The nucleotypic effects of cellular DNA content in cartilaginous and ray-finned fishes

David C. Hardie and Paul D.N. Hebert

Abstract: Cytological and organismal characteristics associated with cellular DNA content underpin most adaptionist interpretations of genome size variation. Since fishes are the only group of vertebrate for which relationships between genome size and key cellular parameters are uncertain, the cytological correlates of genome size were examined in this group. The cell and nuclear areas of erythrocytes showed a highly significant positive correlation with each other and with genome size across 22 cartilaginous and 201 ray-finned fishes. Regressions remained significant at all taxonomic levels, as well as among different fish lineages. However, the results revealed that cartilaginous fishes possess higher cytogenomic ratios than ray-finned fishes, as do cold-water fishes relative to their warm-water counterparts. Increases in genome size owing to ploidy shifts were found to influence cell and nucleus size in an immediate and causative manner, an effect that persists in ancient polyploid lineages. These correlations with cytological parameters known to have important influences on organismal phenotypes support an adaptive interpretation for genome size variation in fishes.

Key words: evolution, genome size, DNA content, cell size, erythrocyte size, fishes, nucleotypic effect.

Résumé : Des caractéristiques cytologiques et de l'organisme entier, lesquelles sont associées avec le contenu en ADN, sous-tendent la plupart des interprétations adaptivistes de la variation quant à la taille des génomes. Puisque les poissons constituent le seul groupe de vertébrés chez lequel les relations entre la taille du génome et certains paramètres cellulaires clés sont incertains, les corrélations entre les caractéristiques cytologiques et la taille du génome ont été examinées chez ce groupe d'espèces. Il existait une corrélation positive très significative entre les volumes de la cellule et du noyau chez les érythrocytes ainsi qu'entre ceux-ci et la taille du génome chez 22 espèces de poissons cartilagineux et chez 201 espèces de poissons à nageoires rayonnées. Les régressions sont demeurées significatives à tous les niveaux taxonomiques ainsi que chez tous grands groupes de poissons. Les résultats ont cependant révélé que les poissons cartilagineux possédaient des ratios cytogénomiques plus élevés que les poissons à nageoires rayonnées, tout comme le font les poissons d'eau froide par rapport aux poissons d'eau chaude. Des accroissements de la taille des génomes dus à des changements de ploïdie influençaient directement et immédiatement la taille de la cellule et du noyau, un effet qui persiste chez les anciennes lignées évolutives polyploïdes. Ces corrélations avec des paramètres cytologiques connus pour exercer des influences importantes sur le phénotype de l'organisme viennent appuyer une interprétation adaptative de la variation de la taille du génome chez les poissons.

Mots clés : évolution, taille du génome, contenu en ADN, taille de la cellule, taille des érythrocytes, poissons, effets nucléotypiques.

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Introduction

Early observations that cellular DNA amounts varied in a non-random fashion both within and among groups of organisms (Mirsky and Ris 1951; Vendrely 1955) were soon followed by the discovery that cell and nuclear sizes varied in concert with DNA content in protists (Shuter et al. 1983), plants (Martin 1966), and animals (Olmo and Morescalchi 1975). Although prokaryotic genome sizes are much less variable, they are also strongly associated with cell size (Cavalier-Smith 1978 and references therein). Since this early work, the cytological correlates of genome size have been confirmed in many groups across a 200 000 fold range of genome size (Gregory 2001*b* and references therein), such that the relationships between C value and both nucleus and cell size rank among the most fundamental rules of eukaryote cell biology (Cavalier-Smith 1993).

Other than shifts in ploidy level, differences in amounts of non-coding DNA account for most genome size diversity (Cavalier-Smith 1985*b*), and five main explanations have been advanced to explain this fact. The earliest of these hypotheses proposed that non-coding DNA consists of extinct genes (Ohno 1972), or "junk DNA", which accumulates in the genome until constrained by selection. This term was more recently extended to include any genetic elements that increase in the genome by chance and lack a coding or regulatory function (Pagel and Johnstone 1992). Second, the "selfish DNA" theory argues that self-replicating DNA segments like transposable elements persist and increase within

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the genome solely for their own benefit, and account for much non-coding DNA (Doolittle and Sapienza 1980; Orgel and Crick 1980). More recently, the "mutational equilibrium" model proposed that genome size varies in a lineage until the loss of DNA through frequent small deletions is equal to the rate of DNA increase owing to long insertions (Petrov 2002). Under the "nucleoskeletal" theory, DNA content is secondarily selected owing to selection on cell and nuclear size (Cavalier-Smith 1978). Lastly, Bennett (1971) proposed the "nucleotypic" theory that DNA content affects cellular parameters in a causative manner, and is therefore subject to secondary selection via selection on cytological and organismal phenotypes. Since each of these theories identifies factors that might contribute to genome size diversity, a pluralistic approach may provide the best explanation of genome size evolution. Chipman et al. (2001) provide a clear statement of this prospect — "One of the problems in many attempts at explaining the evolution of genome size is the search for a single evolutionary model that holds for all taxa. We believe the situation is more complicated. Changes in genome size are probably the result of a complex interaction of heritable factors…random factors…and adaptive factors…". Nonetheless, the view that DNA content exerts causative "nucleotypic" effects on cellular and related organismal characteristics explains commensurate changes in cell size after both increases and decreases in genome size. As such, the nucleotypic theory best addresses observed relationships between nucleus, cell, and genome sizes (Gregory 2001*a*). However, this theory requires that cell size and genome size be associated in a causative manner and that cell size itself be of adaptive significance.

DNA content and cell size: cellular phenotypes

A positive relationship between cell and genome size has been identified in every group of organisms where it has been examined (Cavalier-Smith 1985*a*) except in fishes. Boveri's classic experiments first demonstrated this in urchins, where manipulations of chromosome number generated changes in cell size (Mirsky and Ris 1951). Importantly, this study demonstrated that genome size affects cell size in a causative manner, a result reinforced by later studies that showed immediate cell size increases owing to shifts in ploidy levels and in supernumary B chromosomes (Nurse 1985). This fact is critical to the resolution of the C value paradox, as it provides evidence against the coincidental junk- and selfish-DNA hypotheses, as well as against the coevolutionary nucleoskeletal hypothesis, as none of these can account for the immediate, causative effects of genome size changes on cell size (Gregory 2001*a*).

Since cell size appears universally associated with genome size, and because nuclear volume is a function of the content and degree of folding of the genetic material (Cavalier-Smith 1978), it is not surprising that nuclear size is related to genome size in a strongly positive manner. Many other studies have extended evidence of both nucleus and cell size correlations with genome size (and with each other) for different cell types in a broad range of organisms (Cavalier-Smith 1985*a*). Thus it is not surprising that cytological correlates of genome size extend to nuclear volume, cell volume, cell surface area (Olmo and Odierna 1982), and nuclear surface area (Olmo 1983) in a positive manner and negatively to cell metabolic rate (Smith 1925; Goniakowska 1970) and both mitotic (Van't Hof and Sparrow 1963) and meiotic (Bennett 1971) division rates.

DNA content and cell size: organismal phenotypes

Given the ubiquitous and apparently causative relationship between genome size and a diversity of cytological parameters, the adoption of an adaptive interpretation for observed patterns of genome size variation requires only that these cellular characteristics extend in some way to the organismal level, so that they (and by turn, DNA content) are subject to selection. That cell volume is subject to selection is hardly debatable owing to its many physiological and developmental implications (Gregory 2001*a*). Most obviously, cell size affects body size in organisms with a fixed or constrained number of cells, resulting in strong selection on cell size via its effect on body size (Gregory et al. 2000). In fact, some organisms are known to exploit the causative effects of genome size on cytological parameters, undergoing endopolyploidy "on demand" to increase cell size in certain tissues such as defensive or secretory structures (Perdix-Gillot 1979; Beaton and Hebert 1997). The negative association between cell size and division rates clearly subjects the former to strong selection, particularly during development and reproduction, when mitotic and meiotic rates are paramount. This relationship is best established in the amphibians, where a large volume of literature outlines developmental correlates of cell (and genome) size (Chipman et al. 2001 and references therein), including negative associations with developmental rate (Camper et al. 1993) and complexity (Roth et al. 1997). Negative developmental and growth rate correlates of cell size have also been identified in both protozoan and eukaryotic unicells (Shuter et al. 1983), in plants (Van't Hof and Sparrow 1963), and in invertebrates (Bier and Müller 1969), as well as in some vertebrate groups (Cavalier-Smith 1985*b*). Cavalier-Smith first implicated *r*-selection (organisms adapted for ephemeral environments, exhibiting rapid development, early maturity, high fecundity, short lifespan, low parental care, and high reproductive effort) vs. *K*-selection (organisms adapted for stable, highly competitive environments, exhibiting slow development, late maturity, low fecundity, long lifespan, high parental care, and smaller reproductive effort) as an explanatory factor for cell and genome size variation among organisms (Cavalier-Smith 1980), an idea that was extended by Szarski (1983), who coined the terms "wasteful" vs. "frugal" evolutionary strategies. Along these axes, organisms with high metabolic and developmental rates (*r*-selected or wasteful) were expected to possess smaller cells and (or) genomes and vice versa. Since a 10% shortening of development time matches a 100% increase in fertility (and therefore fitness) (MacArthur and Wilson 1967), it is clear that the developmental effects of cell and genome size are subject to strong selection. Lastly, metabolic constraints on erythrocyte size are imposed by respiratory gas exchange requirements, thereby subjecting blood cell size to strong selection to match the physiological requirements of different animals (Snyder and Sheafor 1999). Thus, because genome and cell size are clearly related, and because cell size itself affects organismal phenotypes, it can be concluded that cell

size and any genetic mechanisms that influence it should be subject to strong selection pressures (Gregory 2001*a*).

DNA content and cell size: the vertebrates

Cytogenomic ratios are relatively stable among eukaryotes, from unicells to higher vertebrates (Shuter et al. 1983). The existing literature concerning cytological and related organismal correlates of genome size is dominated by studies on amphibians owing, in part, to their tremendous genome size variation and diversity of developmental and life history strategies. Both nucleus and cell size are positively related to genome size across this highly variable group (1.9–240 pg/N) (Olmo 1973; Olmo 1983). Although the relationship persists over the entire class, there is slight variation in the slope of the regression for the anurans (1.9–38 pg/N), which have smaller genomes than the urodeles (26–240 pg/N), the latter being more strongly allometric than the former (Gregory 2001*b*). Although much less variable than those of amphibians, reptilian genome sizes (2.2–10.8 pg/N) correlate in a strong and highly significant manner with both nucleus and cell size (Olmo and Odierna 1982). However, unlike the amphibians, this relationship appears not to differ among orders (Gregory 2001*b*).

The genome sizes of birds and mammals are, for the most part, constrained to very narrow limits, probably owing to limitations on cell size diversity imposed by homeothermy, a fact that has hampered efforts to investigate cellular and organismal correlates of genome size in these groups (Vinogradov 1995; Gregory 2001*b*). However, cytological correlations have been found within rodents (Walker et al. 1991; Gallardo et al. 1999) and mammals at large (Gregory 2001*b*). This last study reported a strong positive relationship between DNA content and erythrocyte diameter in 70 mammalian species despite their DNA-free red blood cells (RBCs). Given that erythrocytes are of primary importance to vertebrate physiology (Snyder and Sheafor 1999), it is not surprising that genome size is negatively correlated to metabolic rate in mammals (Vinogradov 1995). This, coupled with the fact that genome size affects even anucleate erythrocytes, further highlights the adaptive significance of DNA content via its cytological effects.

The narrow range of DNA content long discouraged attempts to investigate its relationship with cell size in birds, other than to say that reported values were consistent with the general relationships in vertebrates (Commoner 1964; De Smet 1981). Although not without difficulty (Vinogradov 1995), recent studies have revealed a positive cytogenomic relationship in this highly constrained group and, as in the mammals, extended the relationship (in a negative fashion) to metabolic rate (Vinogradov 1997; Gregory 2002).

Two main groups of fishes are considered in this study: the cartilaginous fishes, or chondrichthyans, and the ray-finned fishes, or actinopterygians. The latter group is further subdivided into the "chondrosteans" (bichirs and sturgeons) and the "teleosteans" (modern bony fishes). It is interesting to note that fishes demonstrate, with the exception of chondrichthyans, polyploids and a very few anomalous actinopterygians, a narrow range of genome size variation (~3 fold) similar to that in homeothermic vertebrates (Hardie and Hebert[3]). Perhaps this is why, as in birds and mammals, cytological correlates of genome size have been elusive in fishes, except to say that they fit on the overall vertebrate regression (Olmo 1983) and that several small-scale studies have observed the same general positive trend (Pedersen 1971; Banerjee et al. 1988). Beyond weak evidence for these relationships in fishes, some recent studies have reported the lack of an association among cytological parameters and genome size in both bony (Lay and Baldwin 1999) and cartilaginous (Chang et al. 1995) fishes, but the methods used in these studies have been criticized (Gregory 2001*a*, 2001*b*). Recently, a preliminary study of erythrocytes from approximately 50 fish species showed a highly significant positive relationship between cell and genome size over a 175-fold range of DNA content across jawless, bony, cartilaginous, and dipnoan fishes (Gregory 2001*b*). However, this study was limited to data compiled from the literature, which can lower accuracy when methodologies are inconsistent or erroneous (Licht and Lowcock 1991). As such, this study could not test the relative strength and nature of the relationship within different fish groups, nor was the relationship with nucleus size examined. Thus, it was concluded that a "detailed analysis using consistent measures of nucleus, cell, and genome sizes (is needed)" (Gregory 2001*b*).

This study tests whether both nuclear and cell sizes correlate in a strongly allometric and significant fashion with DNA content in chondrichthyan and actinopterygian fishes. If this is the case, then cellular DNA content can be considered to be of adaptive significance in fishes, since any significant changes in genome size would result in phenotypic changes that are clearly subject to selection. Moreover, the taxonomic and geographic breadth of this study provides an unprecedented opportunity to compare cytogenomic correlates in different fish groups, while the inclusion of neo- and paleo-polyploid taxa allows testing of the causality of these relationships.

Materials and methods

Dry cell and nuclear areas were measured in 223 fish species including 22 chondrichthyan, 3 chondrostean, and 198 teleostean fishes. Details of specimen collection are provided elsewhere (Hardie and Hebert[3]). Cell and nuclear areas were measured in one individual per species with the exception of eight species that showed high levels of intraspecific DNA content variation suggesting ploidy differences. In these cases, both a "normal" diploid and the ploidy variant were analyzed. These included diploid and triploid shortnose and Atlantic sturgeon (*Acipenser brevirostrum* and *Acipenser oxyrhynchus*), Atlantic salmon (*Salmo salar*), finescale dace (*Phoxinus neogaeus*), one-spot squeaker catfish (*Synodontis notatus*), and tetraploid green jobfish (*Aprion virescens*). In addition, large and small genome-size variants of the blue devil (*Chrysiptera cyanea*) and golden toadfish (*Lagocephalus lunaris*) were examined. Intraspecific variation in cell and nuclear areas and genome size was formally quantified in five species: beta (*Betta splendens*; $n = 6$), goldfish (*Carassius auratus*; $n = 12$), rainbow trout (*Oncorhynchus mykiss*; $n = 6$), fish doctor

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(*Gymnelus viridis*; $n = 6$), and sandy damselfish (*Pomacentrus nagasakiensis*; $n = 6$).

Dry cell and nuclear areas were measured by digital image analysis of air-dried blood smears prepared following standard protocols (Fig. 1) (Hardie et al. 2002). Blood smears for cell area measurements were stained using Wright-Giesma stain from Sigma-Aldrich (WG-16) following the product instructions. Nuclear area measurements were taken from a second set of blood smears from the individuals that were Fuelgen-stained for genome quantification (see below; Fig. 1). Relative areas of at least 50 cells and 100 nuclei from a minimum of 5 different fields were measured, and transformed to actual area in square micrometers using a spatial calibration slide (Edmund Scientific, Barrington, N.J.).

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 Fucshin Special for Flagella (B-0904, Lot 90K3681, Sigma Chemical, St. Louis, Mo.), and the protocol included a 24-h fixation in 85 methanol : 10 formalin (37%) : 5 glacial acetic acid, a 2-h room temperature hydrolysis in 5 Normal (N) hydrochloric acid, and a 2-h stain time.

Blood smears from standard species (Siamese fighting fish, *Betta splendens*; goldfish, *Carassius auratus*; chicken, *Gallus domesticus*; rainbow trout, *Oncorhychus mykiss;* and northern leopard frog, *Rana pipiens*) were collected as closely as possible to the time of sampling of unknowns, and all blood smears were stored in the dark for at least 2 months before staining to minimize errors owing to age differences (Hardie et al. 2002). Slides were numerically coded and stained in batches of 100.

Measurements were made at 40× (cell area) and 100× (nuclear area and IOD) objective magnifications using a Leica DM LS compound microscope mounted with an Optronics DEI-750 CE three-chip CCD camera connected via a BQ6000 frame-grabber board to a Pentium II 300 MHz PC running Windows 98 and using Bioquant True Color Windows 98 version 3.50.6 image analysis software package (R&M Biometrics, Nashville, Tenn.). The linearity of the correlation between integrated optical density (IOD) and known cellular DNA content of the 5 standard species was tested for every staining run, and yielded highly significant and linear regressions ($r^2 > 0.95$, $P < 0.0001$) in every case. Genome sizes of unknowns were calculated based on the mean IOD of the two *G. domesticus* smears included in each run, rather than from the standard curve, since the "known" DNA content of other standard species are mostly estimates based on *G. domesticus*, and, as such, include measurement errors. This method yields reliable and accurate estimates of cellular DNA content (Hardie et al. 2002; Hardie and Hebert, in review).

The lack of a well-established phylogeny for fishes precluded rigorous analyses treating phylogenetic nonindependence of data (i.e., phylogenetically independent contrasts). Although phylogenetically independent contrasts rarely contradict species level regressions (Ricklefs and Starck 1996), all data were analyzed at the specific, generic, familial, and ordinal levels (Gregory 2000) following Nelson's (1994) hierarchical classification. This method accounts for taxonomic bias in the data by redistributing variation evenly at successive taxonomic levels. Relationships among DNA content, nucleus, and cell sizes were analyzed by least-squares regression and Pearson's correlation analysis of log-transformed data. A student *t* test (two-tailed; H_0: slopes and intercepts equal) was used to compare regression slopes and intercepts (elevation) among groups, whereas paired *t* tests were used to compare cytogenomic ratios between ploidy levels. Statistical analyses and calculations were carried out using Excel 2000 (Microsoft Corp., Redmond, Wash.) and Sigmaplot version 4.0 (SPSS Inc., Chicago, Ill.).

Results

Intraspecific variation

Intraspecific variation in erythrocyte area was consistently low in the freshwater species *B. splendens* (coefficient of variation (c.v.) = 0.03), *C. auratus* (c.v. = 0.03), and *O. mykiss* (c.v. = 0.02), as well as in the tropical marine species *P. nagasakiensis* (c.v. = 0.03) and the Arctic marine species *G. viridis* (c.v. = 0.04). Although slightly more variable, measurements of erythrocyte nuclear area were also consistent within species: *B. splendens* (c.v. = 0.03), *C. auratus* (c.v. = 0.06), and *O. mykiss* (c.v. = 0.06), and *P. nagasakiensis* (c.v. = 0.05) and *G. viridis* (c.v. = 0.06).

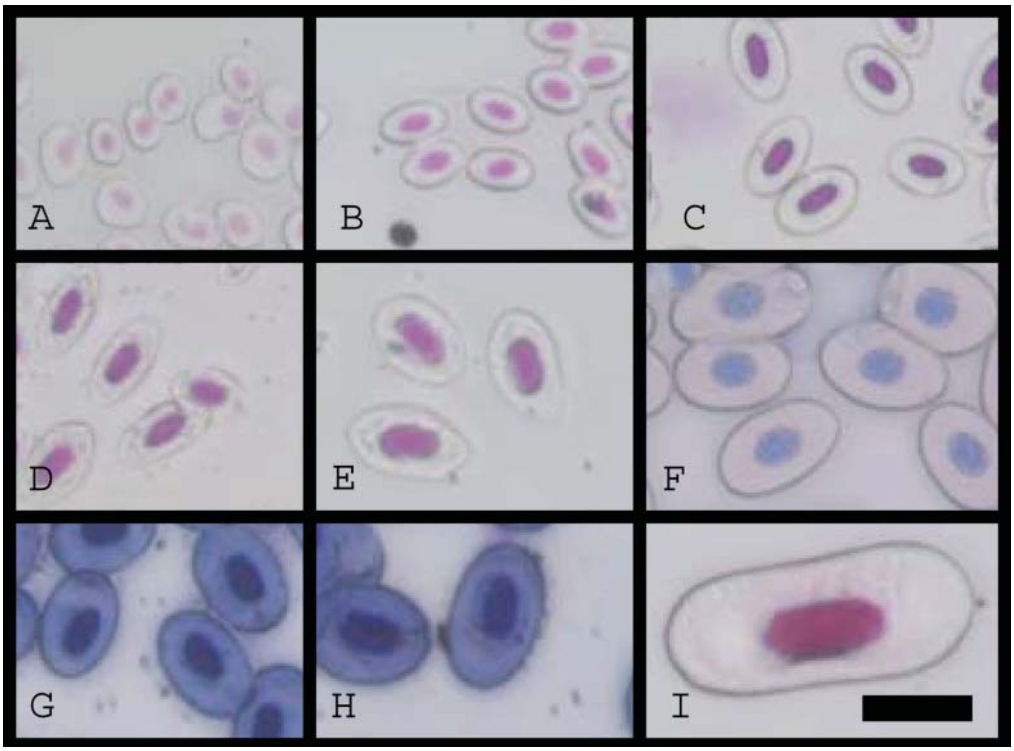
Cell and genome size

Dry erythrocyte area (CS) showed a highly significant correlation across a >40-fold range of genome size (GS) in 223 fish species (Table 1; Fig. 2A). The correlation was weakest for the perciforms ($r^2 = 0.36$), but became successively stronger for diploid ray-finned fishes ($r^2 = 0.41$), pooled ray-finned fishes ($r^2 = 0.61$), polyploids alone ($r^2 = 0.76$), cartilaginous fishes alone ($r^2 = 0.81$), and for all data combined ($r^2 = 0.82$). The relationship was significantly more strongly allometric for diploid actinopterygians ($b = 0.65$) than for polyploids ($b = 0.56$) (Fig. 2B; *t* test, $P < 0.03$). Similarly, the regression was steeper in cartilaginous ($b = 0.75$) than in actinopterygian ($b = 0.61$) fishes, but not significantly so (Fig. 2A; *t* test, $P = 0.14$); nor was the elevation (*y* intercept) significantly greater (*t* test, $P > 0.5$). All regressions were consistent and highly significant at the generic, familial, and ordinal levels (Table 1; all $P < 0.0001$). However, there was a trend for polar and bathypelagic (i.e., cold-water) chondrichthyan and actinopterygian fishes to have larger cells (large positive CS–GS residuals), and warm-water species to have smaller cells (large negative CS–GS residuals), than predicted from the CS–GS relationship for their respective classes. Seventeen of the 20 most positive actinopterygian outliers were extreme coldwater species, while 18 of the 20 most negative were tropical species. The 10 most positive and negative residuals were, without exception, "cold" and "hot" species, respectively. Similarly, 8 of the 10 most positive chondrichthyan outliers were cold-water species, while 9 of the 10 most negative were tropical, and the 5 most extreme in each direction were again "cold" and "hot" without exception.

Nuclear and genome size

Strongly significant positive correlations between RBC nuclear area (NS) and genome size were apparent across the

Fig. 1. Photomicrographs (× 400 magnification, scale bar = 20 μm) of Feulgen-stained erythrocytes of (A) Siamese fighting fish (*Betta splendens*, 1.3pg/N) compared with (B) chicken (*Gallus domesticus*, 2.5 pg/N), (C) rainbow trout (*Oncorhynchus mykiss*, 4.8 pg/N), (D) diploid and (E) triploid Atlantic salmon (*Salmo salar*, 6.5 and 9.8 pg/N), (F) spiny dogfish shark (*Squalus acanthias*, 11.6 pg/N), (G) diploid and (H) triploid shortnose sturgeon (*Acipenser brevirostrum*, 13.2 and 18.6 pg/N), and (I) Australian lungfish (*Neoceratodus forsteri*, 105.5 pg/N). These illustrations clearly demonstrate the strong positive associations between genome size and both nucleus and cell area, even in neopolyploids.



combined dataset and within each subset (Table 1; Fig. 3A). The association with nuclear area was strongest across the entire dataset (r^2 = 0.84), becoming progressively weaker for chondrichthyans (r^2 = 0. 82), followed by polyploid (r^2 = 0.78), pooled (r^2 = 0.65), diploid (r^2 = 0.45), and perciform (r^2 = 0.36) actinopterygian regressions. In contrast to the CS–GS regression, the relationship was steeper in polyploid (b = 0.78) than in diploid actinopterygians (b = 0.67), but not significantly so (Fig. 3B, t test, $P > 0.25$). All regressions were highly significant ($P < 0.0001$) at the specific, generic, familial, and ordinal levels with little variation in the nature of the relationship (Table 1). A similar trend was identified in the NS–GS outliers as was noted in the CS–GS relationship. Coldwater species tended to have larger nuclei (large positive NS–GS residuals) than their genome size predicted, while warm-water species were smaller (large negative NS-GS residuals). As a result, 14 of the 20 most extreme positive actinopterygian outliers were coldwater species, while 18 of the 20 most extreme negatives were tropical. In this case, the pattern was weaker in the chondrichthyans, with only 6 of the 10 most extreme outliers in each direction following the large–cold and small–warm distribution for nucleus size.

Cell and nucleus size

Cellular and nuclear areas of erythrocytes were significantly correlated in ray-finned fishes, cartilaginous fishes, and all species combined (Fig. 4A). The correlation was strongest in the combined dataset (r^2 = 0.87) and in cartilaginous fishes (r^2 = 0.86), and was weaker for polyploid (r^2 = 0.71), pooled (r^2 = 0.70), diploid (r^2 = 0.56), and perciform (r^2 = 0.39) actinopterygians. The relationship was steeper in diploid (b = 0.77) than in polyploid (b = 0.61) actinopterygians, but again not significantly so (Fig. 4B; $P > 0.25$). These relationships persisted at the generic, familial, and ordinal level with only minor variations (Table 1), and were highly significant in every case (all $P < 0.0001$).

Cytological effects of intraspecific genome size variation

There was no significant difference in cytogenomic (CS/GS) nor nucleogenomic (NS/GS) ratios between conspecific individuals for those species showing large-scale variation in genome size (Table 2; paired t test, $P > 0.09$). This included aquaculture species in which genome sizes indicated triploidy in some individuals (*Acipenser brevirostrum*, *A. oxyrhynchus*, *Salmo salar*), aquarium and wild-caught species that included triploids (*Synodontis notatus*, *Phoxinus neogaeus*) and tetraploids (*Aprion virescens*), and wild-caught species where the cause of large-scale genome size differences was either unknown (*Lagocephalus lunaris*) or possibly sex based (*Chrysiptera cyanea*).

Discussion

The present results clearly establish that cell and nuclear size both correlate strongly with genome size and with each other in ray-finned and cartilaginous fishes. As these relationships remain significant at each taxonomic level, it is ap-

Table 1. Coefficients of determination (r^2; $P < 0.0001$), intercepts ($y(0)$) and slopes (b) of least-squares regression analysis of log-transformed erythrocyte cell (CS) and nuclear (NS) areas and genome sizes (GS) for 223 species of chondrichthyan, chondrostean, and teleostean fishes.

			CS vs. GS		NS vs. GS		CS vs. NS	
Data set	*n*	Taxonomic level	r^2	$y(0)$, b	r^2	$y(0)$, b	r^2	$y(0)$, b
All species	223	Species	0.82	1.62, 0.74	0.84	0.92, 0.78	0.87	0.81, 0.90
		Genus	0.78	1.63, 0.75	0.81	0.93, 0.77	0.85	0.80, 0.91
		Family	0.80	1.63, 0.75	0.86	0.94, 0.77	0.88	0.75, 0.95
		Order	0.87	1.64, 0.73	0.90	0.95, 0.77	0.92	0.77, 0.92
Chondrichthyans	22	Species	0.81	1.70, 0.75	0.82	0.87, 0.90	0.86	1.08, 0.77
		Genus	0.76	1.77, 0.67	0.76	0.97, 0.79	0.80	1.11, 0.76
		Family	0.76	1.82, 0.61	0.82	0.96, 0.79	0.79	1.20, 0.70
		Order	0.91	1.83, 0.60	0.92	0.98, 0.78	0.77	1.24, 0.67
Actinopterygians (all)	201	Species	0.61	1.66, 0.61	0.65	0.96, 0.64	0.70	0.89, 0.83
		Genus	0.51	1.66, 0.63	0.57	0.96, 0.64	0.64	0.88, 0.83
		Family	0.53	1.66, 0.63	0.65	0.97, 0.63	0.71	0.75, 0.95
		Order	0.61	1.68, 0.59	0.67	1.00, 0.57	0.80	0.72, 0.97
Actinopterygians (diploid)	178	Species	0.41	1.65, 0.65	0.45	0.95, 0.67	0.56	0.95, 0.77
		Genus	0.42	1.65, 0.69	0.48	0.95, 0.69	0.58	0.91, 0.81
		Family	0.46	1.64, 0.73	0.55	0.96, 0.68	0.65	0.75, 0.95
		Order	0.43	1.65, 0.71	0.44	0.99, 0.62	0.69	0.72, 0.96
Actinopterygians (polyploid)	23	Species	0.76	1.68, 0.56	0.78	0.81, 0.78	0.71	1.24, 0.61
		Genus	0.54	1.73, 0.47	0.64	0.81, 0.78	0.36	1.52, 0.39
		Family*	0.51	1.76, 0.40	0.82	0.74, 0.85	0.75	1.35, 0.52
		Order	—	—	—	—	—	—
Order Perciformes	95	Species	0.36	1.64, 0.56	0.36	0.95, 0.60	0.39	1.14, 0.57

*$P < 0.03$.

parent that these relationships exist independent of phylogenetic context. The persistence of the relationships across the perciform order, despite only a 3-fold genome size range (1.06–3.30 pg/N; mean = 1.76), provides further support. The apparent decrease in the strength of each relationship as the analyses were narrowed from the full dataset to the diploid actinopterygians was probably artefactual. First, there is less relative error inherent in the measure of large cells and nuclei, a fact that likely accounts for the higher strength of regressions in the chondrichthyans and polyploids. This effect should extend to genome size estimates, since nuclear areas are used directly in their estimation. Probably most important though, the range of cell, nuclear, and genome sizes used in each regression should greatly influence the strength of the observed relationships, since a greater range results in more interspecific (real) variation relative to intraspecific variation and measurement error, which remain unchanged. This fact undoubtedly explains why r^2 values were highest in the full dataset in every case, followed by the chondrichthyans, polyploids, pooled actinopterygians, and diploid actinopterygians. Regardless, this study demonstrates that cell, nuclear, and genome size are all strongly positively associated in this group, to such a degree that the claim by Van't Hof and Sparrow (1963) that "…(the relationships) enable the estimation of any two of the above three variables, if the third variable is known" appears to hold as true in fishes as in other groups. Of course, since only portions of the variation in each is described by variation in the other(s), direct measurement is preferable to extrapolation from any one of these variables, but in the special case where cell or nuclear sizes can be measured from fossilized cells, the strength, ubiquity, and general constancy of these relationships could enable the estimation of otherwise unknowable characters.

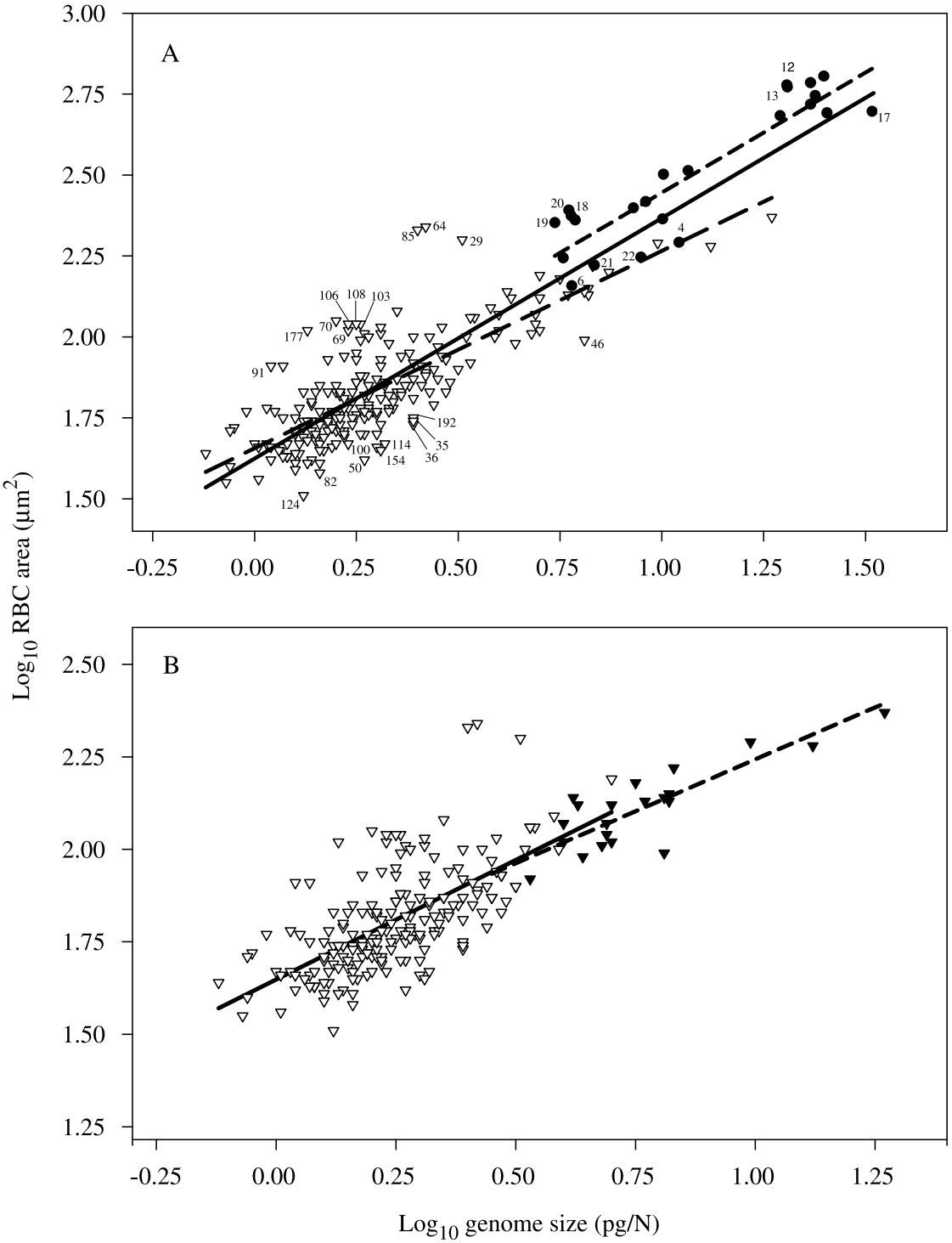
Intraspecific variation

The low intraspecific variation observed in erythrocyte cell and nuclear area measurements for the five species examined in detail demonstrates that small sample sizes are sufficient for qualitative studies of cytological morphology. For studies of cytological variation across few species or very narrow ranges, such as within single orders of fishes or constrained groups like the birds, a higher sample size is desirable. However, in most cases, intraspecific variation and measurement error are negligible relative to interspecific variation in cell and nuclear area.

Nucleus and genome size

Outwardly, the association between nucleus and genome size appears intuitive, and to a certain extent, it is, since the size of a nucleus is, by physical necessity, a function of the amount of DNA that it contains, with any changes in nuclear size independent of DNA content occurring as a result of variation in the degree of folding of the genetic material (Cavalier-Smith 1978). However, the nucleotypic effect of bulk DNA content on cellular and organismal characters may rely on a stepwise relationship between DNA content, nuclear size, cell size, and the organismal phenotype, each affecting the other by turn and as such each step must be verified. This study demonstrates that DNA content effects

Fig. 2. Relationship between dry erythrocyte area and genome size for (A) 223 species of chondrichthyan (●, short-dashed line, r^2 = 0.81, $P < 0.0001$) and actinopterygian (∇, long-dashed line, $r^2 = 0.61$, $P < 0.0001$) fishes (combined regression line solid, $r^2 = 0.82$, $P < 0.0001$). The 20 most extreme (10 positive, 10 negative) actinopterygian and 10 most extreme chondrichthyan (5 positive, 5 negative) outliers are numbered corresponding to data labels in the Appendix. (B) One hundred seventy-eight species of diploid (∇, solid line, r^2 = 0.41, $P < 0.0001$) and 23 species of polyploid (▼, dashed line, $r^2 = 0.76$, $P < 0.0001$) actinopterygians.

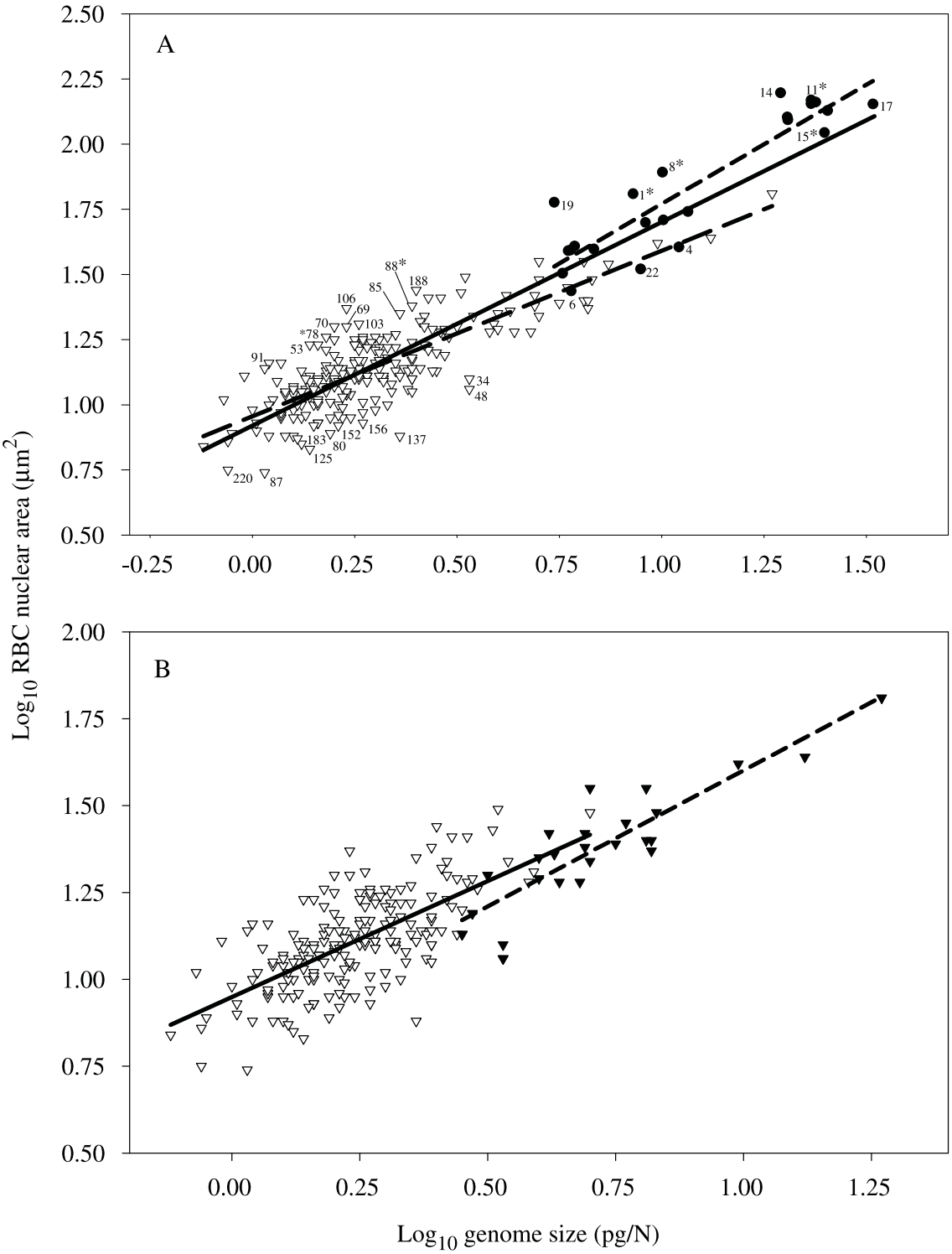


erythrocyte nuclear size in fishes, and supports that this relationship is causal, as evidenced by the constancy of nucleogenomic ratios in neopolyploids relative to those of their diploid progenitors.

Cell and nucleus size

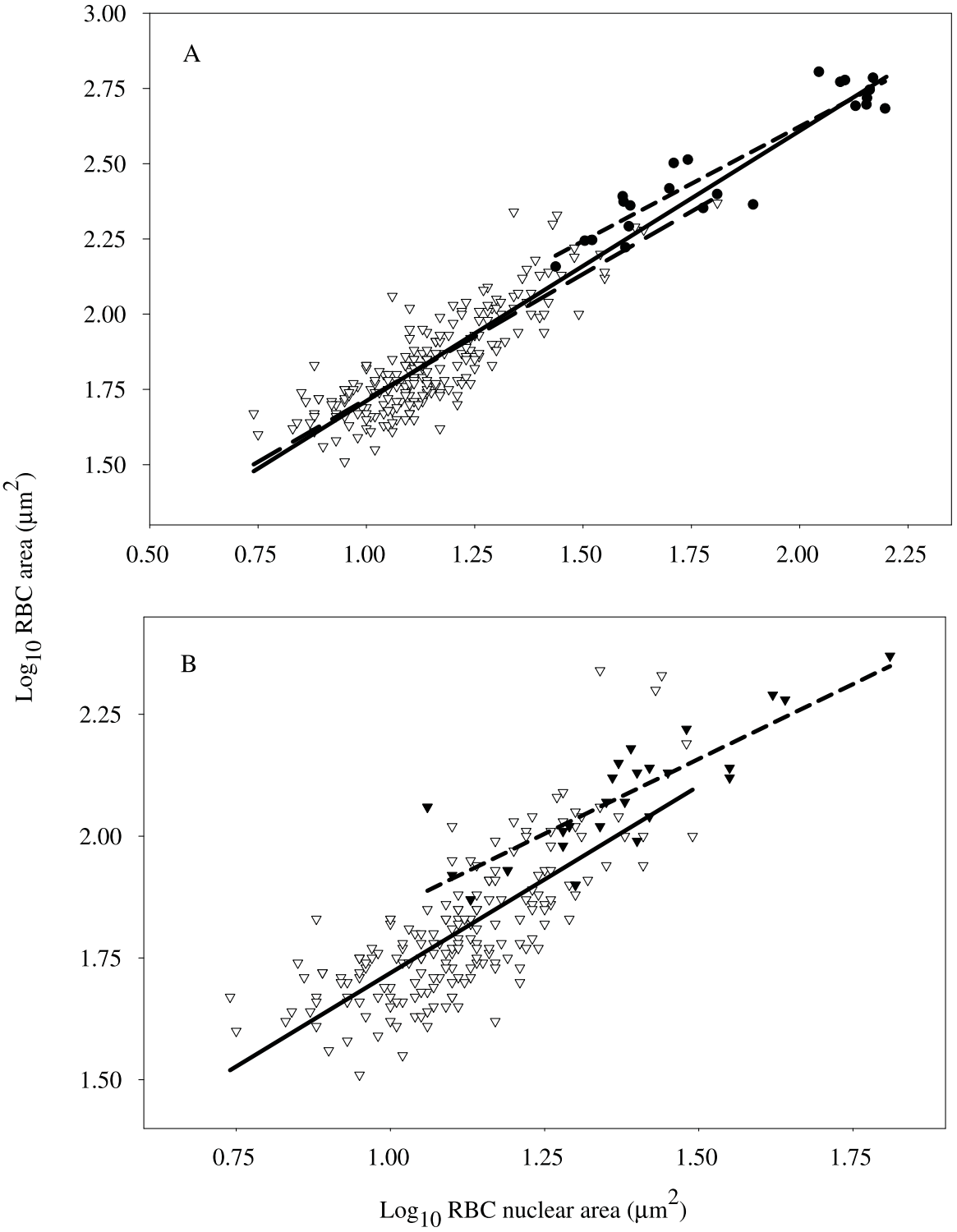
The next "step", the relationship between cell and nuclear size, may provide the link by which genome size exerts cellular and organismal effects, and as such, exposes it to selec-

Fig. 3. Relationship between nuclear area and genome size for (A) 223 species of chondrichthyan (●, short-dashed line, $r^2 = 0.82$, $P < 0.0001$) and actinopterygian (∇, long-dashed line, $r^2 = 0.65$, $P < 0.0001$) fishes (combined regression line solid, $r^2 = 0.84$, $P < 0.0001$). The 20 most extreme (10 positive, 10 negative) actinopterygian and 10 most extreme chondrichthyan (5 positive, 5 negative) outliers are numbered corresponding to data labels in the Appendix. Outliers that do not fit the large–cold, small–warm trend are indicated with an asterisk. (B) One hundred seventy-eigt species of diploid (∇, solid line, $r^2 = 0.45$, $P < 0.0001$) and 23 species of polyploid (▼, dashed line, $r^2 = 0.78$, $P < 0.0001$) actinopterygians.



tion. An earlier failure (Lay and Baldwin 1999) to identify a significant association between erythrocyte nuclear and cell size in fishes is suspect, both intuitively and owing to methodological inconsistencies between measurements of cell volume (measured wet) and nuclear volume (calculated from dry areas), as previously suggested (Gregory 2001*a*). The

Fig. 4. Relationship between dry erythrocyte cell and nuclear area for (A) 223 species of chondrichthyan (●, short-dashed line, $r^2 = 0.86$, $P < 0.0001$) and actinopterygian (∇, long-dashed line, $r^2 = 0.70$, $P < 0.0001$) fishes (combined regression line solid, $r^2 = 0.87$, $P < 0.0001$). (B) One hundred seventy-eight species of diploid (∇, solid line, $r^2 = 0.56$, $P < 0.0001$) and 23 species of polyploid (▼, dashed line, $r^2 = 0.71$, $P < 0.0001$) actinopterygians.



breadth of the current study (more than four times that of Lay and Baldwin's sample size) and the measurement of dry areas of both cell and nucleus size probably accounts for the identification of a strong association between erythrocyte cell and nuclear size in this study. The fact that this association persisted across all species, as well as within each taxonomic subset, powerfully supports the conclusion that nucleus and cell size are strongly associated in the

Table 2. Cytogenomic relationships between conspecific individuals of fish species with highly variable genome size.

Taxon	Data ID	Genome size (pg/N)	RBC area (μm^2)	RBC nuclear area (μm^2)	CS/GS	NS/GS
Acipenser brevirostrum						
diploid	24a	13.22	192.74	43.82	14.58	3.31
triploid	24b	18.63	234.10	64.60	12.56	3.47
Acipenser oxyrhynchus						
diploid	25a	4.38	96.47	18.88	22.02	4.31
triploid	25b	6.61	141.23	23.63	21.38	3.58
Phoxinus neogaeus						
diploid	38a	2.81	94.19	15.91	33.46	5.65
triploid	38b	4.29	132.19	23.12	30.82	5.39
Salmo salar						
diploid	59a	6.55	135.25	25.33	20.66	3.87
triploid	59b	9.80	196.97	42.14	20.10	4.30
Synodontis notatus						
diploid	45a	1.88	55.95	10.27	29.74	5.46
triploid	45b	2.82	74.05	13.50	26.31	4.80
Chrysiptera cyanea						
female	159a	1.64	54.00	9.11	32.97	5.56
male	159b	2.13	66.12	10.11	31.08	4.75
Aprion virescens						
diploid	132a	1.37	62.43	11.69	45.72	8.56
tetraploid (?)	132b	2.61	78.22	17.17	29.96	6.58
Lagocephalus lunaris						
small GS	222a	0.88	50.91	7.30	58.08	8.33
large GS	222b	1.45	58.24	12.98	61.55	13.71

Note: Neither cytogenomic (CS/GS) nor nucleogenomic (NS/GS) ratios differ significantly between conspecifics (paired *t* test, $P > 0.09$ and $P > 0.4$, respectively).

fishes, as in other groups (Cavalier-Smith 1978; Olmo and Odierna 1982; Olmo 1983; Cavalier-Smith 1991; Gregory and Hebert 1999; Gregory 2000; Gregory 2001*b*).

Cell and genome size

Given the universality of the CS–GS relationship across the protist, plant, and animal kingdoms, it would be surprising to find this relationship absent in fishes. That said, an adaptive interpretation of fish genome size variation depends critically upon a strong and causative association with cell size. In light of recent suggestions that this is not the case (Chang et al. 1995), the strong positive associations identified in this study are important. Chang et al. (1995) reported that erythrocyte sizes in one group of cartilaginous fishes, the batoids, were only 50% larger than those of chicken erythrocytes although their genome sizes were five-times larger. This result is dubious (Gregory 2001*a*), given that chicken erythrocytes fit inside the nuclei of most cartilaginous fishes (Fig. 1). Their failure to detect an association between DNA content and erythrocyte size was probably the result of small sample sizes (15 species) and inaccurate sizing of erythrocytes. Unfortunately, since only five batoid fish species were included in the present study, no comment can be made on the CS–GS relationship in this group. However, given the strong and highly significant nature of the CS–GS relationship across the 22 cartilaginous fishes in this study, it is likely that the analysis of dry erythrocyte area across a broader range of genome sizes would reveal the relationship in batoid fishes, as in other groups. With this single proviso, it can be concluded that cell and genome size are strongly associated in fishes.

Constancy of cytogenomic ratios in neopolyploids

Although the strength of the CS–GS association presented here demonstrates that these are correlated in fishes, causality is more difficult to establish. To conclude causation, cell sizes must respond immediately to genome size changes (Mirsky and Ris 1951; Nurse 1985; Benfey 1999). The stability of cytogenomic and nucleogenomic ratios between conspecific individuals showing large-scale variations in DNA content strongly supports the conclusion that genome size affects cell size in a causative manner. The fact that neotriploids of *Acipenser brevirostrum*, *A. oxyrhynchus*, and *S. salar* showed increases in their cell size relative to their diploid conspecifics supports causality, and is consistent with an earlier report (Benfey 1999) of this relationship in *O. mykiss*. Further support is provided by the stable ratios in five other species with variable genome size, although less strongly, since the basis and temporal history of these genome size divergences are less certain.

In a previous study, Pedersen (1971) concluded that cell sizes in fishes return to their diploid values after polyploidization events, since he found that *C. auratus* and *O. mykiss* cell volumes were only 1.09- and 1.93-fold larger, respectively, than those of *Pleuronectes americanus* (1.6 pg/N), contrary to his predictions from the 2- (3.4 pg/N) and 4-fold (6.3 pg/N) larger genomes of these ancient tetraploids. This conclusion is problematic for two reasons.

First, *P. americanus* is a poor choice as a representative diploid ancestor of trout and goldfish and is more properly regarded as a highly specialized descendant of their diploid ancestor. Secondly, his assumption that cell volumes should double after tetraploidization is unfounded, as genome size and cell volume are not related in a direct (1:1) manner. The present study showed that dry cell areas were 1.66- and 2.04-times larger in *C. auratus* and *O. mykiss*, respectively, relative to *P. americanus*, which are greater differences than those reported by Pedersen (1971) for cell volumes. It was Pedersen's misguided expectation that cell volume should double after tetraploidization that led him to conclude that cell volumes had returned to diploid sizes in goldfish and trout. It is much more relevant to note that the cytogenomic ratios for *C. auratus* and *O. mykiss* do not depart far from the CS–GS regression for 209 bony fishes (residuals –0.6 for both), indicating that cell size changes caused by polyploidization are dictated by the species' total DNA content, an effect that appears constant over time. Furthermore, the constancy of cytogenomic ratios for the eight neopolyploid species in this study suggests that the effects of genome size on cell size are particularly strong in fishes, and (or) that cell size is less strongly constrained in this group. Despite constant cytogenomic ratios within species of variable genome size, the lowered slope of the CS–GS regression for ancient (i.e., natural) polyploids relative to that of the diploids suggests, as might be expected, that cell size becomes more constrained with greater absolute increases in genome size. This is consistent with the fact that cell sizes in lungfishes, although massive, are smaller than predicted from the cytogenomic ratios of actinopterygians (Gregory 2001*b*). Thus, these results support the conclusion that the strong cytogenomic associations identified in this study are achieved via the nucleotypic effect of DNA content, which may be particularly significant for fishes, as cell sizes may be less strictly constrained than in other vertebrates.

Variation among fish groups

The allometric relationship between cell and genome size can be represented by the equation $V = kC^{\alpha}$, where V is cell size (volume, area, or diameter), k is a constant that varies depending on the cell-size parameter considered, C is genome size, and α is the slope of the log–log relationship between cell size and genome size (Gregory 2001*a*). The CS–GS slopes observed in this study ($\alpha = 0.5$–0.8) are, in general, consistent with those reported for other vertebrates including anuran amphibians ($\alpha \cong 0.8$; data from Horner and Macgregor 1983), birds ($\alpha \cong 0.6$; Gregory 2002), mammals ($\alpha \cong 0.6$; Gregory 2000), and reptiles ($\alpha \cong 0.9$; Gregory 2001*b*). However, issues of phylogenetic non-independence can greatly influence the nature of these regressions (Harvey and Pagel 1991), such that comparisons of α values can only be given moderate weight (Gregory 2001*a*).

However, the difference, shown in Fig. 2A, may be biologically significant even if not statistically so, since it is consistent with trends in a different dataset (Gregory 2001*b*). In particular, the low metabolic rates, slow development, and growth rates of cartilaginous fishes (Compagno 1984) may favour larger cells relative to ray-finned fishes. Negative associations between cell size, genome size, and metabolic rate processes have been identified both at the cellular level (Smith 1925; Goniakowska 1970) and in homeothermic vertebrates (Vinogradov 1995; Vinogradov 1997; Gregory 2002). Since RBCs function primarily to provide a surface for gas transport and exchange mediated by haemoglobin, it is not surprising that animals with high metabolic rates tend to maximize total cell surface area while minimizing diffusion distance by minimizing cell size (Holland and Forster 1966). The negative association between erythrocyte size and aerobic swimming performance is well established in fishes (Wilhelm Filho et al. 1992) and, given that elasmobranch fishes are thought to have about five-fold lower metabolic rates than teleostean fishes (Brett 1970), it is no surprise that the latter tend to have larger cells than the former, independent of genome size differences. Likewise, developmental rates, which are negatively related to cell and genome size in many groups (Bennett 1971), are also very slow in elasmobranchs (Compagno 1984). Although cell division and developmental rate associations with cell size have not been identified in fishes, their existence at the cellular level and in other closely allied vertebrates suggests that these factors may play a role in explaining the large cells of the chondrichthyans. These trends follow suggestions that *K*-selected species should have larger cell and (or) genome sizes than their *r*-selected counterparts (Cavalier-Smith 1978; Szarski 1983). Because of their large body size, low metabolic rate, slow growth, delayed maturity, and low fecundity, chondrichthyans are *K*-selected relative to the *r*-selected actinopterygians. Despite the fact that there is very little overlap between the two groups, it is unlikely that the larger cells of the chondrichthyans are simply an artefact of their larger genome size range, since species at the top end of CS–GS regressions tend to have lower CS/GS ratios, not higher (present study; Gregory 2001*b*). This trend is suggestive of differential selection pressures and constraints on cell size between these groups, given that they occur independently of changes in genome size, which may be a driving force behind the drastically diverged genome sizes of cartilaginous and bony fishes.

Hot and cold: cytogenomic ratios vary with temperature

The present study has revealed that cold-water species have larger cells and nuclei than warm-water species with the same genome size. This result is interesting on several fronts, as it highlights the adaptive significance of cell size. The trend for larger cells in cold-water rather than in warm-water species suggests that temperature effects may account for some of the remaining variation in cell sizes independent of genome size. That this relationship extends to some degree to the nuclear level is perplexing, and suggests that cell and nuclear sizes may be tightly linked, even when the variation in both is independent of genome size. This provides some support for the idea that large cells require large nuclei for balanced growth, as suggested by Cavalier-Smith (1978), but does not discount that his nucleoskeletal theory can not account for the observed causal influence of genome size on cell size presented in this study and others. It should be noted that the trend is slightly less strong in the NS–GS regression, possibly because measurement error is greater in nucleus- than in cell-area measurements, owing to their smaller size. That no strong trend is evident in the NS–CS

regression is due, of course, to the fact that both erythrocyte cell and nuclear area increase in concert at lower temperatures relative to genome size.

The observed trend is consistent with various ontogenetic, metabolic, and ecological differences between warm- and cold-water fish groups, very much in parallel with the discussion of larger cells in cartilaginous relative to bony fishes. It has been well documented that fishes develop more slowly at lower temperatures (Johnston 1990). Given the well-established correlations between cell size and developmental rate (Vaughn and Locy 1969; Sessions and Larson 1987; Chipman et al. 2001), it is not surprising that cell sizes tend to be smaller in warm-water than in coldwater-water fishes, as required by the rapid development, maturation, and growth of the former.

Reduced temperatures slow molecular diffusion and enzyme reaction rates, such that cold-water fishes have much slower metabolisms than warm-water species (Johnston 1990). Whether this difference persists independent of temperature has been the subject of debate (Scholander et al. 1953), but it now appears that coldwater fishes have lower metabolic rates independent of any "metabolic cold adaptation" (Holeton 1974). The difference in cytogenomic ratios between cold- and warm-water species then, may be due in part to the lower metabolic rates in the former, relative to the higher oxygen-diffusion and -carrying capacities required by the latter's higher metabolic rates. It has been suggested that low metabolic rates may be adaptive in cold-dwelling poikilotherms in response to low temperature and the associated high oxygen concentration, as well as the highly seasonal nature of food supplies, which require lengthy periods of near dormancy (Johnston 1990). It is interesting to note that other species that undergo drastic seasonal resource limitations, such as lungfishes, also have massive cells and genomes. In addition, the high oxygen concentration of frigid waters allows cold-water fishes a degree of metabolic compromise. For example, the Antarctic icefish, *Chaenocephalus aceratus*, has nearly done away with erythrocytes, relying instead on its low metabolism and the high oxygen levels of cold waters to meet its respiratory demands (Barber et al. 1981). In parallel with the higher cytogenomic ratios of cartilaginous fishes relative to bony fishes, this pattern also supports views of cell and genome size variation across the *r*–*K* continuum, as cold- and deep-water fishes are clearly more *K*-selected than are *r*-selected tropical species.

Furthermore, these results support "Bergmann's rule", which states that smaller cell and individual size is in some way adaptive at higher temperatures. This rule was originally proposed to explain clinal variation in the body size of endothermic animals, with larger individuals occurring at higher latitudes and altitudes (Mayr 1963). However, similar clinal patterns have since been observed in ectotherms including amphibians (Berven 1982), houseflies (Bryant 1977), and fruitflies (David and Bocquet 1975; Coyne and Beecham 1987) and this trend derives, at least in part, from increased cell size in individuals from colder climates (Patridge et al. 1994). Cold acclimation also increases the cross-sectional area of both skeletal (Jones and Siddel 2002) and cardiac (Rodnick and Siddel 1997) muscle cells in striped bass (*Morone saxatilis*), but this is distinct from the adaptive advantage of large cells in cold climates (and vice versa). Thus, the large cells and nuclei of cold-water fishes relative to warm-water fishes with the same genome size provide the first adaptive example of Bergmann's rule in this group, and reveals a factor (other than DNA content) that contributes to cell size variation. Further evidence that large cells are selected for in frigid settings (or selected against in tropical species) is the relative success of large-celled polyploid fishes (and other animals) at high latitudes, and their paucity in tropical waters. For example, the ancient polyploid salmoniform fishes dominate northern freshwaters. Similarly to the elevated cytogenomic ratios of cartilaginous fishes, this trend highlights differential selection pressures on cell size at different temperatures, and its implications for environmental influences on genome evolution are considered elsewhere (Hardie and Hebert, in review).

The results of this study do not rule out the possibility that the accumulation of "junk" or "selfish" DNA or that the cumulative deletion of small genomic elements contribute to genome size diversity. In fact, the strength and ubiquity of the associations among genome, nucleus, and cell sizes demonstrate how such gradual processes could reach a threshold, beyond which further accumulation (or deletion) would be selected against owing to their effects on cell size and related phenotypic characters. However, only the nucleotypic theory accounts for the persistence of similar correlations after significant decreases and quantum shifts in DNA content (Gregory 2001*a*) and, as such, remains the most parsimonious theory of genome size evolution.

The strong positive associations among genome, nucleus, and cell sizes and the evidence for the causal nature of these relationships reaffirm that the nucleotypic effects of DNA content entrain cytological characters in fishes. Consequently, since significant changes in DNA content result in commensurate changes in nucleus and cell size, interpretation of patterns of fish genome size variation can and should be approached from an adaptive perspective.

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Appendix A

Table A1. Erythrocyte cell and nuclear areas and genome sizes for 223 fish species including 22 chondrichthyans (C) and 201 actinopterygians.

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Superclass Gnathostomata										
Class Chondrichthyes										
Order Orectolobiformes										
Ginglymostomatidae (nurse sharks)										
Nebrius ferrugineus	1	C	250.48	64.46	8.51	29.44	7.57	0.006	0.103	–0.080
Orectolobidae (wobbegons)										
Orectolobus ornatus	2	C	318.06	51.17	10.09	31.52	5.07	0.054	–0.065	0.101
Hemiscyllidae (bamboo sharks)										
Chiloscyllium punctatum	3	C	261.88	50.01	9.12	28.70	5.48	0.003	–0.035	0.024
Hemiscyllium ocellatum	4	C	195.86	40.31	11.02	17.78	3.66	–0.184	–0.203	–0.031
Order Carcharhiniformes										
Carcharhinidae (requiem sharks)										
Carcharhinus brachyurus	5	C	175.36	31.92	5.72	30.64	5.58	–0.021	–0.045	–0.002
Carcharhinus melanopterus	6	C	143.91	27.33	6.01	23.95	4.55	–0.123	–0.132	–0.036
Sphyrnidae (hammerhead sharks)										
Sphyrna lewini	7	C	229.69	40.63	6.13	37.50	6.63	0.074	0.033	0.035
Order Lamniformes										
Lamnidae (mackerel / white sharks)										
Isurus oxyrinchus	8	C	231.39	78.07	10.04	23.04	7.77	–0.082	0.121	–0.177
Order Squaliformes										
Dalatiidae (sleeper sharks)										
Etmopterus brachyurus	9	C	556.70	144.96	23.77	23.42	6.10	0.021	0.047	–0.001
Etmopterus granulosus	10	C	492.36	134.46	25.43	19.36	5.29	–0.054	–0.012	–0.029
Centroscymnus crepidater	11	C	609.98	147.57	23.16	26.34	6.37	0.069	0.065	0.033
Centroscymnus coelolepis	12	C	599.60	127.09	20.26	29.60	6.27	0.105	0.054	0.075
Centroscymnus owstoni	13	C	591.67	124.07	20.33	29.10	6.10	0.098	0.042	0.077
Centroscymnus plunketi	14	C	482.56	157.33	19.51	24.73	8.06	0.023	0.161	–0.090
Oxynotus bruniensis	15	C	639.02	110.68	24.98	25.58	4.43	0.065	–0.090	0.148
Squalidae (dogfish sharks)										
Squalus acanthias	16	C	326.42	55.17	11.60	28.15	4.76	0.021	–0.087	0.087
Order Squatiniformes										
Squatinidae (angel sharks)										
Squatina australis	17	C	497.46	142.49	32.81	15.16	4.34	–0.131	–0.088	–0.044
Order Rajiformes										
Rhinobatidae (guitarfishes)										
Aptychotrema rostrata	18	C	236.70	39.20	5.99	39.53	6.55	0.094	0.027	0.060
Rajidae (skates)										
Bathyraja parmifera	19	C	225.29	59.84	5.46	41.25	10.96	0.103	0.247	–0.101
Leucoraja ocellata	20	C	246.45	39.01	5.91	41.70	6.60	0.116	0.030	0.079

Table A1. (*continued*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Dasyatidae (rays)										
Himantura signifer	21	C	166.70	39.57	6.83	24.42	5.80	–0.100	–0.021	–0.095
Dasyatis bennetti	22	C	176.42	33.16	8.88	19.86	3.73	–0.160	–0.203	–0.012
Class Actinopterygii										
Subclass Chondrostei										
Order Polypteriformes										
Polypteridae (bichirs)										
Polypterus palmas	23	D	158.87	34.72	7.39	21.50	4.70	0.014	0.032	0.034
Order Acipenseriformes										
Acipenseridae (sturgeons)										
Acipenser brevirostrum	24a	P	192.74	43.82	13.22	14.58	3.31	–0.058	–0.027	0.031
(triploid)	24b	P	234.10	64.60	18.63	12.56	3.47	–0.060	0.048	–0.021
Acipenser oxyrhynchus	25a	P	96.47	18.88	4.38	22.02	4.31	–0.066	–0.082	0.030
(triploid)	25b	P	141.23	23.63	6.61	21.38	3.58	–0.006	–0.106	0.125
Subclass Neopterygii										
Division Teleostei										
Order Anguilliformes										
Anguillidae (freshwater eels)										
Anguilla rostrata	26	D	101.88	16.71	2.03	50.19	8.23	0.165	0.068	0.110
Chlopsidae (false morays)										
Ophichthus cephalozona	27	D	99.34	20.24	3.92	25.37	5.17	–0.015	–0.020	0.025
Muraenidae (morays)										
Sideria picta	28	D	123.73	18.99	3.81	32.45	4.98	0.081	–0.044	0.140
Synaphobranchidae (cutthroat eels)										
Diastobranchus capensis	29	D	201.00	26.75	3.24	62.09	8.26	0.333	0.151	0.225
Muraenesocidae (pike congers)										
Muraenesox cinereus	30	D	87.77	22.62	2.27	38.68	9.97	0.065	0.166	–0.068
Order Clupeiformes										
Chirocentridae (wolf herrings)										
Chirocentrus dorab	31	D	57.50	12.49	1.63	35.17	7.64	–0.024	0.011	–0.040
Clupeidae (herrings)										
Sardinella gibbosa	32	D	59.94	15.09	2.13	28.18	7.09	–0.077	0.015	–0.087
Nematalosa come	33	D	62.94	10.85	1.76	35.84	6.18	–0.002	–0.068	0.050
Order Cypriniformes										
Cyprinidae (carps)										
Carassius auratus	34	P	82.69	12.72	3.35	24.67	3.79	–0.059	–0.192	0.120
Epalzeorhychos frenatum	35	D	55.17	14.73	2.44	22.61	6.03	–0.153	–0.033	–0.119
Epalzeorhychos bicolor	36	D	53.20	12.65	2.46	21.62	5.14	–0.163	–0.103	–0.070
Labeo chrysophekadion	37	D	60.03	11.98	2.20	27.23	5.44	–0.083	–0.091	–0.004
Phoxinus neogaeus	38a	D	94.19	15.91	2.81	33.46	5.65	0.040	–0.041	0.087
(triploid)	38b	P	132.19	23.12	4.29	30.82	5.39	0.080	0.005	0.104

Table A1. (*continued*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Semotilus corporalis	39	D	74.07	14.99	2.46	30.11	6.09	–0.023	–0.023	0.003
Gyrinocheilidae (algae-eaters)										
Gyrinocheilus aymonieri	40	D	40.43	7.57	1.25	32.42	6.07	–0.107	–0.139	–0.007
Catostomidae (suckers)										
Catostomus catostomus catostomus	41	P	137.82	26.57	4.18	32.97	6.36	0.106	0.071	0.074
Catostomus commersoni	42	P	105.44	21.97	5.05	20.88	4.35	–0.062	–0.060	0.020
Order Characiformes										
Characidae (characins)										
Colossoma macropomum	43	D	71.90	18.08	3.00	23.93	6.02	–0.088	0.000	–0.073
Order Siluriformes										
Plotosidae (eel-tailed catfishes)										
Euristhmus lepturus	44	D	114.00	21.90	3.49	32.66	6.28	0.075	0.042	0.060
Mochokidae (squeakers)										
Synodontis notatus	45a	D	55.95	10.27	1.88	29.74	5.46	–0.070	–0.117	0.025
“ (triploid)	45b	P	74.05	13.50	2.82	26.31	4.80	–0.060	–0.111	0.045
Callichthyidae (armoured catfishes)										
Corydoras metae	46	P	98.71	25.09	6.43	15.36	3.90	–0.159	–0.070	–0.060
Loricariidae (armoured catfishes)										
Farlowella acus	47	P	84.53	15.65	2.97	28.44	5.27	–0.012	–0.064	0.055
Liposarcus pardalis	48	P	113.83	11.43	3.38	33.70	3.38	0.081	–0.232	0.293
Hypostomus plecostomus	49	P	79.06	19.84	3.16	24.98	6.27	–0.060	0.027	–0.067
Order Gymnotiformes										
Rhamphichthyidae (sand knifefishes)										
Rhamphichthys rostratus	50	D	41.83	14.79	1.86	22.46	7.94	–0.200	0.043	–0.239
Order Esociformes										
Esocidae (pikes)										
Esox lucius	51	D	73.84	16.63	2.22	33.24	7.49	0.001	0.042	–0.030
Esox niger	52	D	75.30	13.72	1.83	41.12	7.49	0.066	0.020	0.046
Order Osmeriformes										
Osmeridae (smelts)										
Osmerus mordax	53	D	61.20	17.10	1.37	44.58	12.45	0.049	0.186	–0.118
Order Salmoniformes										
Salmonidae (salmonids)										
Subfamily Coregoninae										
Coregonus clupeaformis	54	P	118.22	23.95	4.88	24.24	4.91	–0.006	–0.014	0.037
Prosopium cylindraceum	55	P	105.51	19.47	3.96	26.62	4.91	–0.001	–0.046	0.062
Subfamily Salmoninae										
Oncorhynchus tschawytscha	56	P	109.19	26.35	4.91	22.26	5.37	–0.036	0.026	–0.026
Oncorhynchus keta	57	P	131.08	35.16	4.99	26.27	7.05	0.038	0.150	–0.054
Oncorhynchus mykiss	58	P	101.65	19.19	4.80	21.18	4.00	–0.060	–0.107	0.060
Salmo salar	59a	P	135.25	25.33	6.55	20.66	3.87	–0.026	–0.076	0.080

Table A1. (*continued*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
(triploid)	59b	P	196.97	42.14	9.80	20.10	4.30	0.031	0.036	0.057
Salvelinus alpinus (Labrador)	60a	P	164.56	30.27	6.72	24.47	4.50	0.058	–0.002	0.104
Salvelinus alpinus (Somerset Is.)	60b	P	135.35	28.09	5.84	23.16	4.81	0.005	0.006	0.039
Salvelinus namaycush	61	P	150.45	24.55	5.68	26.49	4.32	0.067	–0.042	0.139
Salvelinus fontinalis	62	P	137.53	35.09	6.43	21.40	5.46	–0.009	0.080	–0.034
Subfamily Thymallinae										
Thymallus arcticus	63	P	116.93	22.40	3.98	29.37	5.63	0.049	0.014	0.062
Order Stomiiformes										
Stomiidae (barbeled dragonfishes)										
Idiacanthus atlanticus	64	D	218.07	21.71	2.61	83.61	8.32	0.428	0.118	0.340
Order Aulopiformes										
Synodontidae (lizardfishes)										
Synodus sageneus	65	D	67.32	16.32	2.68	25.16	6.10	–0.088	–0.018	–0.062
Order Myctophiformes										
Neoscopelidae (neoscopelid lanternfishes)										
Neoscopelus macrolepidotus	66	D	153.71	29.93	5.04	30.50	5.94	0.108	0.080	0.074
Order Gadiiformes										
Macrouridae (grenadiers / rattails)										
Caelorinchus innotabilis	67	D	67.35	12.35	1.59	42.49	7.79	0.052	0.008	0.038
Caelorinchus maurofasciatus	68	D	67.07	12.82	1.50	44.67	8.54	0.065	0.040	0.021
Coryphaenoides serrulatus	69	D	105.73	20.05	1.70	62.15	11.79	0.224	0.199	0.053
Mesobius antipodum	70	D	112.19	20.07	1.58	70.98	12.70	0.272	0.218	0.083
Macrourus berglax	71	D	62.17	12.82	1.89	32.92	6.79	–0.036	–0.023	–0.019
Phycidae (phycid hakes)										
Gaidropsarus ensis	72	D	86.95	13.81	1.66	52.46	8.33	0.150	0.045	0.106
Gadidae (cods)										
Gadus morhua	73	D	76.51	17.33	1.86	41.18	9.33	0.060	0.113	–0.037
Boreogadus saida	74	D	89.24	12.65	1.77	50.53	7.16	0.142	–0.014	0.150
Merlucciidae (hakes)										
Urophycis tenuis (musicki)	75	D	72.90	17.76	1.78	40.90	9.97	0.052	0.136	–0.065
Moridae (morid cods)										
Halargyreus johnsonii	76	D	102.21	18.26	1.88	54.36	9.71	0.190	0.133	0.077
Order Lophiiformes										
Lophiidae (goosefishes)										
Lophius americanus	77	D	84.21	17.70	2.06	40.92	8.60	0.085	0.098	0.005
Antennariidae										
Tetrabrachium ocellatum	78	D	84.62	18.19	1.53	55.35	11.90	0.165	0.190	–0.003
Order Mugiliformes										
Mugilidae (mullets)										
Myxus elongatus	79	D	51.14	8.91	1.68	30.47	5.31	–0.080	–0.145	0.034
Liza vaigiensis	80	D	52.82	7.78	1.55	34.16	5.03	–0.052	–0.186	0.094

Table A1. (*continued*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Order Beloniformes										
Belonidae (needlefishes)										
Platybelone argalus platyura	81	D	74.71	18.20	2.00	37.33	9.09	0.031	0.114	–0.063
Order Cyprinodontiformes										
Aplocheilidae (rivulines)										
Pachypanchax playfairii	82	D	37.63	8.58	1.46	25.79	5.88	–0.173	–0.127	–0.079
Poeciliidae										
Poecilia mexicana	83	D	60.97	13.40	2.76	22.08	4.85	–0.134	–0.105	–0.035
Order Beryciformes										
Diretmidae (spinyfins)										
Diretmichthys parini	84	D	106.75	19.01	2.92	36.61	6.52	0.094	0.033	0.080
Order Zeiformes										
Oreosomatidae (oreos)										
Neocyttus rhomboidalis	85	D	213.83	27.68	2.52	84.91	10.99	0.430	0.231	0.247
Zeidae (dories)										
Zenopsis nebulosus	86	D	100.99	23.88	2.47	40.95	9.68	0.107	0.177	–0.033
Order Gasterosteiformes										
Syngnathidae (seahorses and pipefishes)										
Syngnathus scovelli	87	D	46.96	5.46	1.07	43.73	5.09	–0.004	–0.234	0.169
Corythoichthys intestinalis	88	D	101.04	25.42	2.68	37.66	9.47	0.082	0.182	–0.058
Hippocampus abdominalis	89	D	81.26	14.44	1.17	69.46	12.34	0.212	0.160	0.060
Fistulariidae (cornetfishes)										
Fistularia petimba	90	D	51.76	12.93	1.38	37.53	9.38	–0.031	0.066	–0.099
Macroramphosidae (snipefishes)										
Centriscops humerosus	91	D	81.62	14.36	1.10	74.02	13.02	0.230	0.179	0.060
Centriscidae (shrimpfishes)										
Aeoliscus strigatus	92	D	52.75	7.74	0.90	58.62	8.60	0.095	–0.033	0.094
Order Scorpaeniformes										
Scorpaenidae (scorpionfishes / firefishes)										
Helicolenus percoides	93	D	70.46	12.93	1.92	36.76	6.74	0.024	–0.023	0.041
Helicolenus barathri	94	D	65.95	13.36	1.92	34.29	6.94	–0.006	–0.003	–0.005
Sebastes atlanticus	95	D	81.20	14.78	2.02	40.21	7.32	0.065	0.018	0.051
Sebastes polyspinis	96	D	58.58	17.36	1.96	29.94	8.87	–0.062	0.101	–0.147
Sebastes alutus	97	D	59.73	12.82	1.90	31.36	6.73	–0.046	–0.023	–0.029
Scorpaena cardinalis	98	D	87.20	25.53	2.86	30.47	8.92	0.004	0.163	–0.118
Scorpaenopsis oxycepgus	99	D	70.87	11.49	2.40	29.58	4.80	–0.037	–0.137	0.083
Pterois volitans	100	D	45.79	10.47	1.97	23.19	5.30	–0.179	–0.126	–0.074
Platycepgidae (flatheads)										
Platycepgus indicus	101	D	51.54	11.93	1.59	32.52	7.53	–0.068	–0.002	–0.074
Cottidae (sculpins)										
Icelus spatula	102	D	67.11	9.93	1.60	41.84	6.19	0.046	–0.089	0.113

Table A1. (*continued*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Triglopsis quadricornis	103	D	109.58	20.44	1.84	59.54	11.10	0.226	0.190	0.065
Hemitripteridae (goosefishes)										
Hemitripterus americanus	104	D	84.27	14.79	1.79	46.98	8.25	0.122	0.056	0.071
Agonidae (poachers)										
Podothecus acipenserinus	105	D	53.99	16.33	1.51	35.87	10.85	–0.035	0.140	–0.162
Cyclopteridae (lumpfishes)										
Cyclopteropsis jordani	106	D	109.64	23.23	1.71	64.17	13.60	0.244	0.269	0.015
Eumicrotremus spinosus	107	D	98.97	16.74	1.90	52.02	8.80	0.174	0.087	0.100
Liparidae (snailfishes)										
Liparis tunicatus	108	D	108.82	16.92	1.76	61.78	9.61	0.232	0.116	0.132
Order Perciformes										
Moronidae (temperate basses)										
Morone saxatilis	109	D	57.33	12.23	1.90	30.15	6.43	–0.066	–0.043	–0.032
Serranidae (groupers)										
Cephalopholis cyanostigma	110	D	82.95	17.53	2.45	33.82	7.15	0.027	0.037	0.003
Epinephelus cyanopodus	111	D	64.82	13.91	2.05	31.56	6.77	–0.035	–0.012	–0.024
Epinephelus merra	112	D	73.76	14.42	2.23	33.05	6.46	0.001	–0.018	0.020
Epinephelus spilotoceps	113	D	56.34	11.18	1.51	37.33	7.41	–0.015	–0.020	–0.009
Plectopomus leopardus	114	D	47.12	12.66	2.08	22.68	6.09	–0.181	–0.058	–0.130
Aulacocephalus temmincki	115	D	49.70	16.04	1.99	24.94	8.05	–0.139	0.064	–0.192
Opistognathidae (jawfishes)										
Opistognathus (unknown sp.)	116	D	95.06	18.08	2.12	44.82	8.52	0.123	0.095	0.047
Priacanthidae (bigeyes)										
Priacanthus tayenus	117	D	46.47	10.98	1.68	27.63	6.53	–0.126	–0.061	–0.080
Apogonidae (cardinalfishes)										
Apogon savayensis	118	D	71.44	17.99	1.60	44.62	11.23	0.072	0.168	–0.075
Apogon kallopterus	119	D	66.46	14.70	1.62	41.08	9.08	0.036	0.081	–0.039
Apogon guamensis	120	D	70.03	13.96	2.59	27.04	5.39	–0.056	–0.076	0.016
Apogon endetakaena	121	D	70.61	12.99	2.07	34.16	6.29	–0.001	–0.048	0.041
Apogon cookii	122	D	66.93	13.62	2.23	30.07	6.12	–0.039	–0.048	0.005
Sillaginidae (whitings)										
Sillaginodes punctata	123	D	50.68	10.90	1.26	40.30	8.67	–0.017	0.021	–0.050
Sillago analis	124	D	32.34	9.01	1.33	24.32	6.78	–0.219	–0.081	–0.166
Echeneidae (remoras)										
Echeneis naucrates	125	D	42.16	6.79	1.37	30.82	4.97	–0.121	–0.214	0.044
Coryphaenidae (dolphinfishes)										
Coryphaena hippurus	126	D	42.56	11.06	1.21	35.17	9.14	–0.074	0.034	–0.120
Carangidae (jacks)										
Carangoides aurochs	127	D	38.74	9.56	1.27	30.43	7.51	–0.127	–0.039	–0.111
Carangoides fulvoguttatus	128	D	48.63	9.96	1.30	37.27	7.63	–0.039	–0.031	–0.027
Carangoides gymnostethus	129	D	45.45	8.89	1.18	38.42	7.52	–0.038	–0.050	–0.016

Table A1. (*continued*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Seriola hippos	130	D	46.03	10.25	1.53	30.00	6.68	–0.112	–0.066	–0.065
Leiognathidae (slipmouths and ponyfishes)										
Leiognathus decorus	131	D	40.37	11.43	1.35	29.88	8.46	–0.125	0.022	–0.157
Lutjanidae (snappers)										
Aprion virescens	132a	D	62.43	11.69	1.37	45.72	8.56	0.059	0.026	0.025
(tetraploid?)	132b	D	78.22	17.17	2.61	29.96	6.58	–0.022	0.008	–0.018
Lutjanus fluviflamma	133	D	56.36	11.26	2.47	22.84	4.56	–0.143	–0.153	–0.009
Lutjanus sebae	134	D	57.79	11.62	1.59	36.32	7.31	–0.018	–0.012	–0.015
Lutjanus vitta	135	D	57.70	9.47	2.01	28.68	4.71	–0.079	–0.166	0.059
Lutjanus carponotatus	136	D	62.37	11.19	2.19	28.54	5.12	–0.063	–0.121	0.041
Caesio cuning	137	D	67.28	7.56	2.31	29.14	3.27	–0.045	–0.304	0.213
Gerreidae (mojarras)										
Gerres subfasciatus	138	D	46.74	7.54	1.21	38.58	6.22	–0.034	–0.126	0.053
Haemulidae (sweetlips and grunts)										
Diagramma labiosum	139	D	49.30	9.67	1.67	29.49	5.79	–0.100	–0.105	–0.019
Lethrinidae (emperors)										
Lethrinus atkinsoni	140	D	63.95	12.54	2.44	26.24	5.15	–0.083	–0.103	0.010
Lethrinus nebulosus	141	D	65.57	13.00	2.28	28.75	5.70	–0.055	–0.074	0.011
Lethrinus rubrioperculatus	142	D	71.09	13.65	2.34	30.37	5.83	–0.031	–0.050	0.016
Nemipteridae (monocle breams and spinecheeks)										
Scolopsis monogramma	143	D	51.41	13.47	1.53	33.69	8.83	–0.055	0.060	–0.115
Mullidae (goatfishes)										
Mulloidichthys vanicolensis	144	D	42.44	11.15	1.21	35.10	9.22	–0.074	0.044	–0.129
Upeneichthys lineatus	145	D	51.11	11.86	1.27	40.25	9.34	–0.007	0.051	–0.065
Drepanidae (sicklefishes)										
Drepane punctata	146	D	54.62	10.43	1.44	37.83	7.23	–0.013	–0.037	0.006
Chaetodontidae (butterflyfishes)										
Chaetodon lunulatus	147	D	54.87	12.23	1.39	39.41	8.78	–0.001	0.046	–0.052
Chaetodon rainfordi	148	D	44.22	11.64	1.50	29.56	7.78	–0.109	0.007	–0.125
Pomacanthidae (angelfishes)										
Centropyge bicolor	149	D	48.13	11.60	1.40	34.31	8.27	–0.067	0.009	–0.087
Teraponidae (tiger perches and grunters)										
Pelates quadrilineatus	150	D	52.53	8.83	1.55	33.86	5.69	–0.052	–0.126	0.044
Terapon jarbua	151	D	46.60	9.96	1.29	36.25	7.75	–0.053	–0.025	–0.047
Terapon puta	152	D	51.28	8.33	1.61	31.91	5.18	–0.074	–0.169	0.059
Pomacentridae (damselfishes)										
Amphiprion akindynos	153	D	57.56	14.55	1.77	32.47	8.21	–0.048	0.046	–0.090
Amphiprion clarkii	154	D	44.92	12.89	2.05	21.90	6.29	–0.195	–0.042	–0.159
Premnas biaculeatus	155	D	59.77	16.38	1.82	32.90	9.01	–0.034	0.090	–0.112
Chromis analis	156	D	50.29	8.50	1.86	27.09	4.58	–0.120	–0.197	0.041
Chromis viridis	157	D	59.50	9.24	1.85	32.15	4.99	–0.050	–0.157	0.078

Table A1. (*continued*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Dascyllus aruanus	158	D	55.70	9.00	1.74	32.08	5.18	–0.052	–0.158	0.074
Chrysiptera cyanea (male)	159a	D	66.12	10.11	2.13	31.08	4.75	–0.037	–0.165	0.103
(female)	159b	D	54.00	9.11	1.64	32.97	5.56	–0.054	–0.129	0.046
Dischistodus prosopotaenia	160	D	55.58	14.21	1.52	36.52	9.33	–0.025	0.080	–0.102
Hemiglyphidodon plagiometopon	161	D	72.42	16.94	1.78	40.60	9.50	0.052	0.116	–0.048
Pomacentrus nagasakiensis	162	D	59.95	11.19	1.71	35.09	6.55	–0.016	–0.051	0.021
Labridae (wrasses)										
Coris batuensis	163	D	46.54	12.49	1.60	29.05	7.80	–0.108	0.018	–0.130
Choerodon cepgotes	164	D	59.39	14.44	2.01	29.60	7.19	–0.069	0.014	–0.080
Choerodon fasciatus	165	D	73.19	12.36	2.09	34.95	5.90	0.009	–0.068	0.068
Halichoeres biocellatus	166	D	67.03	13.55	1.32	50.94	10.30	0.101	0.099	0.005
Novaculichthys taeniourus	167	D	46.33	8.48	1.44	32.16	5.88	–0.083	–0.127	0.011
Scaridae (parrotfishes)										
Scarus schlegeli	168	D	67.39	19.59	2.94	22.90	6.66	–0.112	0.036	–0.128
Zoarcidae (eelpouts)										
Bothrocara pusillum	169	D	56.34	15.50	1.60	35.19	9.68	–0.028	0.108	–0.125
Macrozoarces americanus	170	D	50.65	12.61	1.82	27.76	6.91	–0.114	–0.020	–0.100
Gymnelus viridis	171	D	97.92	14.90	1.83	53.36	8.12	0.176	0.050	0.131
Lycodichthys dearbornii	172	D	119.11	18.51	2.22	53.66	8.34	0.211	0.092	0.138
Notothenoidae (notothenoids)										
Dissostichus mawsoni	173	D	107.51	15.67	2.05	52.54	7.66	0.185	0.048	0.147
Trematomus bernacchii	174	D	89.19	13.50	2.39	37.35	5.66	0.063	–0.067	0.125
Pinguipedidae (sandperches)										
Parapercis cylindrica	175	D	42.96	9.18	1.17	36.63	7.83	–0.068	–0.040	–0.054
Parapercis hexoptgma	176	D	58.70	10.37	1.12	52.61	9.29	0.084	0.033	0.036
Uranoscopidae (stargazers)										
Kathetostoma canaster	177	D	104.61	12.71	1.36	76.80	9.33	0.285	0.062	0.220
Tripterygiidae (triplefins)										
Ucla xenogrammus	178	D	60.13	11.75	1.65	36.54	7.14	–0.010	–0.025	0.005
Blenniidae (blennies)										
Crossosalarius macrospilus	179	D	65.14	10.73	1.65	39.36	6.48	0.020	–0.065	0.068
Ecsenius yaeyamaensis	180	D	48.38	11.25	1.36	35.66	8.29	–0.055	0.012	–0.079
Ecsenius mandibularis	181	D	50.66	13.30	1.65	30.74	8.07	–0.090	0.025	–0.117
Petroscirtes lupus	182	D	60.30	10.47	1.30	46.31	8.04	0.057	–0.005	0.046
Petroscirtes mitratus	183	D	54.80	7.06	1.33	41.11	5.29	0.011	–0.181	0.148
Petroscirtes fallax	184	D	55.97	9.25	1.17	47.82	7.90	0.052	–0.030	0.058
Plagiotremus rhinorhynchos	185	D	59.77	13.80	1.06	56.27	13.00	0.106	0.166	–0.054
Callionymidae (dragonets)										
Callionymus limiceps	186	D	53.63	14.95	2.04	26.26	7.32	–0.115	0.018	–0.129
Gobiidae (gobies)										
Neogobius melanostomus	187	D	80.93	20.87	2.54	31.83	8.21	0.004	0.104	–0.073

Table A1. (*continued*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Istigobius rigilius	188	D	100.36	30.76	3.30	30.38	9.31	0.027	0.204	–0.125
Cryptocentrus leptocepgus	189	D	79.81	19.58	2.76	28.92	7.10	–0.024	0.055	–0.058
Gobiodon citrinus	190	D	58.22	16.48	2.13	27.28	7.72	–0.087	0.055	–0.130
Oplopomus oplopomus	191	D	76.04	20.00	2.65	28.67	7.54	–0.032	0.078	–0.087
Paragobiodon xanthosomus	192	D	54.76	13.89	2.48	22.09	5.60	–0.153	–0.063	–0.094
Signigobius biocellatus	193	D	68.03	13.17	1.73	39.36	7.62	0.028	0.012	0.013
Valenciennea longipinnis	194	D	66.67	17.80	1.86	35.83	9.56	0.000	0.123	–0.105
Scatophagidae (scats)										
Scatophagus multifasciatus	195	D	55.01	10.83	1.39	39.54	7.78	–0.001	–0.014	–0.002
Siganidae (rabbitfishes)										
Siganus doliatus	196	D	51.96	11.34	1.33	38.97	8.51	–0.009	0.019	–0.039
Siganus fuscescens	197	D	45.05	12.20	1.15	39.16	10.60	–0.042	0.097	–0.142
Acanthuridae (surgeonfishes / unicornfishes)										
Acanthurus grammoptilus	198	D	49.53	11.76	1.50	33.10	7.86	–0.069	0.007	–0.085
Zebrasoma scopas	199	D	58.40	12.53	1.45	40.38	8.67	0.017	0.043	–0.030
Sphyraenidae (barracudas)										
Sphyraena obtusa	200	D	41.97	10.01	1.09	38.60	9.21	–0.060	0.019	–0.097
Scombridae (tunas and mackerels)										
Thunnus alalunga	201	D	75.31	12.99	1.82	41.32	7.12	0.066	–0.010	0.071
Sarda australis	202	D	56.08	13.84	1.63	34.42	8.50	–0.034	0.051	–0.084
Belontiidae (gouramies)										
Betta splendens	203	D	56.86	8.92	1.27	44.80	7.03	0.033	–0.069	0.074
Order Pleuronectiformes										
Psettodidae (psettodid flounders)										
Psettodes erumei	204	D	68.22	10.03	1.41	48.30	7.10	0.083	–0.051	0.113
Scophthalmidae (scophthalmid flounders)										
Scophthalmus maximus	205	D	53.80	13.44	1.73	31.17	7.78	–0.072	0.022	–0.095
Paralichthyidae (large-tooth flounders)										
Pseudorhombus jenynsii	206	D	45.61	7.63	1.09	41.94	7.01	–0.020	–0.101	0.043
Pleuronectidae (righteye flounders)										
Hippoglossus hippoglossus	207	D	70.52	16.93	1.45	48.62	11.67	0.097	0.173	–0.058
Reinhardtius evermanni	208	D	54.06	12.27	1.43	37.81	8.58	–0.023	0.033	–0.062
Lepidopsetta bilineata	209	D	44.38	9.97	1.44	30.89	6.94	–0.103	–0.057	–0.067
Limanda ferruginea	210	D	59.03	13.72	1.54	38.45	8.94	–0.002	0.064	–0.064
Limanda aspera	211	D	67.10	12.42	1.62	41.39	7.66	0.046	0.001	0.038
Pleuronectes americanus	212	D	50.45	8.28	1.40	36.09	5.92	–0.047	–0.131	0.049
Soleidae (soles)										
Soleichthys heterorhinos	213	D	40.71	10.16	1.46	27.96	6.98	–0.143	–0.047	–0.115
Order Tetraodontiformes										
Triacanthidae (triplespines)										
Tripodichthys angustifrons	214	D	36.51	8.03	1.02	35.92	7.89	–0.102	–0.062	–0.074

Table A1. (*concluded*).

Taxon	Data ID	Group	RBC area (μm^2)	RBC nuclear area (μm^2)	Genome size (pg/N)	CS/GS	NS/GS	CS–GS residuals	NS–GS residuals	CS–NS residuals
Balistidae (triggerfishes)										
Abalistes stellatus	215	D	43.91	7.36	1.29	34.15	5.72	–0.083	–0.155	0.031
Sufflamen fraenatus	216	D	43.92	11.49	1.27	34.52	9.03	–0.077	0.041	–0.127
Balistapus undulatus	217	D	54.48	9.14	1.34	40.62	6.81	0.005	–0.078	0.056
Monacanthidae (filefishes)										
Pseudomonacanthus peroni	218	D	35.43	10.53	0.86	41.19	12.24	–0.063	0.109	–0.184
Tetraodontidae (puffers)										
Arothron manilensis	219	D	46.74	9.47	0.99	47.10	9.54	0.014	0.025	–0.031
Canthigaster valentini	220	D	40.26	5.60	0.87	46.27	6.43	–0.019	–0.167	0.091
Canthigaster bennetti	221	D	43.86	6.94	0.75	58.13	9.20	0.057	–0.039	0.056
Lagocephalus lunaris	222a	D	50.91	7.30	0.88	58.08	8.33	0.091	–0.057	0.109
(triploid?)	222b	D	58.24	12.98	1.43	40.73	9.07	0.126	0.167	–0.039
Tetraodon nigroviridis	223	D	46.12	8.46	1.02	45.11	8.28	–0.002	–0.032	0.001

Note: Actinopterygians are identified as diploid (D) or polyploid (P) following Klinkhardt et al. (1995). Taxonomy follows Nelson (1994). Cytogenomic (CS/GS) and nucleogenomic (NS/GS) ratios are given, as well as residuals from regressions of erythrocyte cell (CS) and nuclear (NS) areas against genome size (GS) and against each other (CS/NS). Sample size is 1 for all cell and nuclear area measurements. Genome size measurements are from Hardie and Hebert[3].