Genome-size evolution in fishes

David C. Hardie and Paul D.N. Hebert

Abstract: Fishes possess both the largest and smallest vertebrate genomes, but the evolutionary significance of this variation is unresolved. The present study provides new genome-size estimates for more than 500 species, with a focus on the cartilaginous and ray-finned fishes. These results confirm that genomes are smaller in ray-finned than in cartilaginous fishes, with the exception of polyploids, which account for much genome-size variation in both groups. Genome-size diversity in ray-finned fishes is not related to metabolic rate, but is positively correlated with egg diameter, suggesting linkages to the evolution of parental care. Freshwater and other eurybiotic fishes have larger genomes than their marine and stenobiotic counterparts. Although genome-size diversity among the fishes appears less clearly linked to any single biological correlate than in the birds, mammals, or amphibians, this study highlights several particularly variable taxa that are suitable for further study.

Résumé : Les poissons possèdent à la fois les plus grands et les plus petits génomes des vertébrés, mais la signification évolutive de cette variation n'est pas encore comprise. Nous présentons des estimations de la taille du génome de plus de 500 espèces, en particulier de poissons cartilagineux et de poissons à nageoires à rayons. Nos résultats confirment que la taille du génome est plus petite chez les poissons à nageoires à rayons que chez les poissons cartilagineux, à l'exception des polyploïdes qui expliquent une partie importante de la variation de la taille du génome dans les deux groupes. La diversité de la taille du génome chez les poissons à nageoires à rayons n'est pas reliée au taux métabolique; il y a cependant une corrélation positive avec la taille des oeufs, ce qui laisse croire qu'il y a des liens avec l'évolution des soins parentaux. Les poissons d'eau douce et les poissons eurybiontes ont de plus grands génomes que leurs équivalents marins et sténobiontes. Bien que la diversité de la taille du génome chez les poissons semble moins clairement liée à une seul facteur biologique comme c'est le cas chez les oiseaux, les mammifères et les amphibiens, notre étude identifie plusieurs taxons particulièrement variables en vue d'études futures.

[Traduit par la Rédaction]

Introduction

Among vertebrates, genome-size measurements are available for approximately 400 amphibians (~8%), 320 mammals (~7%), 300 reptiles (~4%), 160 birds (~2%), and 900 fishes (~3%) (Gregory 2002*a*). Genome size is negatively associated with growth and development rate in varied organisms (e.g., Shuter et al. 1983; Chipman et al. 2001), while plants (Bennett 1976) and invertebrates (Cavalier-Smith 1978; Beaton and Hebert 1988) with larger genomes tend to exhibit broader ecological tolerance than those with lower DNA content. While genome size is negatively associated with metabolic rate in birds and mammals (Gregory 2002*a*), the evolutionary significance of genome-size diversity among reptiles and fishes is unresolved.

Genome-size diversity in fishes

Since the first broad studies of genome-size evolution among 275 bony fish species (Hinegardner 1968; Hinegardner and Rosen 1972), most work has concentrated on narrow taxonomic assemblages, with a strong bias towards freshwater species (data from Gregory 2001). Although each of these studies added taxonomic breadth to the data, these results have not been assembled to provide a broader overview of genome-size diversity in the fishes.

Low DNA content in ancestral chordates (e.g., Seo et al. 2001), and the multiplicity of certain gene families in vertebrates, which occur as single copies in their ancestral lineages, suggest that genome-duplication events have been important in the evolution of vertebrate genomes (e.g., Amores et al. 1998). The explosive speciation of teleosts may have been spurred by additional genome duplications after the divergence of the tetrapods (Amores et al. 1998), and additional "recent" polyploidization events account for the large genomes of many chondrichthyans (cartilaginous fishes) (Stingo and Rocco 2002) and chondrosteans (sturgeons and bichirs) (Blacklidge and Bidwell 1993), as well as certain actinopterygian (ray-finned) fishes (Uyeno and Smith 1972). Although fish genome sizes span the entire range of vertebrate DNA contents, being smaller than the smallest bird genome and bigger than the largest salamander genome, intraspecific variation, aside from ploidy shifts, is very low within most diploid fishes (e.g., Wolf et al. 1969; Tiersch and Goudie 1993).

Hinegardner and Rosen (1972) found that specialized fishes had smaller genomes than more generalized forms, and this trend was supported in later studies (Cimino 1974). This pattern may reflect decreases in chromosome number and ge-

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nome size in specialized species (Gold 1979), or constraints on genome size linked to the heightened developmental complexity of specialized species (Gregory 2002*b*).

Xia's (1995) model relating body temperature and gene duplication predicts that poikilotherms will have larger genomes in cold than in warm environments. Hardie and Hebert (2003) identified a trend towards larger cells in deep- and cold-water fishes, and a close correlation between genome and cell size in this group, results consistent with the finding that genome size increases with habitat depth among argentinoid fishes (Ebeling et al. 1971).

Species with large genomes also appear to survive better across a broader range of environmental variables (Gregory and Hebert 1999), and this pattern may also apply to fishes. This was first suggested as an explanation for the success of salmonid and catostomid fishes across broad latitudinal and salinity ranges (Allendorf and Thorgaard 1984). Nikolsky (1976) suggested that fishes in stenobiotic settings, such as marine and tropical waters, occupy narrow ecological ranges, while species from freshwater and temperate/polar climes occupy broad niches. He proposed that specialization in bony fishes has involved a move towards a narrower ecological amplitude and a decrease in chromosome number and genome size, which is supported by the small genomes of specialized fishes in stable environments (Uyeno and Smith 1972; Banerjee et al. 1988) and the larger genomes of fishes in broader niches (Ebeling et al. 1971).

The present study

This study provides genome-size estimates for more than 500 fish species. It focuses first on the range and variance of genome sizes within and among taxa, and this is followed by a consideration of the adaptive significance of these patterns. The taxonomic distribution of genome-size variance and the relative contribution of polyploidy are discussed, with a focus on chondrichthyan and actinopterygian fishes, as all new data are from these groups. Lastly, the evolution of fish genomes along physiological, developmental, and ecological gradients are considered.

Materials and methods

Sampling

Genome sizes were estimated in 1848 individuals representing 506 species from 155 families of marine and freshwater fishes, using Feulgen image analysis densitometry of erythrocyte nuclei from air-dried blood smears prepared as described elsewhere (Hardie et al. 2002). Blood smears from standard species (Siamese fighting fish, *Betta splendens*; goldfish, *Carassius auratus*; chicken, *Gallus domesticus*; rainbow trout, *Oncorhynchus mykiss*, and northern leopard frog, *Rana pipiens*) were collected as closely as possible to the time of sampling of unknowns.

Prior genome-size estimates for 54 agnathan, chondrichthyan, sarcopterygian, and cichlid fish species were also included to provide more comprehensive taxonomic coverage, producing a total of 560 species (Table D1[3]), using genome sizes from Gregory (2001) and karyotypes from Klinkhardt et al. (1995).

Metabolic rate data, corrected for body size and temperature (mg $O_2 \cdot min^{-1} \cdot kg^{-1}$ at 20 °C) and averaged over all species in a family for which data were available, were compiled from FishBase (2000) for 24 families for standard metabolism and for 37 families for routine metabolism. Egg-size data were compiled for 88 species of actinopterygian fishes (Coad et al. 1995; FishBase 2000). Reproductive modes were classified according to Baylis (1981), while taxonomy followed Nelson (1994).

Feulgen staining

Feulgen-stain preparation, dye manufacturer, lot number, and staining protocol were optimized as described in Hardie et al. (2002). The dye used was Sigma Basic Fuchsin Special for Flagella (B-0904, Lot 90K3681), and the protocol included 24 h fixation in 85 methanol : 10 formalin (37%) : 5 glacial acetic acid, 2 h room-temperature hydrolysis in 5 $mol \cdot L^{-1}$ hydrochloric acid, and 2 h staining time. Slides were stained in 1000-mL containers, with each run of 100 slides including at least one individual of each standard species and two individuals each of *G. domesticus* and *O. mykiss*. The strength of the correlation between staining intensity (integrated optical density) and the known genome sizes of the five standard species was tested for every run, and yielded highly significant and linear regressions ($r^2 > 0.95$, $P < 0.0001$). Genome sizes for unknowns were calculated based on the mean integrated optical density of the two *G. domesticus* smears), rather than from the standard curve (for justification see Hardie et al. 2002), using a "known" chicken genome size of 2.5 pg DNA per nucleus ($pg \cdot N^{-1}$) (Tiersch and Chandler 1989) for smears obtained from pathogen-free white leghorn hens from the Alma Poultry Research Station, Guelph, Ontario.

Data analysis

The Shapiro–Wilk test was used to test for normality of distributions. All data were log-transformed when required to achieve normal or near-normal distributions. Analysis of variance was used to compare means among groups, while the Kruskal–Wallis test was used to compare medians where outliers and (or) non-normality were a concern. Fisher's least significant difference test (multiple comparisons) and independent *t* tests (pairwise comparisons) were used to identify significant differences among group means. *F* tests were used to compare coefficients of variation (CVs) as described in Zar (1996). Data falling farther than 1.5 interquartile distances from the nonparametric 50th percentile range of any data set were identified as outliers. Least-squares regression and Pearson's correlation analysis were used to assess the strength of the relationships between genome size and both metabolic rate and egg diameter. While sufficient egg-size data were available to test this relationship at all taxonomic levels from species to order, metabolic-rate data were scarce, and could only be analyzed at the family level for families for which both genome-size and metabolic-rate data were

[3] Supplementary data for this article are available on the Web site or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0S2, Canada. DUD 3604. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_eshtml.

available. The total variance attributable to each taxonomic level was determined by top-down variance component analysis (Sokal and Rohlf 1969). Statistical analyses were carried out using Excel + Analyze-It 2000 (Microsoft Corporation 2000), Sigmaplot version 4.0 (SPSS Inc. 1998), and Statgraphics Plus version 5.0 (Manugistics 1999).

The biological significance of some patterns of genome-size diversity for which sample sizes were unbalanced or too low to be tested statistically are nonetheless discussed to identify groups deserving of more thorough analysis in the future. Furthermore, phylogenetic relationships among fishes are poorly established, precluding analyses treating phylogenetic non-independence of data (i.e., phylogenetically independent contrasts).

Results

Genome-size diversity among fish classes

Significant differences in both median (Kruskal–Wallis test, $P < 0.05$) and mean (analysis of variance, $P < 0.05$) genome sizes ($\log_2$-transformed) occurred among the five fish classes. In fact, differences among classes accounted for more than 93% of the total variance in genome size among fishes (Fig. 1; Table 1). Log-transformation of genome sizes normalized data for all classes (Shapiro–Wilk test, $P > 0.67$) except the actinopterygians, sarcopterygians, and lampreys ($P < 0.05$). Fisher's least significant difference test confirmed that the means for all five classes differed significantly from each other ($P < 0.05$). Since the data for the hagfishes, lampreys, and sarcopterygian fishes were taken from the literature, these groups are not discussed further, except to note the significantly larger genomes of the direct-developing hagfishes (6.6 pg·N^{-1}) relative to the metamorphosing lampreys (3.0 pg·N^{-1}), and the massive genomes of the lungfishes (80.9–225.6 pg·N^{-1}) relative to the coelacanth (7.2 pg·N^{-1}). Genome sizes of species in the three classes of primitive fishes (hagfishes, lampreys, chondrichthyans) appeared to vary in a quantum series, with multiples of 2.9 pg·N^{-1} in the hagfishes and lampreys and multiples of 1.45 pg·N^{-1} in the chondrichthyans.

Genome-size diversity among chondrichthyans

The distribution of chondrichthyan genome sizes extended across a broad range (~30 pg·N^{-1}, CV 55%), and was significantly skewed around a mean of 11.8 pg·N^{-1} (Shapiro–Wilk test, $P < 0.0001$). Variance among orders within this class (42.5%) and among families within orders (41.7%) accounted for similar amounts of the total genome-size variance, while variance among (8.1%) and within (3.7%) genera was much less important (Table 1). A high degree of variance in genome size among chondrichthyan families and orders was evident (Table 1), but its statistical significance could not be tested because some orders were represented by single families. A large component of the total variance within orders was due to broad ranges within such orders as the Carcharhiniformes (6.1–13.0 pg·N^{-1}), Squaliformes (11.6–22.5 pg·N^{-1}), and Rajiformes (5.6–10.8 pg·N^{-1}). Variable families contributing to the intergeneric variance component included the hound sharks (Triakidae; 8.6–17.3 pg·N^{-1}) and the rays (Dasyatidae; 8.3–13.4 pg·N^{-1}).

Genome-size diversity among actinopterygians

Genome sizes in actinopterygians ranged from a low of 0.8 pg·N^{-1} in the pufferfish *Canthigaster benneti* to a high of 13.8 pg·N^{-1} in the shortnose sturgeon, *Acipenser brevirostrum*, and showed a significant deviation from normality (Shapiro–Wilk test, $P < 0.0001$) around a mean of 2.0 pg·N^{-1}. The CV in genome size among species (52%) did not differ significantly from that in chondrichthyans (F test, $P > 0.05$). This study largely examined marine species, and the addition of previous data for freshwater taxa (from Gregory 2001) raised the mean to 2.4 pg·N^{-1}, although it was still significantly negatively skewed (Shapiro–Wilk test, $P < 0.0001$).

Genome-size variation among actinopterygians

Variance among actinopterygian orders accounted for 80% of the total variance in this class, while families and genera did not contribute further variance, and variance among congeneric species and intraspecific variance contributed approximately 16.7% and 3.1% to the total variance, respectively (Table 1). The high variance among orders was mostly due to the inclusion of orders dominated by polyploids, such as the Acipenseriformes, Salmoniformes, and Cypriniformes. Some polyploid genera, such as *Acipenser* (4.4–13.8 pg·N^{-1}), also contributed to variance at the generic level, while neopolyploid specimens of *Acipenser brevirostrum*, *Acipenser oxyrhynchus*, *Salmo salar*, and *Phoxinus neogaeus* contributed to intraspecific variance. The exclusion of these polyploid orders halved the ordinal contribution to total variance, producing a genome-size distribution with a closer approach to normality, with each higher taxonomic level contributing successively more variance (Table 1). Although the distribution of actinopterygian genome sizes still deviated from normality when the three polyploid orders were excluded (Shapiro–Wilk test, $P < 0.0001$), the data were significantly less variable (CV 32%, F test, $P < 0.05$) around a significantly lower mean of 1.8 pg·N^{-1} (t test, $P < 0.005$). All significant actinopterygian outliers were large-genome species, and most were polyploid (Fig. 2).

Genome-size diversity among conspecifics was ordinarily low, with a CV less than 10% for 387 of 398 species (Fig. 3). Three high outliers derived from the detection of neotriploids in aquaculture strains (*A. brevirostrum*, *A. oxyrhynchus*, *S. salar*) and intraspecific ploidy differences in several other species (e.g., *Synodontis notatus* and *P. neogaeus*). Other cases where single individuals had genome sizes deviating significantly (one-sample t test, all $P < 0.05$) from those of their conspecifics included the green jobfish (*Aprion virescens*), golden toadfish (*Lagocephalus lunaris*), the blue devil (*Chrysiptera cyanea*), gelatinous snailfish (*Liparis fabricii*), orange roughy (*Hoplostethus atlanticus*), and broadbill swordfish (*Xiphias gladius*).

Members of most fish families showed little variation in genome size, with CVs lower than 20% (Fig. 4). However, 12 of the 87 families had CVs greater than 20%, including representatives from three classes: Myxini, Chondrichthyes, and Actinopterygii. High CVs in the Myxinidae reflected the non-overlapping ranges of genome size between members of the subfamilies Myxininae (8.6–9.2 pg·N^{-1}) and Eptatretinae (4.6–6.9 pg·N^{-1}). Chondrichthyan families with broad ranges of genome size included the requiem sharks (Carcharhinidae, 5.7–9.9 pg·N^{-1}), stingrays (Dasyatidae, 6.8–13.4 pg·N^{-1}),

Fig. 1. Distribution of genome sizes among 560 species belonging to five classes of fishes. (*a*) The Myxini (7 species; open bars) and Cephalaspidomorphi (8 species; solid bars). (*b*) The Chondrichthyes (47 species). (*c*) The Sarcopterygii (5 species). (*d*) The Actinopterygii (493 species). Data derived from this study (506 species) and published data (54 species) were used to ensure coverage of all major taxonomic groups.

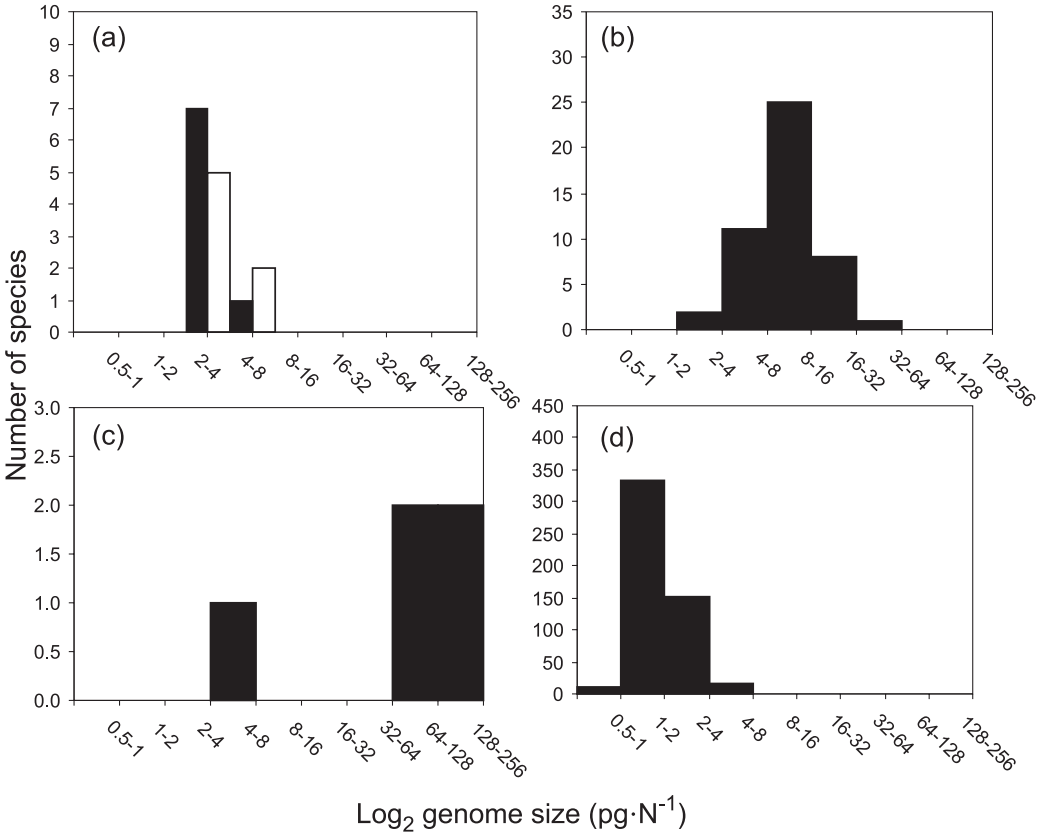


Table 1. Variance component analysis showing the portion of total variability in fish genome size contributed by each taxonomic level.

	Percentage of variance			
Source of variance	Entire data set	Chondrichthyans	All actinopterygians	Diploid actinopterygians
Class	93.78	—	—	—
Order	3.58	42.46	80.15	42.03
Family	2.44	41.71	0.00	31.43
Genus	0.00	8.06	0.00	15.96
Species	0.17	3.74	16.72	7.70
Individual	0.03	0.23	3.13	2.87

Note: Zero values indicate that no variance was added at a given taxonomic level that was not already accounted for at higher levels.

and hound sharks (Triakidae, 8.6–17.3 pg·N^{-1}). Among actinopterygian families, the acipenserids (4.4–13.8 pg·N^{-1}) and cyprinids (2.2–3.4 pg·N^{-1}) had broad ranges of genome size that reflect interspecific differences in ploidy level. By contrast, entirely tetraploid families, such as the salmonids and catostomids, had lower CVs, reflecting narrower ranges. Genome sizes also ranged widely in six diploid actinopterygian families: the pipefishes and seahorses (Syngnathidae, 1.2–2.7 pg·N^{-1}), dories (Zeidae, 1.5–2.5 pg·N^{-1}), dragonets (Callionymidae, 1.4–2.0 pg·N^{-1}), wrasses (Labridae, 1.3–2.8 pg·N^{-1}), filefishes (Monacanthidae, 0.9–1.6 pg·N^{-1}), and silversides (Atherinidae, 1.3–2.1 pg·N^{-1}), all of which had CVs greater than 20%.

Physiological, reproductive, and ecological correlates of genome-size diversity among the actinopterygians

There was no significant relationship between the mean genome size of species in a fish family and either standard (24 families, $r^2 = 0.02$, $P > 0.45$) or routine (37 families, $r^2 = 0.007$, $P > 0.73$) metabolic rates (Fig. 5). However, a strong positive relationship was identified between genome size and egg diameter in actinopterygians (Fig. 6). The relationship persisted at the specific ($r^2 = 0.30$), generic ($r^2 = 0.29$), familial ($r^2 = 0.15$), and ordinal ($r^2 = 0.41$) levels (all $P < 0.007$). Actinopterygian families showing parental care also had larger genomes (mean = 2.48 pg·N^{-1}) than non-guarding open-substratum scatterers (mean = 1.79 pg·N^{-1})

Fig. 2. Box-whisker plot of actinopterygian genome sizes. The box indicates the non-parametric 50th percentile range and is divided at the median. The outer lines indicate the 95th percentile range. Outliers, indicated by points on the graph, are more than 1.5 interquartile ranges (1 interquartile ranges = 0.67 pg) outside the 50th percentile range. Significant outliers include both polyploid (○) and diploid (●) species.

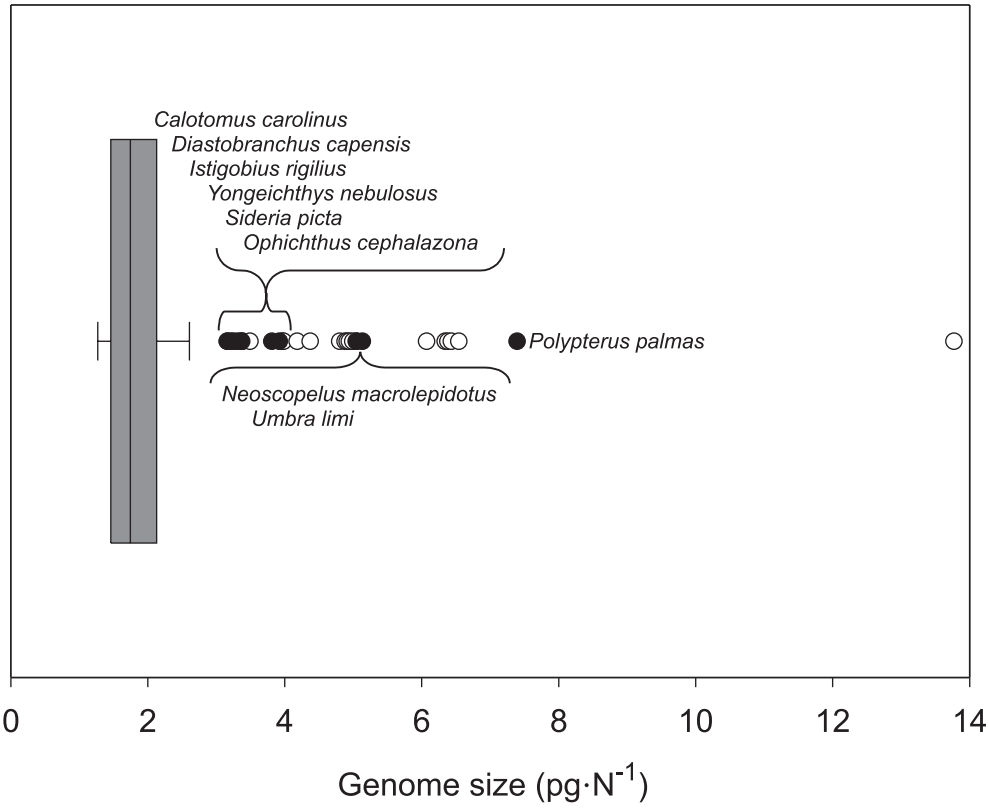


Fig. 3. Relationship between coefficient of variation (CV) and genome size for all 398 fish species from the present study represented by two or more individuals. Species identity is shown for both polyploid (○) and diploid (●) taxa with CVs greater than 10%.

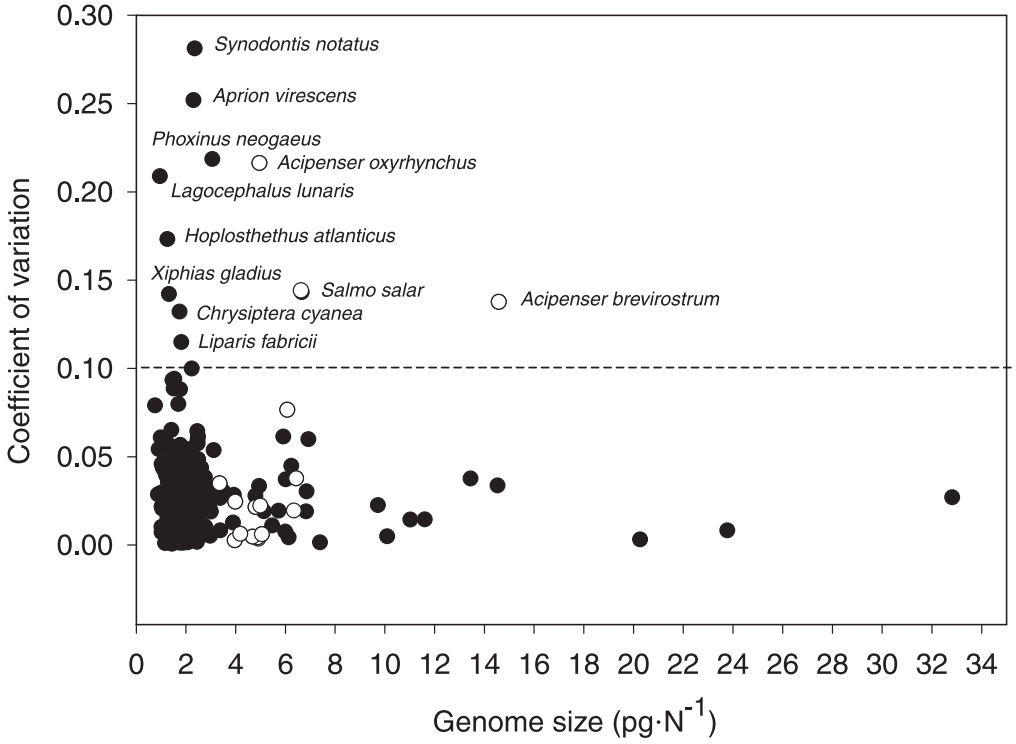


Fig. 4. Relationship between CV and genome size among all 87 fish families represented by two or more species. Identity is shown for 12 families with CVs greater than 20% and for 2 less-variable polyploid families (○).

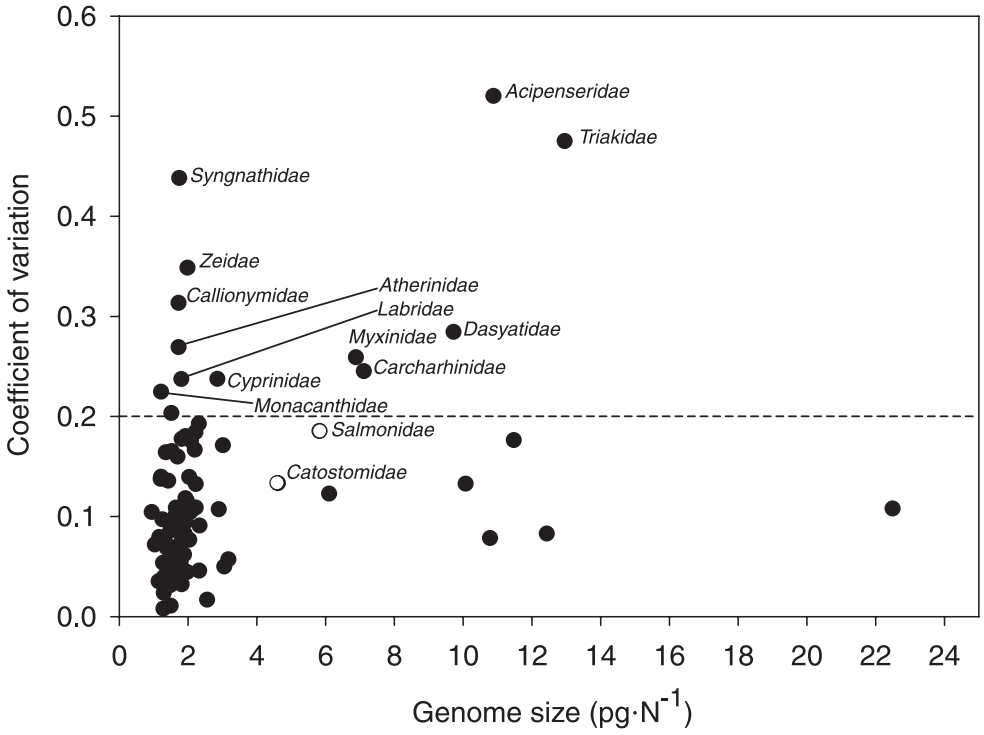


Fig. 5. Relationships between mean standard (●, solid line; $n = 24$, $r^2 = 0.016$, $P > 0.55$) and routine (○, broken line; $n = 37$, $r^2 = 0.002$, $P > 0.81$) metabolic rates and mean genome size for all fish families represented in this study for which metabolic-rate data are available in FishBase (2000).

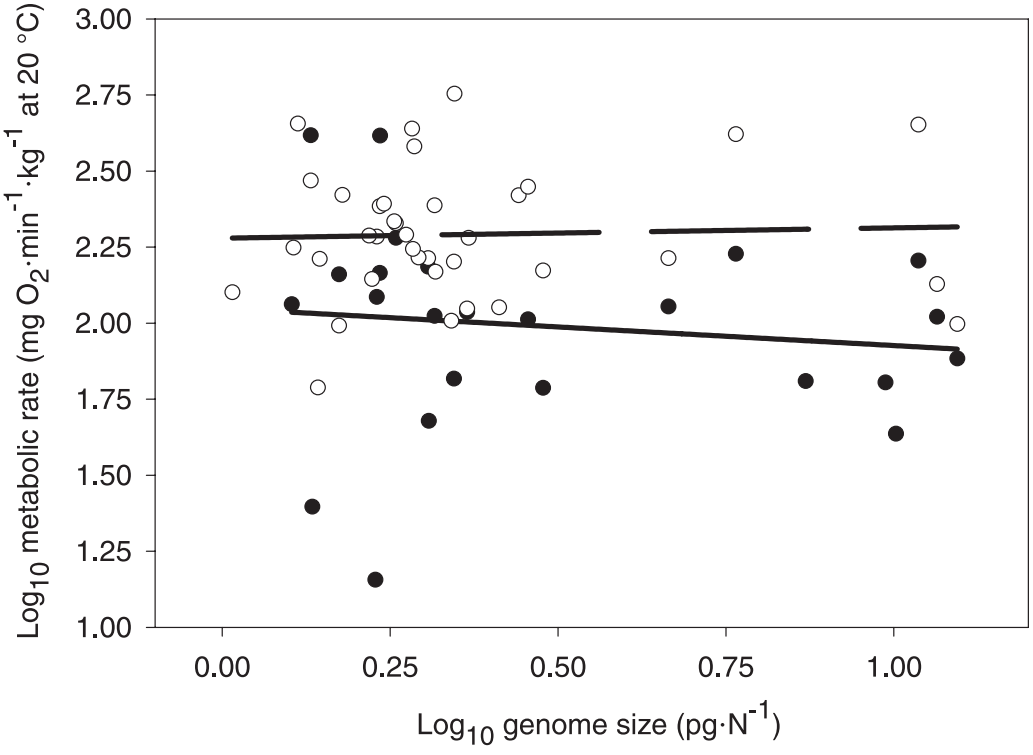
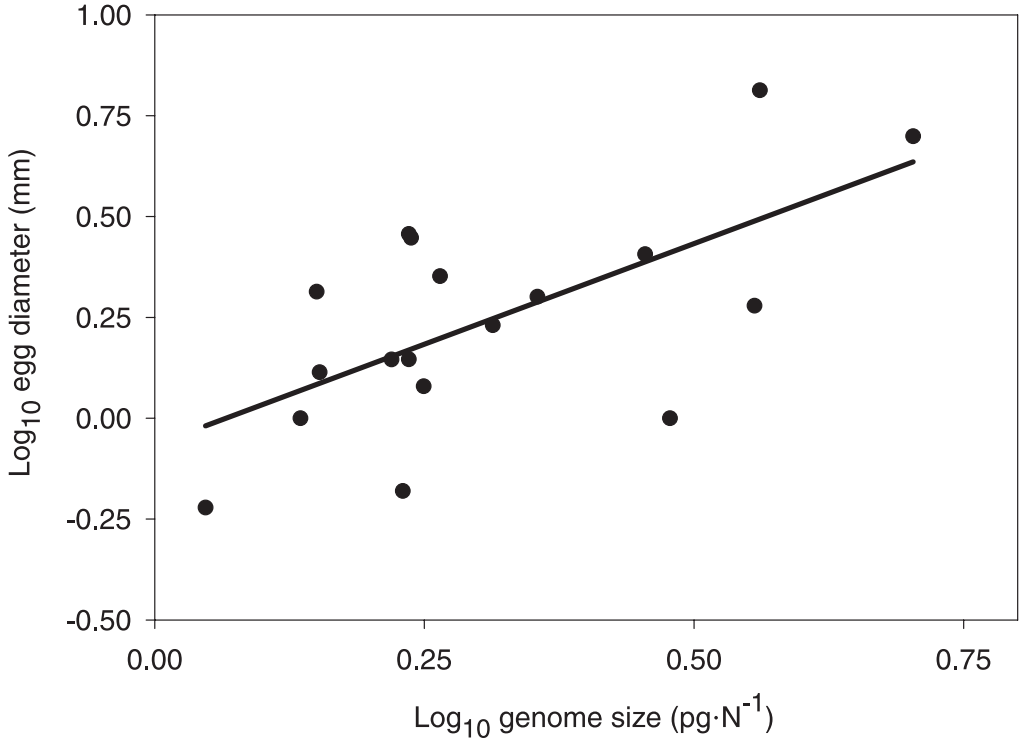


Fig. 6. Relationship between mean egg diameter and genome size for 18 orders of actinopterygian fishes ($r^2 = 0.41$, $P < 0.005$). These results derive from the analysis of 88 species. The regression persists (all $P < 0.007$) at the specific ($r^2 = 0.30$), generic ($r^2 = 0.29$), and familial ($r^2 = 0.15$) levels.



(Table 2; *t* test, $P < 0.0006$). Furthermore, the data provide many specific examples of significantly larger genomes in species that exhibit a higher degree of parental care than their allies (Hardie 2002).

Interestingly marine/catadromous actinopterygians had significantly smaller genomes (mean = 1.77 pg·N^{-1}) than their freshwater/anadromous counterparts (mean = 2.81 pg·N^{-1}; *t* test, $P < 0.0001$). Polyploidy in actinopterygians was limited to freshwater/anadromous taxa, but the significantly larger genomes of freshwater fishes persisted even with their exclusion (freshwater diploid mean = 2.32 pg·N^{-1}; *t* test, $P < 0.0001$).

Discussion

Genome-size diversity among fish classes

This study has confirmed that there are large differences in genome size among the five fish classes. The 36-fold range of genome size among sarcopterygian fishes is due mostly to the remarkably large genomes of the lungfishes, which provide evidence of a major shift in genome size within this class, unrivalled by any other group of animals. Although most of this apparently occurred as a result of polyploidy (Klinkhardt et al. 1995), the low chromosome numbers (~38) of most dipnoans make it unlikely that polyploidy alone accounts for the high DNA contents of this group. The genomic obesity of lungfishes has been linked to aestivation (Cavalier-Smith 1978), but this association will be difficult to validate, given the low species diversity of this group. The non-overlapping genome sizes of the direct-developing hagfishes (4.6–9.2 pg·N^{-1}) and the metamorphosing lampreys (2.6–4.2 pg·N^{-1}) provide a potential example of genome-size constraint linked to developmental complexity.

The present study has revealed evidence that genome sizes in primitive fishes vary in a quantum series (for a more complete discussion see Hardie 2002). This observation justifies further investigation, since the genomes of several other groups of organisms evolved as multiples of some "basal" C value (e.g., Narayan 1985).

Genome-size diversity: chondrichthyans versus actinopterygians

The high mean, broad range, and discontinuous variation of genome sizes of chondrichthyans relative to those of actinopterygians support earlier reports that polyploidization followed by rediploidization characterizes genome-size evolution in this group (Stingo and Rocco 2002). Although large-scale differences in genome size among chondrichthyan orders contribute much variation, intergeneric variation within families can also be significant. In contrast, polyploidy is less common among ray-finned fishes, where diploid chromosome numbers remain remarkably constant around 48, even among species that differ significantly in DNA content (Klinkhardt et al. 1995). The fact that DNA content has evolved differently between cartilaginous and ray-finned fishes is not surprising, given that the classes differ drastically in traits such as physiology, reproduction, and development, and have had longer histories of evolutionary independence than most other vertebrate classes. Nucleotypic limitations of genome size on cellular and organismal phenotypes may explain why the chondrichthyans, with their slow and direct development, viviparity, long life-span, low

Table 2. Mean genome size and reproductive mode for 22 freshwater and 18 marine families of fishes that are primarily larva guarders (L), embryo guarders (E), and bearers (B; includes mouthbrooders).

Family	Mode of parental care	Mean genome size ($pg \cdot N^{-1}$)
Freshwater habitat		
Characidae (characins)	B	3.00
Adrianichthyidae (adrianichthyids)	B	1.95*
Poeciliidae (mollies)	B	2.76
Osteoglossidae (bonytongues)	B, E	1.69
Loricariidae (armoured catfishes)	B, E	3.15
Doradidae (thorny catfishes)	E	3.20*
Siluridae (sheatfishes)	E	2.18*
Cyprinidae (carps)	E	2.85
Notopteridae (knifefishes)	E	3.38
Callichthyidae (armoured catfishes)	E	4.82
Umbridae (mudminnows)	E	5.13
Salmonidae (salmonids)	E	5.27
Fundulidae (topminnows and killifishes)	E	2.58
Eleotridae (sleepers)	E	2.50
Belontiidae (gouramies)	E	1.28
Cichlidae (cichlids)	B, L	2.33
Amiidae (bowfins)	L	2.28
Ictaluridae (North American catfishes)	L	2.08*
Channidae (snakeheads)	L	1.62*
Gasterosteidae (sticklebacks)	L	1.30
Centrarchidae (sunfishes)	L	1.92
Ophidiidae (cusk-eels)	B	1.23
Marine habitat		
Scorpaenidae (scorpionfishes and firefishes)	B	2.21
Syngnathidae (seahorses & pipefishes)	B	1.74
Opistognathidae (jawfishes)	B	2.12
Apogonidae (cardinalfishes)	B	2.03
Hemirhamphidae (halfbeaks)	B	2.22
Embiotosidae (surfperches)	B	1.68*
Cottidae (sculpins)	B, E	1.66
Ariidae (ocean catfishes)	B, E	4.94
Plotosidae (eel-tailed catfishes)	E	3.49
Cyclopteridae (lumpfishes)	E	1.81
Batrachoididae (toadfishes)	E	5.33*
Sparidae (porgies)	E	1.40
Labridae (wrasses)	E	1.80
Zoarcidae (eelpouts)	E	1.93
Stichaeidae (pricklebacks)	E	1.60
Blenniidae (blennies)	E	1.35
Gobiidae (gobies)	E	2.33
Tetraodontidae (puffers)	E	0.94

Note: Classification of reproductive modes follows Baylis (1981). Genome sizes are from this study except for those marked with an asterisk, which are from Gregory (2001).

metabolic rate, and large body size, have evolved larger genomes than the actinopterygians, which generally fall at the other end of the spectrum with respect to these features.

Genome-size diversity among actinopterygians: the role of polyploidy

The current data confirm the fact that much genome-size variation among actinopterygians stems from polyploidy. In fact, the broad ranges of genome sizes of species within the four orders that contain polyploid taxa (Acipenseriformes, Cypriniformes, Siluriformes, Salmoniformes) account for much of the total variation among actinopterygian genomes. The higher CVs of the acipenserid and cyprinid families versus the salmonids and catostomids reflect the fact that species in the former families show ploidy variation (e.g., Blacklidge and Bidwell 1993; Gold and Li 1994), while the latter are all tetraploid (e.g., Allendorf and Thorgaard 1984; Uyeno and Smith 1972). Likewise, the highest CVs at both inter- and

intra-specific levels were largely due to ploidy-level differences. Particularly high CVs were observed among species of *Acipenser*, which show evidence of one to four polyploidization events from a diploid ancestor represented by the extant *Huso dauricus* (Blacklidge and Bidwell 1993). Since sturgeon species with genome sizes around 4.5 $pg \cdot N^{-1}$ are octaploid (~120 chromosomes; Blacklidge and Bidwell 1993), it is likely that *A. oxyrhynchus* (4.4 $pg \cdot N^{-1}$) is also octaploid, while *A. brevirostrum* (13.8 $pg \cdot N^{-1}$) is dodecaploid (~360 chromosomes). The reasons why dramatic ploidy increases have been tolerated in this order are unclear, since the 6-fold range of DNA contents among acipenseriform species is not related to any conspicuous biological correlate.

Genome-size diversity among putative diploid actinopterygians

The mean and range of actinopterygian genome sizes observed in this study are similar to those reported by Hinegardner and Rosen (1972). Once polyploid orders were excluded, the ordinal variability of actinopterygian genome sizes was halved, and family and generic variability became significant. Large interspecific genome-size differences within both the seahorse and pipefish subfamilies account for the high CV of the syngnathid family. Although a four-fold range has previously been reported among different pipefish species (Vitturi et al. 1998), this is the first report of similar differences among seahorses, which were previously thought to have small genomes (Hinegardner 1968; Vitturi et al. 1998). The high CVs discovered within other diploid actinopterygian families, such as the zeids, callionymids, and atherinids, require further investigation, as each of these families was represented by only two species. Previous evidence for intrafamily variation has been limited to polyploid taxa (e.g., Wolf et al. 1969; Allendorf and Thorgaard 1984), while diploid fish families generally have CVs lower than 20% (e.g., Hinegardner and Rosen 1972; Tiersch and Goudie 1993), which is similar to most results in this study.

Divergent actinopterygian genomes

Although most of the positive genome size outliers among actinopterygians were polyploids, some diploid outliers possessed physiological, reproductive, or ecological attributes that may explain their unusual genome sizes. The large genome of the bichir, *Polypterus palmas*, is consistent with suggestions that slowly evolving groups have larger genomes than rapidly evolving ones (Hinegardner and Rosen 1972). Polyploidy is an unlikely explanation in this case, given this group's low chromosome number (36; Klinkhardt et al. 1995). Similarly, the large genome of the mudminnow, *Umbra limi*, is remarkable, since this species has a diploid chromosome number of just 22, while its European congener *Umbra krameri* has 44 chromosomes (Klinkhardt et al. 1995). Moreover, its North American relatives, *Novumbra hubbsi* and *Dallia pectoralis*, have genomes half as large, despite having 48 and 78 chromosomes, respectively (Beamish et al. 1971).

The large genomes of some gobiids, like the large genome of the mouth-brooding catfish, *Arius graffei*, may reflect their high degree of parental care. The high DNA content in the latter species is consistent with an earlier report (Hinegardner and Rosen 1972) of large genomes among ariids, and there are no reports of polyploidy in this family. The large genome of the lanternfish *Neoscopelus macrolepidotus* is consistent with Ebeling et al.'s (1971) report of large genomes in deep-water myctophids, which may be relevant to these species' broad ecological amplitudes, as they undertake extensive vertical migrations daily.

Physiological correlates of genome-size diversity among actinopterygians

At first consideration, the massive genomes of aestivating lungfishes appear to represent a prime example of metabolic constraints on genome size in poikilothermic vertebrates (Cavalier-Smith 1991), as do the large genomes of fishes able to survive in oxygen-depleted waters, or even out of water altogether, such as *U. limi* (present study), as well as the rivulines and killifishes relative to other cyprinodontiforms (~3 vs. ~1.8 $pg \cdot N^{-1}$; Hinegardner and Rosen 1972). However, several extremely active fish groups, including scombrids, xiphids, and salmonids, have genomes similar in size or larger than those of sluggish species such as flatfishes and syngnathids. Furthermore, Pauly et al. (2000) found no evidence for a negative association between DNA content and caudal-fin aspect ratio (as a measure of activity / metabolic rate) in fishes. The lack of a significant relationship between metabolic rate and genome size in fishes agrees with results obtained in studies on other poikilotherms (e.g., Licht and Lowcock 1991). In general, these trends suggest that metabolic constraints on genome size are weak in fishes, except possibly the cartilaginous fishes, which have much lower metabolic rates than bony fishes (Brett 1970).

Reproductive correlates of genome-size diversity among actinopterygians

Given the strong positive association between genome and erythrocyte sizes in fishes (Hardie and Hebert 2003), it is not surprising that egg diameter is positively correlated with genome size in actinopterygians. Moreover, this relationship has particular ecological significance, since the production of large eggs limits fecundity and is associated with parental care (Sargent et al. 1987). As predicted by these associations, a large genome was also typically linked to parental care in fishes. Interestingly, genomes are generally larger in freshwater than in marine fishes, and the former tend to show a higher degree of parental care (Breder and Rosen 1966). These results may provide the first example of a genome-size-driven shift into a "novel niche" among fishes (Gregory and Hebert 1999). Since genome size is clearly related to egg size in fishes, large increases in DNA content might result in intense selection for increased parental care, to compensate for decreased fecundity. This trend is similar to the case of brain and feeding behaviour simplification in some amphibians after the expansion of their genomes (Roth and Wake 2001).

If high DNA content coevolved with parental care in fishes, the paucity of large genomes and the negative skew of DNA contents are consistent with the fact that most fish families (~77%) show no parental care. By comparison, about 17% care for eggs, while 6% extend this care to newly hatched young (Keenleyside 1978). The internal fertilization and slow, direct development of chondrichthyans versus the usual ovi-

parity, external fertilization, and rapid development of actinopterygians provides general evidence for reproductive and developmental correlates of genome-size differences between classes. Thus, as noted in other poikilotherms (e.g., Chipman et al. 2001), nucleotypic effects of genome size on cytological parameters are most strongly exhibited as constraints on early development in fishes. Hence, it is possible that an increase in genome size has driven the evolution of viviparity and parental care in certain fish lineages. However, some continuous quantifiable measure of development rate is needed to test whether genome size limits it directly.

Ecological correlates of genome-size diversity among actinopterygians

The fact that freshwater/anadromous have larger genomes than marine/catadromous actinopterygians supports the prediction that eurybiotic fishes will have larger genomes than stenobiotic fishes (Ebeling et al. 1971), and is consistent with evidence that large-genome organisms have broader ecological tolerances (Bennett 1976; Beaton and Hebert 1988). The present study has revealed other evidence that a high DNA content may be linked to ecological factors in vertically migrating myctophids and taxa routinely exposed to harsh environments, such as umbrids, dipnoans, fundulids, and poeciliids.

The adaptive advantage of gene multiplicity in polyploids has previously been advanced as an explanation for their relative success and frequency in stochastic freshwater systems (Ebeling et al. 1971; Allendorf and Thorgaard 1984). While these factors may explain the persistence of polyploid lineages in freshwater/anadromous fishes, it is also possible that polyploids simply arise more often in these settings. Relative to the marine environment, where few barriers limit gene flow, the freshwater environment is highly dissected, providing more opportunities for population isolation, a factor that may enhance opportunities for the synthesis of polyploids (Otto and Whitton 2000).

Although the larger genomes of freshwater fishes reflect, in part, the greater prevalence of polyploids in these settings, diploid genomes are still larger in freshwater/anadromous than in marine/catadromous actinopterygians. Furthermore, polar marine fishes have smaller-than-expected genomes, which may relate to the fact that their environment, although extreme, is highly stable, barring seasonal fluctuations in productivity. Similarly, the fact that tropical marine taxa have small genomes is consistent with their occupancy of stable environments. Thus, although the precise mechanisms underlying the elevation of genome size in freshwater/eurybiotic versus marine/stenobiotic fishes remain unknown, these results support previous suggestions that genome size and ecological amplitude are positively related in fishes (Ebeling et al. 1971; Nikolsky 1976), as in other groups (Beaton and Hebert 1988).

The relatively small genomes of cold-water fishes conflict with the prediction (Xia 1995) that cold-climate poikilotherms will have larger genomes than their warm-climate counterparts. The small genomes of polar fishes may be explained by development-time constraints imposed by the ephemerality of growth seasons, which may select for high rates of larval development, and therefore small genomes. Moreover, fishes chronically exposed to low temperatures may have evolved compensatory mechanisms (e.g., larger yolk supplies) to overcome environmental challenges. However, the lack of any consistent trend suggests that thermal regime is not strongly related to DNA content in fishes, in contrast to clinal patterns in other groups (Bennett 1976; Xia 1995).

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