		96	*Symphodus ocellatus*	26.2	1.7004	1.7012	0.8	3.1	42.0	1.10 (l)
	Scaridae	98*	*Scarus gibbus*	21.8	1.6984	1.6988	0.4	1.2	39.6	(1.9–2.1)
	Notothenidae	99*	*Trematomus bernachii*	14.5	1.7011	1.7020	0.9	2.5	42.9	1.95 (h)
		100*	*Trematomus borchgrevinki*	23.6	1.7002	1.7010	0.8	2.3	41.8	
		(101)*	*Trematomus centronotus*	11.0	1.7009	1.7017	0.8	2.9	42.5	1.98 (h)
		102*	*Trematomus nicolai*	15.0	1.7010	1.7017	0.7	3.2	42.5	
		(103)*	*Trematomus newnesi*	10.4	1.7005	1.7018	1.3	3.2	42.6	2.13 (h)
		104*	*Trematomus hansoni*	22.2	1.6999	1.7002	0.3	2.1	41.1	1.85 (h)
		105*	*Dissosticus mawsoni*	19.6	1.7003	1.7007	0.4	2.2	41.5	
	Bathydraconidae	106*	*Gymnodraco acuticeps*	24.6	1.7007	1.7012	0.5	3.4	42.1	1.95 (h)
	Blenniidae	107*	*Ophioblennius atlanticus*	17.4	1.7007	.7011	0.4	2.4	41.9	(0.81–1.0)
	Callionymidae	108	*Synchiropus splendidus*	20.6	1.7073	1.7076	0.3	2.4	48.6	
Pleuronectiformes	Bothidae	(109)	*Siacium papillosum*	12.4	1.7024	1.7025	0.1	4.0	43.4	(0.74–1.0)
	Pleuronectidae	110*	*Limanda aspera*	16.7	1.6995	1.7002	0.7	3.2	41.0	(0.65–0.97)
		111	*Limanda ferruginosa*	20.0	1.7012	1.7021	0.9	4.5	42.9	
Tetraodontiformes	Balistidae									
	Balistinae	112	*Melichthys vidua*	21.8	1.7014	1.7022	0.8	2.3	43.1	
		113†	*Balistes capriscus*	24.5	1.7023	1.7024	0.1	2.2	43.3	0.72 (f)
		114	*Rhinecanthus aculeatus*	18.2	1.7047	1.7048	0.1	0.7	45.7	
	Monacanthinae	115†	*Stephanolepis hispidus*	13.9	.7037	1.7043	0.6	3.9	45.2	0.68 (f)
		(116)†	*Aluterus schoepfi*	10.2	1.7038	1.7039	0.1	4.3	44.8	0.64 (f)
	Ostraciidae	117†	*Acanthostracion quadricornis*	38.2	1.7001	1.7008	0.7	2.6	41.6	
	Tetraodontidae	118	*Lagocephalus laevigatus*	19.2	1.7022	1.7026	0.4	3.3	43.5	(0.39–0.50)
		119*	*Arothron diadematus*	19.8	1.7025	1.7036	1.1	2.7	44.5	
		120	*Arothron meleagris*	29.4	1.7034	1.7058	1.4	4.7	46.7	
		121†	*Sphoeroides annulatus*	25.3	1.7047	1.7053	0.6	3.5	46.2	
	Diodontidae	122*	*Diodon holocanthus*	16.3	1.7009	1.7012	0.3	1.4	42.0	0.90

[a] Asterisks indicate values from Hudson et al. (1980); crosses indicate values from Pizon (1983); all other values were obtained in the present work. Figures in parentheses concern DNAs exhibiting low molecular weights. These were not taken into account in Figs. 2–4. *Acipenser sturio* data are from Felix et al. (1956)

[b] *c* values are from (a) Morescalchi and Olmo (1982); (b) Schwartz and Maddock (1986); (c) Hinegardner (1976); (d) Stingo et al. (1980); (e) Sober (1970); (f) Hinegardner and Rosen (1972); (g) Pedersen (1971); (h) Stingo, unpublished; (i) Rasch et al. (1965); (j) Majumdar and McAndrew (1986); (k) Alvarez et al. (1980); (l) Stingo (1986); (m) Cano et al. (1982); (n) Ohno and Atkins (1966). *c* values in parentheses refer to other species belonging in the same family; they are from Hinegardner and Rosen (1972)

Table 4. Properties of DNAs from amphibians and reptiles

Class / Order / Suborder	Family		Species	s20,w s	ρ_0 g/cm³	$\langle\rho\rangle$ g/cm³	A mg/cm³	H %GC	%GC
Amphibians									
Anura	Pipidae	1	*Xenopus laevis*[a]	35.0	1.6991	1.6997	0.6	2.9	40.5
	Leptodactylidae	2	*Leptodactylus pentadactylus*		1.7013	1.7018	0.5		42.6
	Bufonidae	3	*Bufo paracnemis*		1.7028	1.7036	0.8		44.5
	Ranidae	4	*Rana* sp.	39.7	1.7029	1.7033	0.4	2.9	44.2
Caudata	Salamandridae	5	*Pleurodeles waltlii*[a]	27.1	1.7041	1.7047	0.6	3.0	45.6
Reptiles									
Crocodylia	Crocodylidae	6	*Crocodylus niloticus*	37.7	.7046	1.7051	0.5	3.2	46.0
Chelonia									
Cryptodira	Emydidae	7	*Pseudemys nelsoni*	34.4	1.7022	1.7023	0.1	1.3	43.2
	Testudinidae	8	*Testudo graeca*[a]	25.1	1.7027	1.7042	1.5	4.5	45.1
Squamata									
Sauria	Iguanidae	9	*Iguana iguana*[a]	20.5	1.7015	1.7022	0.7	2.7	43.1
	Gekkonidae	10	*Tarentola mauretanica*	23.9	1.7047	1.7057	1.0	2.7	46.6
	Scincidae	11	*Chalcides ocellatus*		1.7039	1.7050	1.1		45.9
	Teiidae	12	*Ameiva ameiva*		1.7014	1.7018	0.4		42.6
	Varanidae	13	*Varanus griseus*		1.7024	1.7029	0.5		43.8
Ophidia	Colubridae	14	*Natrix maura*	23.1	1.7002	1.7018	1.6	3.4	42.6
		15	*Tomodon dorsatus*		1.7014	1.7015	0.1		42.3
		16	*Dromicus porcilogyrus s.*		1.7006	1.7007	0.1		41.5
	Viperidae	17	*Cerastes cerastes*		1.6995	1.7007	1.2		41.5
	Crotalidae	18	*Bothrops neuwiedi p.*		1.6992	1.7029			43.8
					(1.7028)[b]				

[a] Data from Thiery et al. (1976). H values from the present work
[b] Satellite band

ble 3, which also lists haploid genome sizes (*c* values), whenever available.

Figure 1 displays the analytical CsCl profiles of the fish DNAs studied. Only three DNA samples (4, 11, 12; all of them from Chondrichthyes) showed sizable amounts of resolved satellite bands, which were on the right, heavy, GC-rich side of the main band. Minor satellite bands were found in other DNA samples, mainly on the heavy side (samples 83–86, 100, 105, 107, 122), and more rarely on the light side (samples 75, 76, 105, 116). Finally, some other samples showed poorly resolved satellite on the heavy side (samples 6, 7, 33, 46, 56, 89, 102).

Figure 2a displays the distribution of the modal buoyant densities of the fish DNAs studied. The modal buoyant density range covered was 1.696–1.707 g/cm³. In the case of Osteichthyes, the average ρ_0 value, $\bar{\rho}_0$, was 1.7014 g/cm³ and the corresponding standard deviation, σ, was 2.2 mg/cm³. Most values were between 1.699 and 1.704 g/cm³. In the case of Chondrichthyes, $\bar{\rho}_0$ was 1.7035 g/cm³, and 1.1 mg/cm³. Most values were in the 1.702–1.704 g/cm³ range.

Because the histogram in Fig. 2a could be biased by the overrepresentation of species from the same genera or families of fishes, another histogram was constructed in which only one DNA sample per family or genus was taken into account when the other species within the family or the genus, respectively, had the same buoyant density. This histogram (Fig. 2b), separately showing Osteichthyes and Chondrichthyes, was not, however, strikingly different from that of Fig. 2a.

The Asymmetry of CsCl Bands of Fish DNAs

CsCl band asymmetries, A, of fish DNAs were determined for 116 species (see Table 3). The distributions of DNA asymmetries for the fish species investigated are presented in the histogram of Fig. 3a. The majority of fishes showed values around 0.5 mg/cm³ ($\bar{A}$ = 0.65 mg/cm³; σ = 0.54 mg/cm³ in the case of Osteichthyes).

Three remarks are appropriate at this point.

1) Only four out of 122 DNAs, those from *Protopterus* sp., *Urophycis chuss, Cichlasoma meeki,* and *Chromis chromis* (samples 13, 40, 88, 90), exhibited a significant skewness on the GC-poor (left) side of the CsCl band (see Fig. 1 and Table 3). In two cases (samples 40 and 88) the CsCl peaks of DNAs of other species from the same genus or the same family, respectively, showed a positive asymmetry (skewness on the right side). Moreover, a separate analysis of the right and left sides of the CsCl bands of the four DNAs revealed that the left sides also exhibited some skewness (not shown). The ex-

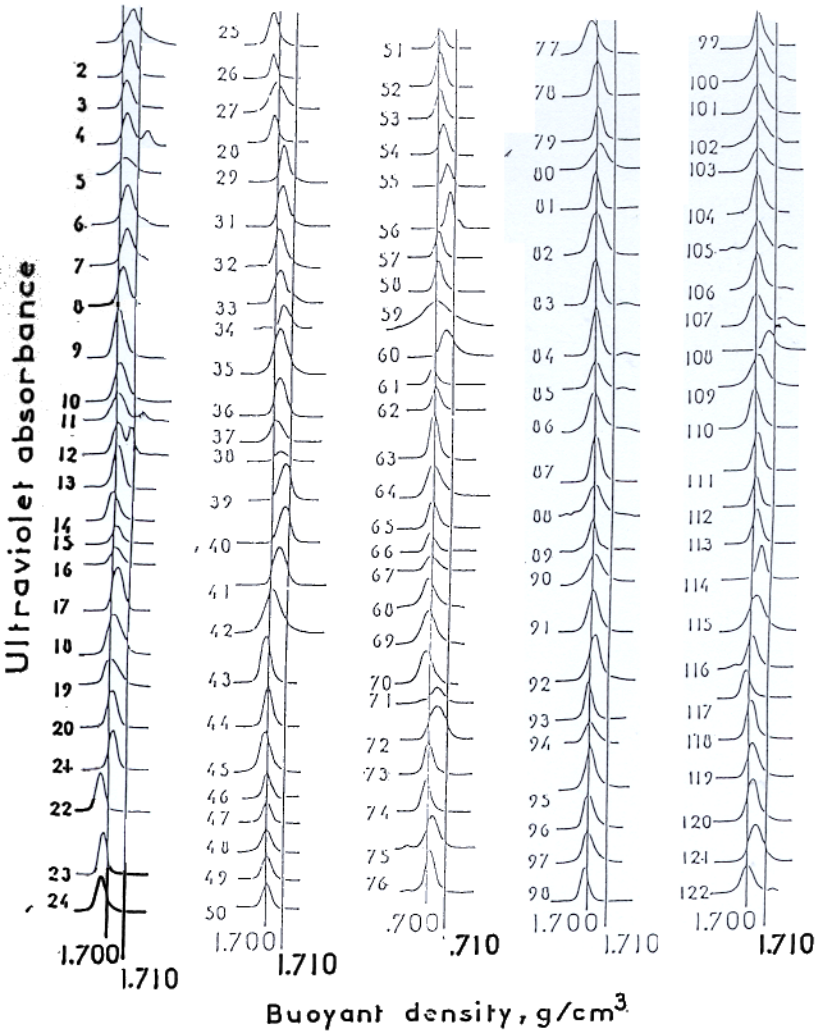

Fig. 1. CsCl band profiles of fish DNAs. See Table 3 for the numbering of samples.

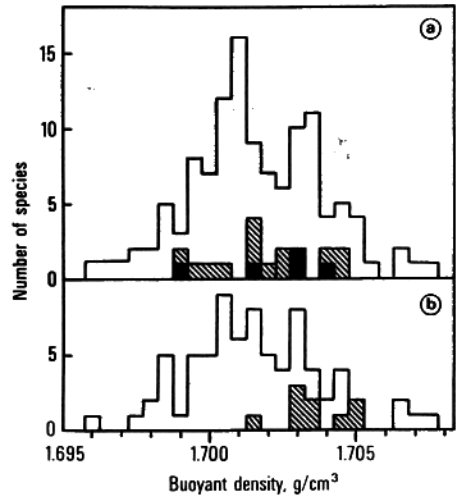

Fig. 2. **a** Plots of modal buoyant densities, ρ_0, of DNAs from fishes (open histogram), amphibians (solid histogram), and reptiles (cross-hatched histogram) against the number of corresponding species. **b** Plots of modal buoyant densities of DNAs from Osteichthyes (open histogram) and Chondrichthyes (cross-hatched histogram) against the number of species; in this case each genus or family was only represented by species characterized by different modal buoyant densities. A window of 0.5 mg/cm³ was used in all histograms.

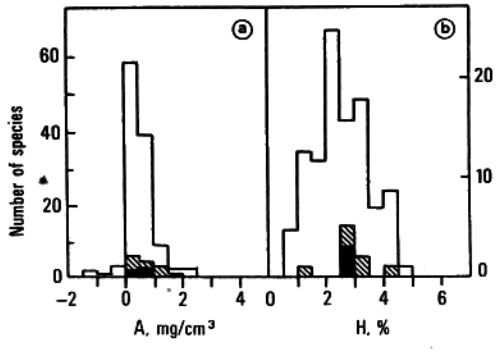

Fig. 3. CsCl band asymmetry **a** and intermolecular compositional heterogeneity **b** of DNAs from Osteichthyes, amphibians, and reptiles (see legend of Fig. 2a for symbols)

ceptional situation of the four DNAs is, therefore, likely to be due to cryptic light satellites rather than to a compositional heterogeneity of main-band DNA.

2) As far as the few high asymmetry values are concerned, it should be pointed out that asymmetries can only be overestimated because of the presence of GC-rich cryptic satellites. Such is the case

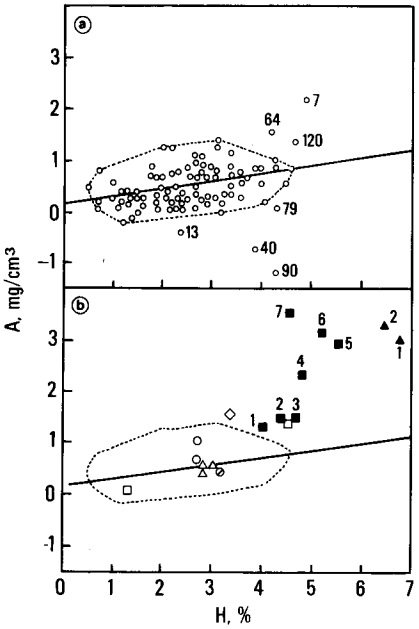

Fig. 4. Plot of CsCl band asymmetry, A, against compositional heterogeneity of DNAs **a** from fishes (numbers refer to Table 3; the straight line is the slope through the points within the delimited area; using all the points would not significantly change the slope); and **b** from amphibians (open triangles), reptiles (crocodile: barred circle; turtles: open squares; lizards: open circles; snake: open lozenge), mammals (closed squares; 1, mouse; 2, rat; 3, hamster; 4, human; 5, cat; 6, hedgehog; 7, dog), and birds (closed triangles; 1, sea gull; 2, chicken).

of *Squatina dumerili* (sample 7), for example. It has been observed, indeed, that most detectable satellites (comprising satellites appearing as separate peaks or as shoulders) are on the GC-rich side of main bands. This makes the presence of cryptic satellites on this side more likely than on the other side. In addition, experiments in which DNAs were centrifuged in the presence of DNA ligands revealed a number of otherwise cryptic heavy satellites (see Fig. 6 and comments on it below).

3) In the case of the two species living at high temperature, *Cyprinodon salinus* and *Oreochromis grahami* (samples 64 and 82), the asymmetry on the GC-rich side appeared, however, not to be due to the presence of cryptic satellite components in main-band DNA (see Fig. 6, below).

The Intermolecular Compositional Heterogeneity of Fish DNAs

Intermolecular compositional heterogeneity values, H, of CsCl bands were determined for 108 fishes (see Table 3). Intermolecular compositional heterogeneities provide a measure of the GC heterogeneity of the DNA fragments under study. Basically, this parameter reflects the spread of GC levels among different fragments in a given DNA preparation (see also Materials and Methods). Figure 3b displays the distribution of H values for different fish species. Values ranged from 0.5% GC for *Aphyosemion striatum* (sample 55) to 4.7% GC for *Arothron meleagris* (sample 120; the higher value of 4.9% for *S. dumerili,* sample 6, was due to a shoulder on the heavy side of the main band, indicative of a satellite; see above). 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 6). Artifactual reasons, like an overestimation of the sedimentation coefficient, may also account for some high values of H; this might be the case for samples 10, 53, 59, 73, 74, and 120. Very low molecular weight samples, not taken into account in Figs. 2–4, also showed high H values (see samples 5, 37, 101, 103, 109, and 116); short-range compositional heterogeneity was, in all likelihood, responsible for this phenomenon. Two DNA samples showing high H values, *C. salinus* (sample 64) and *O. grahami* (sample 82), were studied further; in these cases, the high values proved not to be due to satellites, as shown by the analysis of Fig. 6.

Correlations between CsCl Band Asymmetry and Intermolecular Compositional Heterogeneity

A plot of CsCl band asymmetry versus intermolecular compositional heterogeneity as shown in Fig. 4a for fish DNAs. A contour line separates extreme values, which are biased by the presence of poorly resolved satellite bands, or which show an intrinsically high heterogeneity, not due to satellites (this is the case in *C. salinus,* sample 64). A linear relationship with a correlation coefficient $R = 0.37$ was found using the least-squares approach and the points within the contour line. The slope of the line was significantly different from zero ($P < 0.001$). If outside points were also used, the slope was not significantly changed.

The Correlation between GC Contents of Fish DNAs and the Haploid Genome Size of the Corresponding Species

Figure 5 shows that GC contents of fish DNAs are negatively correlated with haploid genome size, *c,* of the corresponding species; on the average, an increase of 1 pg corresponded to a decrease of about 5% GC. Strong deviations from the correlation were exhibited by all Chondrichthyes, by *Corydoras aeneus* (sample 28), by salmonids (samples 31, 32) and by *Opsanus tau* (sample 43); these species showed *c* values in the 3–7-pg range. Lesser deviations were shown by notothenids (samples 99, 101, 103, 104, and 106) with *c* values close to 2 pg. Three samples (39, 55, and 56) showed a strong deviation along the vertical axis.

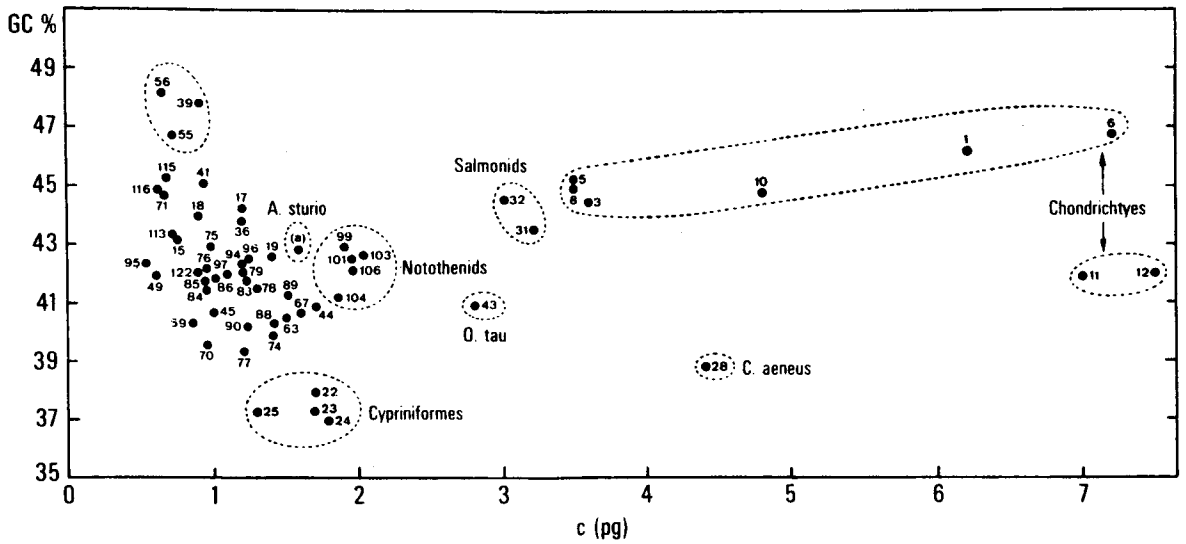

Fig. 5. GC levels of fish DNAs are plotted against the corresponding haploid genome sizes. Data from Table 3 were used. Points surrounded by a broken line are discussed in the text. The high *c* value (50 pg) of *Protopterus* sp. was not included in the figure.

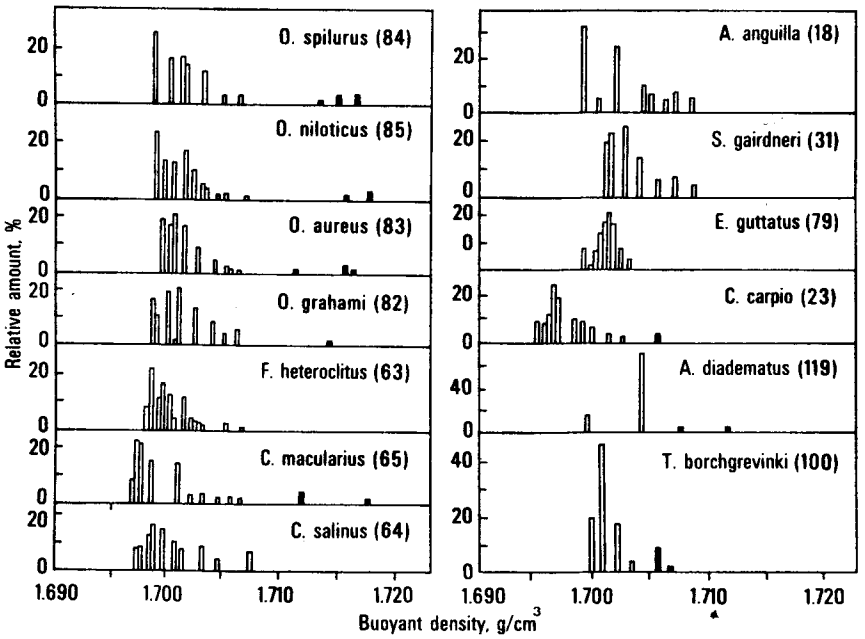

Fig. 6. Histograms showing the relative amounts and the modal buoyant densities in CsCl of DNA fractions obtained by preparative Cs_2SO_4/BAMD density gradient centrifugation from several fish species. Black bars correspond to satellite components.

Fractionation of Fish DNAs in Cs_2SO_4/BAMD Density Gradients

Fractionation in Cs_2SO_4/BAMB was used to analyze in more detail a number of fish genomes. The analyses of DNA fractions, presented as histograms of relative DNA amounts versus modal buoyant densities in CsCl in Fig. 6, provided information on cryptic, or poorly detectable, satellites, which all showed up as GC-rich satellites. When not resolved from the main band, cryptic satellites were sometimes detected by restriction enzyme digestions. For example, DNA from *Salmo gairdneri* (sample 31) showed a typical ladder pattern in GC-rich fractions when digested with restriction endonuclease HpaII (B. Aissani, G. Bernardi, and G. Bernardi, unpublished). Several of these analyses were originally done for different purposes.

1) Some analyses were performed in order to show in more detail differences between the genome of fishes living at high temperatures (40°C), like *O. grahami* (sample 82) or *C. salinus* (sample 64), and their congeners living at lower temperatures.

2) Some analyses were used to confirm the correlation proposed by Cuny et al. (1981) between chromosomal banding patterns and DNA compositional heterogeneity (Medrano et al. 1988). *Anguilla anguilla* and *Epinephelus guttatus* (samples 18 and 79) exhibit different degrees of asymmetry (1.3 and 0.2 mg/cm³, respectively) and compositional heterogeneity (2.1 and 1.2% GC, respective-

ly). These species were shown to be characterized by differences in Giemsa and reverse banding of their metaphase chromosomes; indeed, practically no banding pattern was found in *E. guttatus,* whereas rather distinct patterns could be detected in *A. anguilla.* Another species, *Labeo bicolor* (sample 25), exhibiting intermediate values of asymmetry (0.7 mg/cm³) and heterogeneity (1.8% GC) showed a banding pattern of intermediate intensity and reciprocity.

3) The histograms of DNA fractions of *Cyprinus carpio* (sample 23) and *Arothron diadematus* (sample 119) were used to show the very conspicuous compositional patterns of a very GC-poor DNA (in fact one of the GC-poorest DNAs among all those studied) and of a DNA that belongs to a species exhibiting one of the lowest haploid DNA content ($c = 0.45$ pg) among all fishes studied (Pizon et al. 1984).

4) Finally, in the case of the genome of *Trematomus borchgrevinki,* a typical Antarctic fish, the CsCl bands for the six fractions obtained show (Hudson et al. 1980) that 85% of the DNA is in the range 1.700–1.702 g/cm³ (fractions 1–3). Among higher density fractions, the most abundant, fraction 5, showed, upon digestion with HaeIII, three strong bands indicative of a satellite component.

Buoyant Densities of DNAs from Amphibians and Reptiles

Table 4 reports the modal and mean buoyant densities, ρ_0 and $\langle\rho\rangle$, the asymmetry, A, and the intermolecular compositional heterogeneity, H, of the CsCl bands, as well as the sedimentation coefficients, s20,w, and the GC levels of the DNAs from 5 amphibians and 13 reptiles.

The modal buoyant densities of DNAs from anurans range from 1.6991 g/cm³ for *Xenopus laevis* (sample 1 of Table 4 and Fig. 7), a strictly aquatic species from the ancient superorder Pipoidei, to 1.7013, 1.7028, and 1.7029 g/cm³ for *Leptodactylus pentadactylus, Bufo paracnemis,* and *Rana* sp. (samples 2–4), respectively; these three species belong to the more recent, not strictly aquatic, superorder Ranoidei. The ρ value of DNA from the urodele *Pleurodeles waltlii* (sample 5) is equal to 1.7041 g/cm³. The distribution of modal buoyant densities of DNAs from amphibians is shown in Fig. 2.

The CsCl profiles of DNAs from amphibians (Fig. 7) show no satellite band. Cryptic satellites may exist, however. For instance, in the case of *X. laevis,* the CsCl analysis of fractions from preparative centrifugation in Cs_2SO_4/Ag^+ showed the presence of minor components banding at 1.705 and 1.706 g/cm³ and of a minute amount of a 1.712-g/cm³ component (Thiery et al. 1976). The minor components, which cannot be physically separated by Cs_2SO_4/Ag^+ nor by Cs_2SO_4/BAMD centrifugation, but are enriched in some fractions, are degraded to small fragments by HindII + III degradation (Macaya et al. 1976) and are likely to be responsible for the ladder-like patterns produced upon HpaII digestion in some fractions from Cs_2SO_4/BAMD centrifugation (B. Aissani, G. Bernardi, and G. Bernardi, unpublished). On the other hand, the Cs_2SO_4/Ag^+ fractionation of *P. waltlii* DNA (Thiery et al. 1976) revealed the presence of a component banding at 1.706 g/cm³ and representing about 25% of DNA, whereas the majority of DNA bands at 1.704 g/cm³ (Thiery et al. 1976). It is possible that the heavier component is a satellite, as no such component is found after HindII + III digestion (Macaya et al. 1976), as if it had been degraded to small fragments.

DNAs from reptiles exhibit modal buoyant densities that are in the range 1.6992–1.7047 g/cm³, similar to that shown by DNAs from amphibians (see Fig. 2a). Shoulders on the CsCl bands can be seen in at least three DNAs, those from *Tarentola mauretanica, Natrix maura,* and *Bothrops neuwiedi* (samples 10, 14, 18). Moreover, cryptic satellites might be responsible for the GC-rich shoulders exhibited by the CsCl band profiles of most DNAs.

Asymmetry and Intermolecular Compositional Heterogeneity of DNAs from Amphibians and Reptiles

All amphibian DNAs are characterized by low asymmetries (ranging from 0.4 to 0.8 mg/cm³), the skewness always being on the heavy side of the CsCl bands. Heterogeneities were equal to 2.9–3.0% GC in the three cases analyzed. Both asymmetries and heterogeneities of DNAs from amphibians are close to those exhibited by most fish DNAs (Fig. 3). It is likely, however, that both sets of values for amphibians are overestimated because of the presence of GC-rich cryptic satellites, as in the case of fishes. This is also suggested by previous results on *X. laevis* (Thiery et al. 1976; see above), and by evidence on the existence of three tandemly repeated sequences in the genome of this species (Hummel et al. 1984). Another phenomenon that may affect the buoyant density profiles is the extremely high c value of *P. waltlii* with the possible accompanying amplification of some repeated sequences. The distribution of asymmetries and heterogeneities of DNAs from amphibians is shown in Fig. 3.

The asymmetries of CsCl bands of DNAs from reptiles are close to those shown by DNAs from amphibians. They may, however, be larger in some cases (Fig. 3). In five cases out of the six in which heterogeneity was determined, values were close to those found in DNAs from amphibians, with a higher

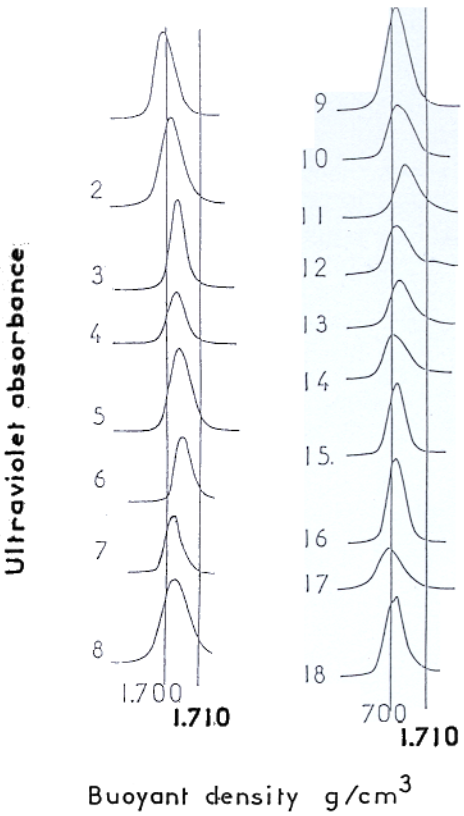

Fig. 7. CsCl band profiles of DNAs from amphibians and reptiles. See Table 4 for the numbering of the samples.

value for *Testudo graeca* (sample 8; Fig. 3). Again, the presence of cryptic heavy satellites may be responsible, at least in part, for the higher asymmetry and heterogeneity values of DNAs from reptiles. Such presence is suggested by the existence of components melting at high temperature in DNAs from some reptiles (Olmo 1981). Asymmetries and heterogeneities of DNAs from reptiles are in the same range as those of most fish DNAs.

Figure 4 shows the plots of CsCl band asymmetry versus heterogeneity as obtained with DNAs from amphibians, reptiles, mammals, and birds and compares them with those of fish DNAs. The DNAs from amphibians, the crocodile, one turtle (*Pseudemys nelsoni*, sample 7), and two lizards show points within the common range of most fish DNAs. The DNAs from another turtle (*T. graeca*) and a snake (*N. maura*) fall outside that range, but this is due, in all likelihood, to the presence of GC-rich unresolved satellites (see above). Finally, the DNAs from mammals and birds fall very much outside the fish values, the DNAs from murids showing, however, the least deviation. The latter finding is not surprising in view of the narrower compositional distribution of DNA fragments from murids (and cricetids) relative to those of all other mammals investigated (Salinas et al. 1986; Zerial et al. 1986; Mouchiroud et al. 1987; Bernardi et al. 1988; Bernardi and Bernardi 1990).

Discussion

The Properties of DNAs from Cold-Blooded Vertebrates

The main conclusion of the present work is that, as previously suggested by investigations on a much smaller number of species (Thiery et al. 1976; Hudson et al. 1980), the genomes of cold-blooded vertebrates do, indeed, share common properties. These are the low degree of intermolecular compositional heterogeneity, the low degree of CsCl band asymmetry, and the relatively wide spread of modal buoyant densities. Other common features concern satellite DNAs, which are mostly cryptic or present in very low amounts (and overwhelmingly GC-rich). All these properties set the genomes of cold-blooded apart from those of warm-blooded vertebrates.

Interestingly, recent thermal denaturation data (Karel and Gold 1987; Gold and Karel 1988) on DNAs from 34 fish species belonging to four orders investigated here and from one species of the order Percopsiformes (superorder Paracanthopterygii), not studied in the present work, agree with the compositional homogeneity and symmetry of fish genomes found here by density gradient centrifugation. Likewise, low compositional heterogeneities had been previously reported (Olmo 1981) in a study of thermal denaturation carried out on DNAs from 23 species belonging to 9 families of reptiles (Chelonia and Squamata). In contrast with the case of fishes, several DNAs from reptiles showed small shoulders and peaks especially on the high-melting side of the curves. Most of such melting components are likely to correspond to satellite DNAs, as they show great variability among species from the same family and even from the same genus.

It should be stressed here that the compositional properties of the genome of reptiles definitely put them in the same category as those of amphibians and fishes. This conclusion is at variance with the suggestions (1) that reptilian genomes may be more similar to those of warm-blooded than to those of cold-blooded vertebrates (Birstein 1982; Olmo et al. 1989); and (2) that "the generalized property of genome uniformity is a singular feature of teleostan fish genomes and not a difference between low and higher vertebrates or between cold-blooded vs. warm-blooded species" (Gold and Karel 1988). We will now examine these suggestions and explain why, in spite of the fact that they were based on correct observations, they are not tenable anymore.

The suggestion by Birstein (1982) was made on the basis of the observation that Giemsa banding, which is very poor or absent in the metaphase chromosomes of fishes and amphibians, is generally obtained in those of reptiles. Reverse fluorescent banding has been recently shown, however (Schmid and

Guttenbach 1988), to be absent not only in the chromosomes of fishes and amphibians, but also in those of reptiles. In the few cases in which R-bands were obtained in reptiles, the contrast was very low compared to those obtained in chromosomes of birds and mammals. Obviously, this finding indicates that the two staining procedures reveal different structural features, and that R-banding, but not necessarily G-banding, parallels DNA heterogeneity in reptiles.

The suggestion by Olmo et al. (1989) was based on an entirely different argument, namely on the fact that the amount of repetitive DNA in reptiles is closer to that present in mammals and birds than to the larger one present in fishes and amphibians. The amount of repetitive DNA is, however, correlated with genome size (Olmo et al. 1989), and the differences between fish DNA, on the one hand, and reptiles, birds, and mammals, on the other hand, found by Olmo et al. (1989), while applying to the particular fish sample used, are not generally valid. If genome size is small, as in the case of *Arothron diadematus* ($c = 0.45$ pg), slow-reassociating DNA may correspond to as much as 87% of DNA (Pizon et al. 1984), a value much higher than those of DNA from reptiles and warm-blooded vertebrates. If one compares the c values of the fish sample used by Olmo et al. (1989) with ours (Fig. 4a), it is clear that a higher genome size range was considered in the first case, thus accounting for the different conclusions drawn.

The suggestion by Gold and Karel (1988) is due to a misunderstanding. Indeed, these authors interpret the thermal denaturation data of Olmo (1981), showing the existence of one or more minor components in DNAs from reptiles, as "falsifying the hypothesis that cold-blooded vertebrates differ from warm-blooded vertebrates by the absence of numerous minor DNA components." In fact, the high melting components seen by Olmo (1981) in turtles, lizards, and snakes are not, in all likelihood, main-band DNA components, but satellite and/or minor components, as indicated by their variability among close species (see above).

The Correlations between Intermolecular Compositional Heterogeneities and CsCl Band Asymmetries

As shown in Fig. 4, the intermolecular compositional heterogeneity is positively correlated with CsCl band asymmetry in all vertebrate genomes. The increase in asymmetry with increasing heterogeneity is, however, much stronger, in the case of warm-blooded than in that of cold-blooded vertebrates. This result is of interest, as intermolecular compositional heterogeneity could very well increase without being associated with any CsCl band asymmetry.

The positive correlation between band asymmetry (skewness on the GC-rich side) and compositional heterogeneity, and the small relative amounts of GC-rich DNA indicate that increases in heterogeneity tend to be associated with regional increases in GC in a small part of the genome and/or with increases in AT in the majority of the genome. In the case of warm-blooded vertebrates, it is well established that regional GC increases in a compartment of the genome are indeed responsible for the appearance of GC-rich isochores, as the genes located in that compartment are more GC rich than their homologs from cold-blooded vertebrates (Bernardi et al. 1988). In the case of cold-blooded vertebrates, and especially in fishes, it is not possible to decide about the predominance of either cause, although the points made in the preceding section favor AT increases through the expansion of intergenic noncoding sequences. It should be stressed, however, that the effect being rather small, one may wonder whether it is not simply due to the higher intermolecular heterogeneity of the GC-rich DNA fragments that are closer to 50% GC (where intermolecular heterogeneity reaches a maximum; Cuny et al. 1981). A final decision must, therefore, wait for the assessment of GC levels in genes located at the two ends of the compositional distribution of fish genomes.

Correlation between GC Content and Haploid Genome Size

The correlation between GC levels and haploid size (c values) was studied here for fish genomes. Because GC levels were determined from $\langle \rho \rangle$ values (calculated without taking into account apparent satellites) and because cryptic satellites seem to be present in very small amounts in fish genomes (see Fig. 6), the data used are more reliable than those from reptiles, where satellite DNAs seem to be present in larger amounts (see below).

The existence of the negative correlation found in Fig. 5 between GC level and haploid size of fish genomes can be understood if one takes into consideration (1) that the correlation concerns a low c value range; in this case, as stressed in the preceding section, intergenic repetitive sequences are present in relatively low amounts; (2) that c values essentially increase or decrease by an increase or decrease of noncoding intergenic sequences (Olmo et al. 1989); and (3) that such intergenic sequences are lower in GC than in coding sequences (Bernardi et al. 1985; Bernardi 1989).

Polyploidy influences the correlation, however. This is evident if one considers that the points that

strongly deviate from the straight line of Fig. 5 are those of two salmonids (*S. gairdneri,* sample 31, *c* = 3.2 pg; *Oncorhynchus kisutch,* sample 32, *c* = 3.0 pg), a batrachoid (*O. tau,* sample 43, *c* = 2.8 pg), a callichthyid (*C. aeneus,* sample 28, *c* = 4.4 pg), the notothenids (*c* = 2 pg), and of Chondrichthyes. Now, in the case of salmonids, evidence for an ancient tetraploidization is available (Allendorf and Thorgaard 1984). In the case of batrachoids, tetraploidization is strongly suggested by the fact that although another species (*Opsanus beta*) from the same genus as *O. tau* exhibits an equally high *c* value (3.0), a species from a different genus of the same family (*Parichthys porosissimus,* sample 44) shows a *c* value that is only about half as large (*c* = 1.7 pg). Likewise, another callichthyid (*Corydoras callichthys*) has a *c* value of 1.7 pg, less than half that of *C. aeneus* (Hinegardner and Rosen 1972). If halved *c* values were taken into consideration in all these cases, they would fit the general relationship of Fig. 5. This suggests that these deviations are due to ancient or recent tetraploidizations (the latter being defined here as those in which close species show halved *c* values). Along the same line, polyploidy appears to be responsible for the deviations of notothenids and (see below) of Chondrichthyes.

In conclusion, two factors are mainly responsible for the correlation between GC level and haploid size of the genomes of cold-blooded vertebrates, namely regional expansion–contraction phenomena and polyploidy. The interplay of these factors, especially the fact that expansion–contraction phenomena may also affect polyploid genomes, makes the relationship of Fig. 5 a rather complex one. This is stressed by the fact that the points from Cypriniformes and *Acipenser sturio,* which are ancient tetraploids (Birstein and Vassiliev 1987) do not show strong deviations. This is also true of other families and species, which also presumably are tetraploids (compare, for example, *c* values of Cyprinodontidae and Aplocheilidae, of Diodontidae and Tetraodontidae, of *Symphodus ocellatus* or *Symphodus cinereus* and *Symphodus mediterraneus*). Indeed, a further complication may arise from partial genome duplication. For instance, *Symphysodon discus* (sample 89) has a larger number of chromosomes and a higher *c* value than closely related species (Thompson 1976; Kornfield 1984), the GC content being the same. Yet another complication may arise from differences in the amounts of satellite DNAs. Indeed, increases of GC with increasing *c* values were observed in 3 turtles of the genus *Testudo* and 10 lizards of the family Cordylidae (Olmo 1981); in both cases, differences in Tm, and therefore in estimated GC content, appear largely to depend upon the presence of minor GC-rich melting components. A reverse relationship was also observed (Olmo 1981) in three snakes belonging to the suborder Ophidia; in this case, the two higher and closer values in GC levels correspond to two species having *c* values that are not far from twice that of the third species; obviously, if this situation corresponded to a tetraploidization, the real relationship between GC level and *c* value would be reversed; this suggestion should be taken into serious consideration, because, as pointed out by Olmo (1981), another ophidian, *Crotalus durissus,* is characterized by a *c* value that is only half the two higher values (De Lucca et al. 1974).

The data of Fig. 5 may be of interest in another respect. Because the lowest reported *c* value for a fish genome is 0.4 pg (for a tetraodontid; Hinegardner and Rosen 1972), *c* values close to this minimum are more likely due to intergenic sequence expansions than to polyploidization. As *c* values reach 1 pg, the likelihood of genome duplication increases. Higher *c* values certainly involve polyploidization, as shown by the case of Chondrichthyes (Olmo et al. 1982; Stingo et al. 1989).

In the plot of Fig. 5 genome duplications shift points horizontally to the right. Some points concerning rather close species, however, show a strong deviation along the vertical axis (compare points 55 and 56 with 49, or 115 and 116 with 113). These effects cannot be due to polyploidy and can barely be due to expansion–contraction phenomena, as the *c* values under consideration are very close to each other and to the lower limit of *c* values for fishes. These deviations are apparently due to another reason, compositional transitions, namely to GC changes caused by directional nucleotide substitutions. This point, as well as other points of this work, will be discussed in detail in the following paper (Bernardi and Bernardi 1990).

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