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Flow cytometric determination of genome size for eight commercially important fish species in China

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Abstract The genome size (*C* value) of eight commercially important fish species in China was measured using flow cytometry. Chicken (*Gallus domesticus*) erythrocytes were used as reference cells. When using propidium iodide (PI) as the fluorescent dye, genome sizes were 1.09±0.08, 2.75±0.12, 1.05±0.05, 1.35±0.11, 0.99±0.05, 0.90±0.08, 0.90±0.07, and 0.88±0.07 pg for Japanese eel (*Anguilla japonica*), mullet (*Myxocyprinus asiaticus*), yellowcheek carp (*Elopichthys bambusa*), blunt snout bream (*Megalobrama amblycephala*), yellow catfish (*Pelteobagrus fulvidraco*), ricefield eel (*Monopterus albus*), mandarin fish (*Siniperca chuatsi*), and snakehead (*Ophicephalus argus*), respectively. However, genome sizes were 1.25±0.00, 3.08±0.02, 1.25±0.00, 1.57±0.01, 0.96±0.01, 1.00±0.01, 0.91±0.01, and 0.89±0.01 pg for these fishes, respectively, when 4′, 6-diamidino-2-phenylindole (DAPI) was used as the fluorescent dye. Regardless of the dye used, the more evolutionarily advanced species had a smaller genome size than those with a lower evolutionary status. For each species, we also measured the size of erythrocytes and their nucleus and evaluated the relationships between erythrocyte size, nucleus size, chromosome number, and genome size. Genome size was positively correlated with erythrocyte nucleus size and chromosome number when using PI as the fluorescent dye, but it was only correlated with erythrocyte nucleus size when DAPI was used.

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Introduction

Genome size (*C* value), measured as the haploid DNA content per cell (in picograms; 1 pg=10^{-12} g), is a distinctive characteristic of a given species, as it is constant among different individuals of the same species. Accordingly, measurement of genome size represents a parameter of great utility for taxonomic studies. Genome size has been related to phylogenetic framework and evolution in vertebrates (Ciudad et al. 2002). In addition, genome size was shown to have positive and negative correlations with several ecophysiological traits. For example, cell and nuclear size, duration of the cell cycle, and duration of development and differentiation (Pagel and Johnstone 1992) appear to correlate positively with genome size. In contrast, metabolic rates, flower size, and impacts of altitude on grasses have been shown to correlate negatively with genome size (Meagher and Crostich 1996; Vinogradov 1997).

Among the vertebrates, the class Osteichthyes (bony fish) contains the largest number of species and the greatest diversity. The genome sizes of nearly 2,000 fish species have been reported (Gregory 2012) and among them are the largest (0.35 pg in the smooth puffer fish *Tetraodon fluviatilis*; Lamatsch et al. 2000) and the smallest (142 pg in the marbled lungfish *Protopterus aethiopicus*; Pedersen 1971) vertebrate genomes. It is well-known that fish genome size is not simply associated with the complexity of the species. Inter- and intraspecific genome size variation is mainly related to ploidy shifts and extensive linear replication of specific chromosomal regions, and most of these repetitive sequences have no protein-coding function. Nevertheless, many researchers have attempted to find common

rules to explain genome size evolution. Some researchers have shown that cell and genome sizes increase with habitat depth of fishes (Ebeling et al. 1971; Hardie and Hebert 2003). Other studies have reported that freshwater and other eurybiotic fishes have larger genomes than their marine and stenobiotic counterparts and that species with large genomes with large egg diameter (which is linked to the evolution of parental care) appear to survive better across a broader range of environmental variables (Gregory and Hebert 1999; Hardie and Hebert 2004). Recently, many researchers have focused on the internal structure of the genome, hypothesizing that introns, intergenic regions, and transposable elements might explain genome size evolution (Vinogradov 1999; Guo et al. 2010).

In the present study, we measured the genome size and red blood cell (RBC) size for the following eight fish species that are commercially important in China: Japanese eel (*Anguilla japonica*), mullet (*Myxocyprinus asiaticus*), yellowcheek carp (*Elopichthys bambusa*), blunt snout bream (*Megalobrama amblycephala*), yellow catfish (*Pelteobagrus fulvidraco*), ricefield eel (*Monopterus albus*), mandarin fish (*Siniperca chuatsi*), and snakehead (*Ophicephalus argus*). To our knowledge, this is the first report of genome size measurement of these teleost fish using flow cytometry. Our major goals were to determine the genome size of these fish, evaluate whether genome size is correlated with RBC size of different genera and species, and provide new information about the evolution of genome size in fish.

Materials and Methods

Fish samples. In total, 211 individuals from eight teleost fish species were studied: *A. japonica* (n=15), *S. chuatsi* (n=16), and *E. bambusa* (n=30) were obtained from Liangzi Lake Fisheries of Wuhan; *M. asiaticus* (n=40) was obtained from the Bird and Flower Market at the Port of Wuhan; and *M. amblycephala* (n=35), *P. fulvidraco* (n=26), *M. albus* (n=30), and *O. argus* (n=19) were bought from the Baishazhou Fish Market of Wuhan. The living animals were transported to the laboratory and maintained in a well-aerated aquarium tank until used.

Cell size analysis. Before the experiment, the fish were anesthetized with MS-222 (tricaine methanesulfonate, Sigma-Aldrich, Co., St. Louis, MO) at 100 mg/L concentration. After length and weight measurements were made, we collected 0.1 mL blood sample from each fish following the method of Gao et al. (2007). A small (pinpoint) amount of blood was used to make blood smears, and two slides were prepared for each fish. Air-dried smears were fixed in methanol for 2–3 min, stained with Wright–Giemsa fluid for 8–10 min, washed, and dried. The stained smears were observed and photographed under a light microscope equipped with a video camera linked to a computer. Image analysis software (Motic Images Advanced 3.2, Motic China Group Co., Xiamen, China) was used to measure blood cells. Length (a) and width (b) of the cell and nucleus were measured for more than 100 red cells from each fish. The volume (V) of both the cell and its nucleus were computed using the following formula for ellipsoids or oblate spheroids: $V = 4/3 \times \pi(a/2) \times (b/2)^2$.

Genome size measurement. To measure the genome size, flow cytometry was performed using a Cell Lab Quanta™ SC machine (Beckman Coulter, Brea, CA) with at least 20,000 events (cells) counted in gate FL1-W (UV light) and FL2-W (488 nm argon ion laser) for each specimen. Genome size of the specimens was calculated using the known genome size of chicken (*Gallus domesticus*) RBCs (1.25 pg; Tiersch and Chandler 1989).

When propidium iodide (PI, Sigma-Aldrich) was used as the fluorescent dye, 0.05 mL blood samples were suspended and washed in 2 mL 0.01 M phosphate-buffered saline (PBS) (NaCl 8.0 g, KCl 0.2 g, Na_2HPO_4 1.42 g, KH_2PO_4 0.27 g, dd H_2O 1,000 mL, pH7.4), and then centrifuged at 68×g (Eppendorf centrifuge 5417R, Germany) for 8 min to isolate leucocytes and erythrocytes. The supernatant was discarded, and the RBCs were resuspended in PBS and diluted to 1.0×10^6 cell/mL for each sample. RBCs were fixed in ice-cold ethanol (70%) for at least 2 h at 4°C. Cells then were centrifuged for 8 min at 68×g and the ethanol was thoroughly decanted. The cell pellet was suspended in PBS and re-centrifuged. Next, 1 mL of PI staining solution (0.1% Triton X-100, 10 μg/mL PI, and 100 μg/mL DNase-free RNase A in PBS) was added, and the mixture was kept in the dark at room temperature for 30 min. The same method was used to process the chicken RBCs. Then, stained fish RBC and chicken RBC samples were added to one sample cup (volume ratio was 1:1), measured in the flow cytometer (FL2-W).

Cell staining with 4′,6-diamidino-2-phenylindole (DAPI, isolation and staining solution-10, Beckman Coulter, Inc.) was simpler, as it did not require fixing cells with ethanol. Fish RBC and chicken RBC samples were added directly to one sample cup, to which DAPI was added; the volume ratio of the three components was 1:1:2. Samples were kept in the dark at room temperature and stained for 5–10 min, and then, the samples were placed in the flow cytometer (FL1-W) for analysis.

Based on the sample's fluorescence histogram, the genome size of each sample was calculated as follows:

$$\text{Sample genome size (in picogram)} = 1.25X/H.$$

where X is the fluorescence channel number of the fish sample and H is the fluorescence channel number of chicken erythrocytes.

Statistical analyses. Statistical analyses were performed using SPSS (version 17.0) and the SAS (version 9.1.3). Data were expressed as mean±SD. One-way analysis of variance was performed to determine if there were differences in DNA contents among species or between male and female fish within a species, and p values of <0.05 were considered to be statistically significant. To assess the strength of the relationships between genome size and both blood cell size and chromosome number, the data were log-transformed, and least squares regression and Pearson's correlation analysis were used. An evolutionary tree was generated using SAS and the system cluster method.

Results

RBC size. The shapes of RBCs in chicken and the eight fish species were similar (Fig. 1). Table 1 summarizes the values

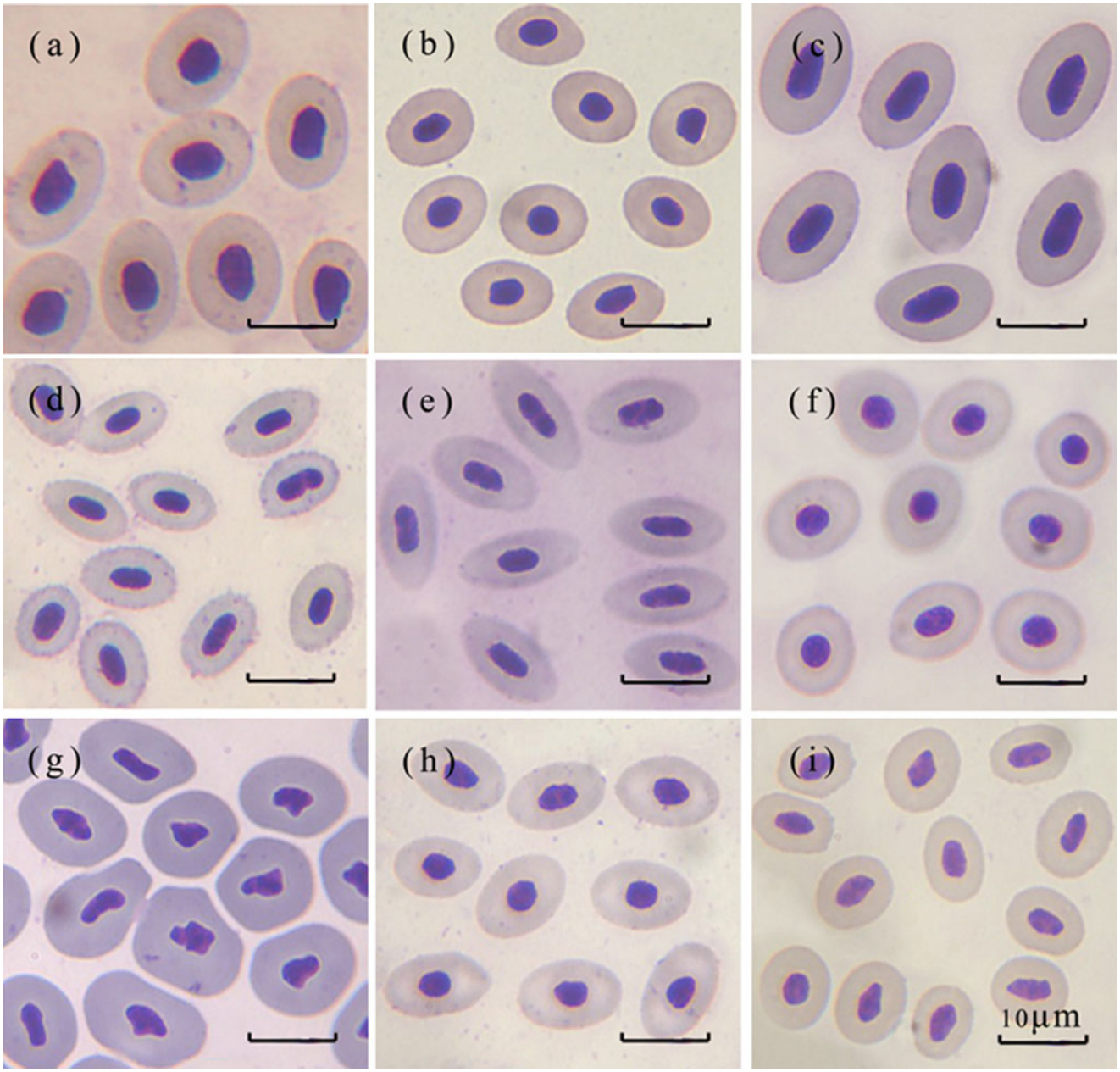


Figure 1. Microstructure of red cells from blood smears of chicken and eight fish species. Cells were stained with Wright–Giemsa. Erythrocytes of (*a*) chicken; (*b*) *A. japonica*; (*c*) *M. asiaticus*; (*d*) *E. bambusa*; (*e*) *M. amblycephala*; (*f*) *P. fulvidraco*; (*g*) *M. albus*; (*h*) *S. chuatsi*; and (*i*) *O. argus*.

Table 1. The erythrocyte and erythrocyte nucleus sizes of eight fish species

Species	Erythrocyte		Erythrocyte nucleus	
	Cell surface area (μm^2)	Cell volume (μm^3)	Nucleus surface area (μm^2)	Nucleus volume (μm^3)
Anguilla japonica	67.17±4.53	340.50±33.70	10.96±0.91	22.50±2.13
Myxocyprinus asiaticus	110.50±7.22	695.18±52.84	19.13±1.02	46.34±3.93
Elopichthys bambusa	61.11±3.81	285.00±23.63	8.36±0.70	13.63±1.33
Megalobrama amblycephala	81.51±6.77	423.20±37.14	12.87±0.89	25.68±2.04
Pelteobagrus fulvidraco	76.84±6.59	460.34±37.55	10.33±0.99	23.22±2.06
Monopterus albus	100.38±6.55	666.98±58.14	9.33±0.72	17.60±1.77
Siniperca chuatsi	76.52±5.75	422.26±33.14	9.39±0.85	19.75±1.79
Ophicephalus argus	56.33±4.53	268.30±19.37	10.18±0.88	17.07±1.44

for erythrocyte size parameters in these species. The mean sizes of the erythrocyte and erythrocyte nucleus in *M. asiaticus* were larger than those in the other fish species, and the values for *O. argus* were the smallest.

Genome size. Figures 2 and 3 show representative fluorescence histograms of the distribution of erythrocytes from the eight fish species compared with the distribution of chicken erythrocytes. All cell distributions were normally distributed and unimodal when stained with PI (Fig. 2) and DAPI (Fig. 3). When DAPI was used as the fluorescent dye, the histograms of the distribution of erythrocytes in *A. japonica* and chicken overlapped (Fig. 3*b*), and the same was true for *E. bambusa* (Fig. 3*d*).

Table 2 shows the relative genome size of individuals from the eight studied species. For the two dyeing methods, *M. asiaticus* had the largest genome size, followed by *M. amblycephala*, *A. japonica*, and *E. bambusa*. In contrast, the genome sizes of *M. albus*, *P. fulvidraco*, *S. chuatsi*, and *O. argus* were relatively low. By comparison, genome size of these fishes was relatively high when DAPI was used as the fluorescent dye except for *P. fulvidraco*, and the DAPI results exhibited relatively low interindividual variation within each species (Table 2). For the fish which gender could be determined, no significant differences in genome size were found between male and female fish. Cluster analysis did not reveal a correlation between complexity of the species and genome size for results obtained using either fluorescent dye (Fig. 4*a*, *b*).

Correlation between genome size and RBCs and chromosomes. For both dyeing methods, linear regression analyses did not support a significant positive relationship between the mean genome size and erythrocyte surface area (r^2=0.3651, p>0.05, Fig. 5*a1*; r^2=0.3340, p>0.05, Fig. 5*b1*) or cell volume (r^2=0.2576, p>0.05, Fig. 5*a2*; r^2=0.2180, p>0.05, Fig. 5*b2*) of these fishes. However, a positive relationship was identified between genome size and erythrocyte nucleus surface area (r^2=0.8706, p<0.01, Fig. 5*a3*; r^2=0.6588, p<0.05, Fig. 5*b*3). Moreover, there was a strong positive relationship between genome size and erythrocyte volume (r^2=0.7742, p<0.01, Fig. 5*a4*; r^2=0.7839, p<0.01, Fig. 5*b4*). When PI was used as the fluorescent dye, chromosome number was positively related to genome size in these fishes (r^2=0.6030, p<0.05, Fig. 5*a5*). In contrast, no obvious relationship between mean genome size and chromosome number was detected when DAPI was used as the fluorescent dye (r^2=0.4853, p>0.05, Fig. 5*b5*).

Discussion

The importance of genome size as a fundamental characteristic of a species is well-known (Alfei et al. 1996). At present, the main methods used to determine genome size are Feulgen densitometry, Feulgen image analysis densitometry, and flow cytometry. Among them, flow cytometry, which offers speed, accuracy, and reproducibility, has become a well-recognized technique for analysis of nuclear DNA content (Filipiak et al. 2011). Common reference standards include human lymphocytes, chicken erythrocytes, and rainbow trout (*Oncorhynchus mykiss*) and leopard frog (*Rana pipiens*) RBCs. Chicken erythrocytes are easy to obtain and have been the most widely used as an internal standard (Vinogradov 1998; Filipiak et al. 2011). Meanwhile, the DNA content of chicken erythrocytes is close to that of these fishes, thus it provides a better reference standard than the other available options (Juchno et al. 2010).

Comprehensive reports on the genome size of fish have been published in the past (Hinegardner and Rosen 1972; Peruzzi et al. 2005; Filipiak et al. 2011). A comparison of our results with those previously reported in the literature (Table 3) showed that genome size of individuals of the same species exhibited some variability, which likely has to do with variability in measurement techniques, reference

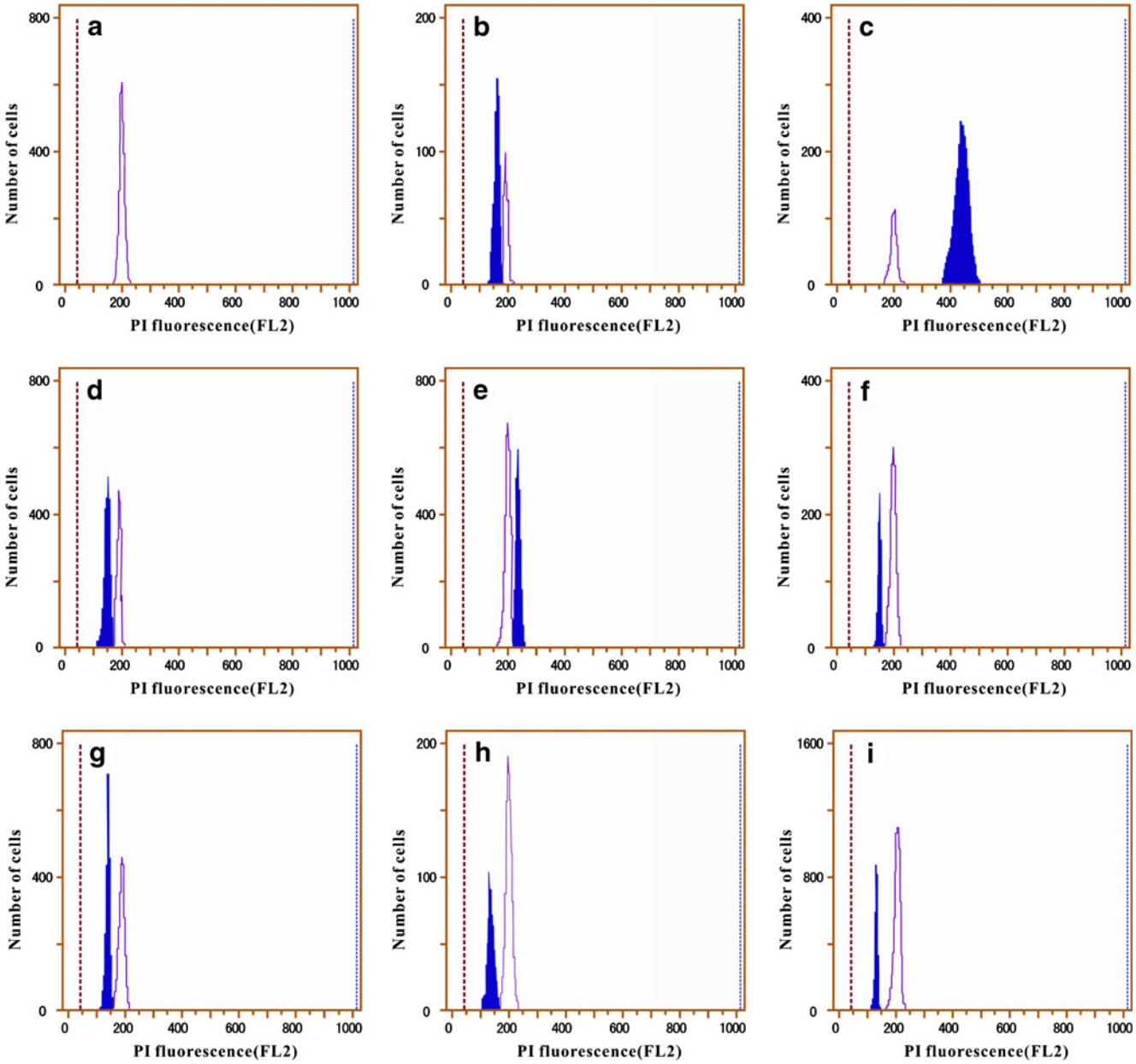


Figure 2. Flow cytometry histograms for samples (in *blue*) and chicken (in *red*) erythrocytes on basis of PI fluorescence dye. (*a*) Chicken; (*b*) *A. japonica*; (*c*) *M. asiaticus*; (*d*) *E. bambusa*; (*e*) *M. amblycephala*; (*f*) *P. fulvidraco*; (*g*) *M. albus*; (*h*) *S. chuatsi*; and (*i*) *O. argus.*

standards, and the DNA fluorochrome used in flow cytometry. Similar variability in results when different methods and reference standards were used has been reported for other species. For example, the DNA content of goldfish (*Carassius auratus*) was 3.4 pg when analyzed by photometry with mouse (*Mus musculus*) as the reference species and 3.54 and 3.75 pg when analyzed by flow cytometry with human (*Homo sapiens*) and chicken as the reference species, respectively (Vinogradov 1998). The DNA content of zebrafish (*Danio rerio*) was 3.4 pg when PI was used as the fluorescence dye (Ciudad et al. 2002), but another study based on DAPI fluorescence yielded a value of 4.6 pg (Lamatsch et al. 2000). In the present study, we used flow cytometry with two different fluorescent dyes to measure the genome size of eight fish species. The genome sizes of these fishes were higher when DAPI was used as the fluorescent dye (except for *P. fulvidraco*). DAPI is an AT base-specific fluorochrome, and the AT content of chicken was 57.73% (Vinogradov 1998). The DAPI data demonstrated that the AT content of these fishes was higher than 57.73%, with the exception of *P. fulvidraco*.

Variations in genome size within and between orders were observed in our analysis. This finding does not suggest a correlation between genome size and organismal complexity

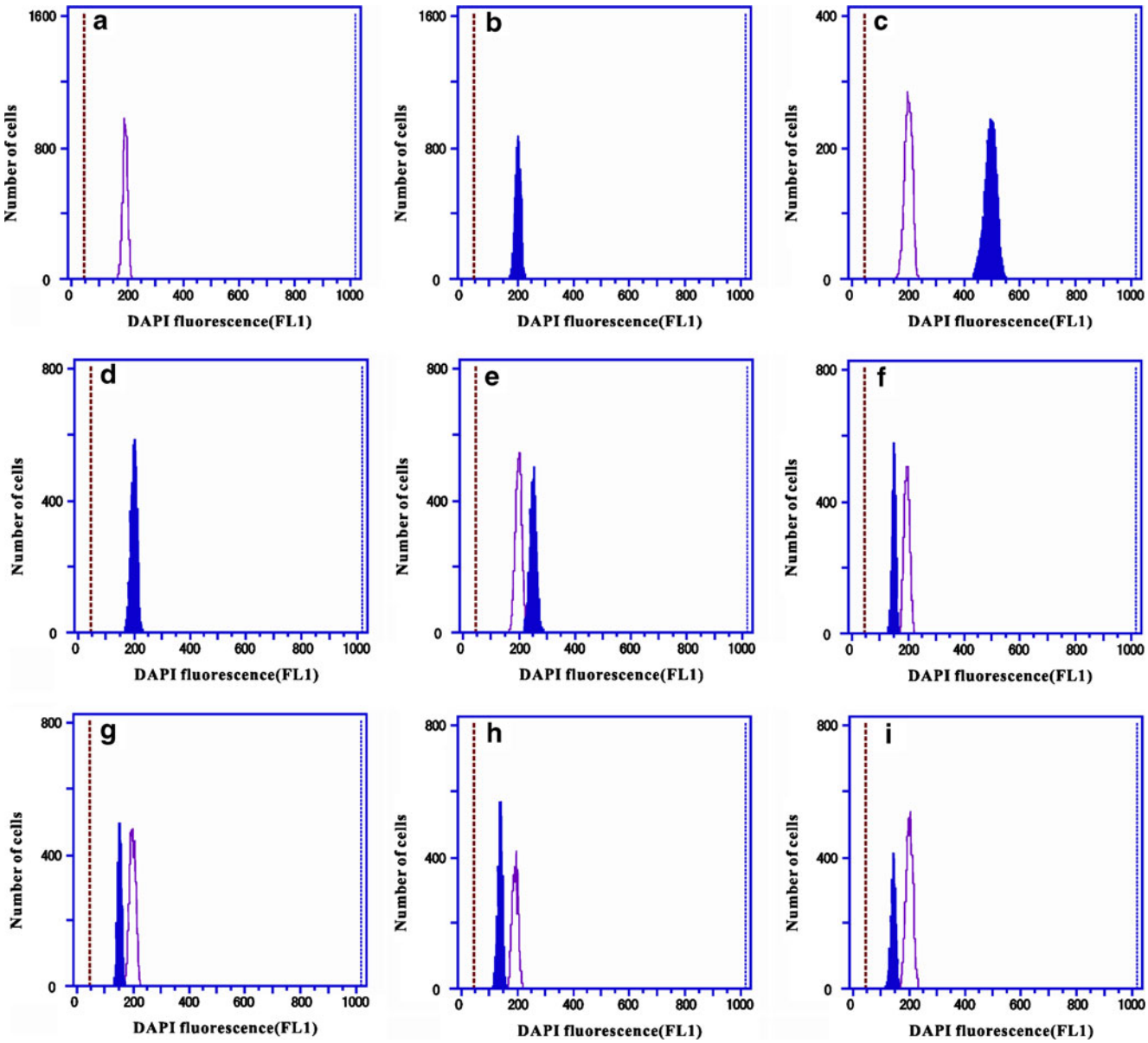


Figure 3. Flow cytometry histograms for samples (in *blue*) and chicken (in *red*) erythrocytes on basis of DAPI fluorescence dye. (*a*) Chicken; (*b*) *A. japonica*; (*c*) *M. asiaticus*; (*d*) *E. bambusa*; (*e*) *M. amblycephala*; (*f*) *P. fulvidraco*; (*g*) *M. albus*; (*h*) *S. chuatsi*; and (*i*) *O. argus.*

(Fig. 4), which is consistent with the *C* value paradox/enigma reported in previous studies (Hardie and Hebert 2004; Guo et al. 2010). For both fluorescent dyes, *M. asiaticus* had the greatest genome size of the species tested; it belongs to the Catostomidae, and the members of which are all polyploid (Venkatesh 2003). However, *M. albus*, *S. chuatsi*, and *O. argus*, which belong to the Percomorpha and have higher evolutionary status than the other species tested, have a smaller genome size, and similar results have been reported in many previous studies (Brainerd et al. 2001; Hickey and Clements 2005). These findings suggest that the overall trend of genome evolution in fish is that the genome has become more contracted. Kraaijeveld (2010) pointed out that small genomes may accumulate mutations and genomic rearrangements more quickly than larger genomes and propagate them more reliably and that mutations and chromosomal rearrangements may also be more stably inherited in smaller genomes.

Genome size has been found to be correlated with nucleus and cell size in vertebrates, including amphibians, reptiles, and birds (Gregory 2001; Hardie and Hebert 2003). Because nuclear volume is a function of the content and degree of folding of the genetic material, it is not surprising that nuclear size is related to genome size in a strongly positive manner. We observed that genome size of the eight

Table 2. Fish species, weight, age, number of individuals, genome size, and chromosome number of eight fish species

Species	Weight (g)	Age (yr)	Fish analyzed M/F/?*	Genome size (pg)		Chromosome number (by reference)
				PI	DAPI	
Anguilla japonica	350.67–754.26	3^+–5^+	7/8/0	1.09±0.08	1.25±0.00	38 (Yang et al. 1999)
Myxocyprinus asiaticus	3.08–105.70	0^+–1^+	10/10/20	2.75±0.12	3.08±0.02	100 (Li et al. 1983a)
Elopichthys bambusa	9.27–125.53	0^+–1^+	8/7/15	1.05±0.05	1.25±0.00	48 (Li et al. 1985b)
Megalobrama amblycephala	245.12–726.75	1^+–2^+	17/18/0	1.35±0.11	1.57±0.01	48 (Zan and Song 1979)
Pelteobagrus fulvidraco	20.61–65.24	1^+–3^+	14/12/0	0.99±0.05	0.96±0.01	52 (Yu et al. 1989)
Monopterus albus	15.19–107.10	1^+–4^+	15/15/0	0.90±0.08	1.00±0.01	24 (Xu et al. 1994)
Siniperca chuatsi	205.21–612.38	2^+–3^+	8/8/0	0.90±0.07	0.91±0.01	48 (Li et al. 1985a)
Ophicephalus argus	5.12–842.51	0^+–2^+	9/9/1	0.88±0.07	0.89±0.01	48 (Zhu et al. 2009)

M male, *F* female, *?* not sexed

fish species tested was correlated with erythrocyte nucleus size but not with erythrocyte size (Fig. 5); in other words, there was no significant direct relationship between erythrocyte size and nuclear size. Chang et al. (1995) measured the relative erythrocyte volume of batoids compared to chicken erythrocytes and analyzed the correlation between DNA contents and cell volumes of the 23 species of batoids, and they found no significant linear correlation (r=0.39, p>0.25). Lay and Baldwin (1999) presented data for nuclear volume (calculated from dry areas) and erythrocyte volume (measured wet) for 52 tropical species of teleost fish and concluded that no significant

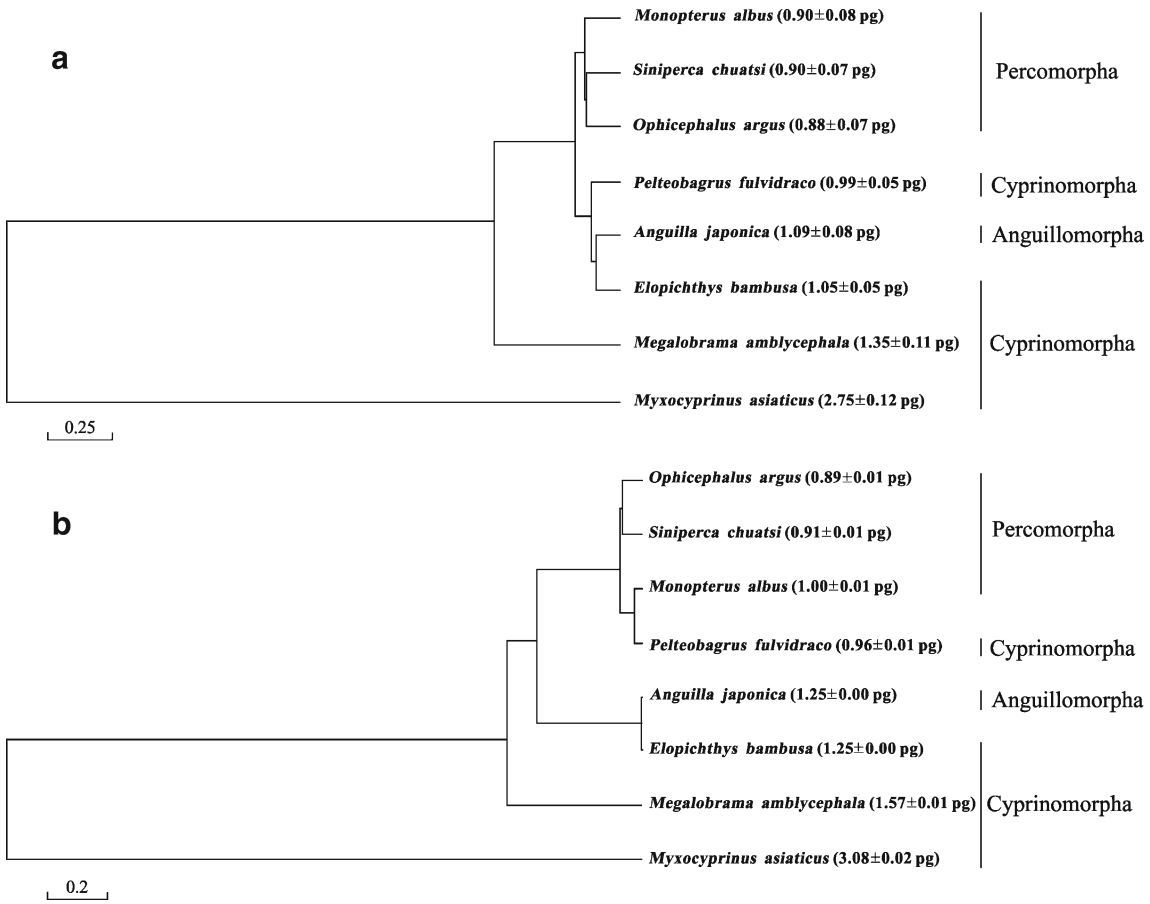
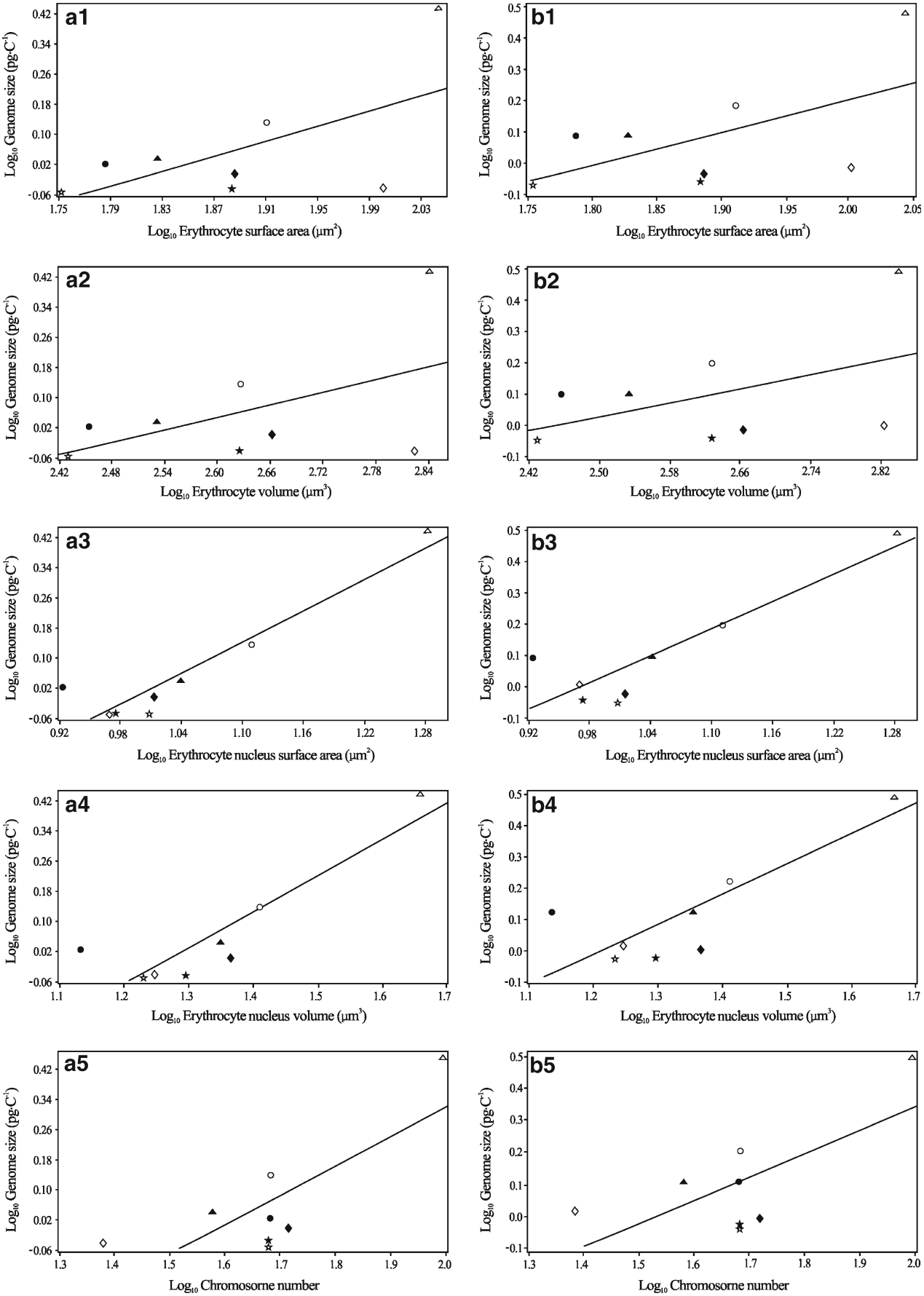


Figure 4. Clustering by single linkage analysis based on genome size of eight fish species: (*a*) genome size based on PI fluorescence dye; (*b*) genome size based on DAPI fluorescence dye.


a1
Log10 Genome size (pg·C-1)
Log10 Erythrocyte surface area (μm2)
b1
Log10 Genome size (pg·C-1)
Log10 Erythrocyte surface area (μm2)
a2
Log10 Genome size (pg·C-1)
Log10 Erythrocyte volume (μm3)
b2
Log10 Genome size (pg·C-1)
Log10 Erythrocyte volume (μm3)
a3
Log10 Genome size (pg·C-1)
Log10 Erythrocyte nucleus surface area (μm2)
b3
Log10 Genome size (pg·C-1)
Log10 Erythrocyte nucleus surface area (μm2)
a4
Log10 Genome size (pg·C-1)
Log10 Erythrocyte nucleus volume (μm3)
b4
Log10 Genome size (pg·C-1)
Log10 Erythrocyte nucleus volume (μm3)
a5
Log10 Genome size (pg·C-1)
Log10 Chromosorne number
b5
Log10 Genome size (pg·C-1)
Log10 Chromosorne number

◀ **Figure 5.** The relationship between genome size and erythrocyte size and chromosome number of the eight fish species. (*a1–a5*, genome size based on PI fluorescence dye; *b1–b5*, genome size based on DAPI fluorescence dye) (*a1*, *b1*) Relationship between genome size and erythrocyte surface area (r^2=0.3651, p>0.05; r^2=0.3340, p>0.05); (*a2*, *b2*) Relationship between genome size and erythrocyte volume (r^2=0.2576, p>0.05; r^2=0.2180, p>0.05); (*a3*, *b3*) Relationship between genome size and erythrocyte nucleus area (r^2=0.8706, p<0.01; r^2=0.6588, p<0.05); (*a4*, *b4*) Relationship between genome size and erythrocyte nucleus volume (r^2=0.7742, p<0.01; r^2=0.7839, p<0.01); (*a5*, *b5*) Relationship between genome size and chromosome number (r^2=0.6030, p<0.05; r^2=0.4853, p>0.05) (*Black up-pointing triangle*, *A. japonica*; *white up-pointing triangle*; *M. asiaticus*; *black circle*, *E. bambusa*; *white circle*, *M. amblycephala*; *black diamond*, *P. fulvidraco*; *white diamond*, *M. albus*; *black star*, *S. chuatsi*; and *white star*, *O. argus*).

relationship exists between these two parameters. Nevertheless, in recent studies conducted using consistent measures of nucleus, cell, and genome sizes, Gregory (2001) and Hardie and Hebert (2003) concluded that the cell and nuclear areas of erythrocytes showed a highly significant positive correlation with each other and with genome size in fishes and explained this by "nucleotypic theory" (DNA content can directly influence cell size). In fact, the relationship between DNA content and erythrocyte size is complicated; the positive correlation has been recognized by some, also not been appreciated by many (Starostová et al. 2008). Therefore, in order to search the common evolution rule of vertebrate genome, more studies are needed to address the internal construction of the genome and cell, such as introns, intergenic regions, cell physiology, the principles of cell cycle regulation, etc.

Previous studies have shown that genome size variation is positively correlated with chromosome number variation (Hinegardner and Rosen 1972; Mank and Avise 2006). However, in this study using two different dyes, the relationship between genome size and the chromosome number was inconsistent. In fact, the relationship was not obvious. For example, *M. albus* has the minimum chromosome number ($2n$=24), but it does not have the smallest genome size (Table 2). In addition, *E. bambusa*, *M. amblycephala, S. chuatsi*, and *O. argus* have the same chromosome number ($2n$=48), but their genome sizes differ. Moreover, *M. amblycephala* has nearly two times more DNA than *O. argus*. Cano et al. (1982) also reported very large genome size differences among eight species of *Blennius* (Blenniidae), and they found no correlation between genome size and chromosome number. Analysis of two species of *Trichomycterus* (Trichomycteridae) showed that although both had the same diploid number ($2n$=54), there were conspicuous differences in their nuclear DNA content (Fenerich et al. 2004). An increase in fish genome size usually is accompanied by an increase in the number of DNA repeats, whereas changes in the number of chromosomes are related mostly to Robertsonian translocation, centromere fusion and inversion, chromosome breakage, and polyploidy. Thus, the reasons for differences in genome size among fish species are complex and not completely related to the chromosome number. However, very large differences in genome size usually are related to chromosome number, and the best example is polyploidy (Lamatsch et al. 2000; Juchno et al. 2010).

Conclusion

In conclusion, this study fills a gap in the literature by providing information about the genome size of eight commercially important fishes in China. It also provides valuable reference data for genetic linkage map construction and genome sequencing of these fishes. The results also showed positive correlations between DNA content and erythrocytes nuclei size in these eight fish species.

Table 3. Literature reports of the genome size of five fish species determined using the Feulgen densitometry method to measure red blood cells

Species	Genome size (pg)	Standard species (genome size, pg)	References
Myxocyprinus asiaticus	2.02	*Cyprinus carpio* (1.70)	Suzuki (1992)
Megalobrama amblycephala	1.12	*Gallus domesticus* (1.25)	Cui et al. (1991)
	1.17	*Cyprinus carpio* (1.70)	Zhou (1984)
	1.20	*Homo sapiens* (3.50)	Li et al. (1983b)
	1.20	*Cyprinus carpio* (1.70)	Suzuki et al. (1995)
Pelteobagrus fulvidraco	0.97	*Cyprinus carpio* (1.70)	Zhou (1984)
	0.99	*Homo sapiens* (3.50)	Li et al. (1983b)
	1.06	*Gallus domesticus* (1.25)	Cui et al. (1991)
Monopterus albus	0.62	*Gallus domesticus* (1.25)	Cui et al. (1991)
	0.78	*Cyprinus carpio* (1.70)	Zhou (1984)
	0.82	*Homo sapiens* (3.50)	Li et al. (1983b)
Siniperca chuatsi	0.80	*Gallus domesticus* (1.25)	Cui et al. (1991)

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