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Haematological characterization of loach *Misgurnus anguillicaudatus*: Comparison among diploid, triploid and tetraploid specimens

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Abstract

The purpose of this study was to determine whether diploid, triploid and tetraploid loach (*Misgurnus anguillicaudatus*) differed in terms of their main haematological and physiological characteristics. Diploid and tetraploid fish were produced by crossing of natural diploids ($2n \times 2n$) and natural tetraploids ($4n \times 4n$), respectively. Triploid fish were produced by hybridization between diploid males and tetraploid females. The blood cells were significantly larger in polyploids, and the volumetric ratios of erythrocytes and leucocytes (thrombocyte and neutrophil) in tetraploids, triploids and diploids were consistent with the ploidy level ratio of 4:3:2. No significant differences were observed in haematocrit among polyploids. The erythrocyte count decreased with increased ploidy level, while total haemoglobin, mean cell volume, mean cellular haemoglobin content, and mean cell haemoglobin concentration all increased with increase in ploidy level. Erythrocyte osmotic brittleness declined in polyploids so that polyploid erythrocytes were more resistant to osmotic stress than diploid ones. Overall, loach with higher ploidy levels showed evidence of some advantages in haematological characteristics.

Keywords: Blood cells; Haematology; *Misgurnus anguillicaudatus*; Ploidy level

1. Introduction

The oriental weather loach *Misgurnus anguillicaudatus* (Cypriniformes; Cobitidae), is a freshwater teleost that inhabits streams, ditches and rice paddy fields, preferably with a soft muddy bottom (Man and Hodgkiss, 1981). This species is widely distributed in Japan, Korea, Taiwan and eastern coasts of Asian continent from the Amur River to North Vietnam (Saitoh, 1989). The loach, for a long time, had been employed as traditional Chinese medicine in folk remedies for treatment of hepatitis, osteomyeitis, carbuncles, inflammations and cancers, as well as for restoration to health in debilities caused by various pathogens and aging (Qin et al., 2002). In Japan, loach has been cited as a main aquaculture species with a high commercial value for a long time. In China, loss of natural ecosystems has caused a major decline in natural production of the loach and artificial culture of loach has been widely developed mainly for commercial export to Korea.

As to the loach, diploid individuals ($2n = 50$) are common in wild populations of Japan (Zhang and Arai, 1999a) and a small number of tetraploids ($4n = 100$) have been found among specimens obtained from a fish dealer (Arai et al., 1991). In China, populations of both diploid ($2n = 50$) and tetraploid ($4n = 100$) loaches have been recorded (Yin et al., 2005). Such natural tetraploid loaches are very useful in producing triploids by crossing them with normal diploids, which is much simpler than chromosomal manipulation. Mass production of triploids by

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hybridization between diploids and tetraploids has not succeeded due to technical difficulties of artificial tetraploidization (Arai, 2001), except for a few salmonids (Chourrout et al., 1986; Chourrout and Nakayama, 1987), *Misgurnus mizolepis* (Nam and Kim, 2004) and *Megalobrama amblycephala* (Zou et al., 2004). In triploid loaches that were produced by hybridization using natural tetraploids, males were sterile but females laid both large-sized triploid and small-sized haploid eggs (Matsubara et al., 1995; Zhang and Arai, 1996). Such reproductive characteristics are different from those observed in artificial triploids induced by the inhibition of the second polar body release. These artificial triploids showed sterility in females but produced aneuploid spermatozoa in males (Zhang and Arai, 1999b). Surprisingly, the occurrence of natural triploid individuals was documented by Ojima and Takaii (1979) who found triploid specimens with 75 chromosomes in natural populations. In China, no natural triploid loach has been reported.

There have been intensive studies of the loach with the major focus on chromosome set manipulation, polyploidy, gynogenesis and genetics (Arai, 2003). In haematological studies, only morphology of peripheral blood cells was reported in Chinese species, with no evaluation of ploidy level (Xiao et al., 2001). Polyploidy is always associated with changes in cell morphology and physiology (Purdom, 1993; Benfey, 1999), which can influence the ecological fitness of individuals. Comparison of haematological indices of diploid and triploid fish have been performed in salmonids (Benfey and Sutterlin, 1984a,b; Benfey et al., 1984; Small and Benfey, 1987; Cogswell et al., 2001), sturgeons (Palíková et al., 1999; Flajšhans and Vajcová, 2000), *Ictalurus punctatus* (Wolters et al., 1982), *Ctenopharyngodon idella* and *Hypophthalmichthys nobilis* (Beck and Biggers, 1983), *Tinca tinca* (Flajšhans, 1997; Svobodová et al., 1998), and *Umbrina cirrosa* (Ballarin et al., 2004). The variable of erythrocyte size of diploids and triploids (Sezaki et al., 1977; Benfey et al., 1984; Flajšhans, 1997; Svobodová et al., 1998) is frequently used as an index to demonstrate ploidy level in some fish species, such as *Cobitis biwa* (Sezaki et al., 1988), *Cobitis taenia* (Boron, 1994) and *Salmo salar* (Benfey et al., 1984).

The purpose of this study was to compare some haematological parameters and morphological features of peripheral blood cells among different cytotypes (diploid, triploid and tetraploid) of loach. This work was done as a preliminary investigation on the effect of ploidy on physiology of the loach. The specific objective of this work was to investigate major blood constituents in diploid, triploid and tetraploid loach with a view to provide information about possible adaptive physiological and behavioural interactions during rearing and husbandry practices.

2. Material and methods

2.1. Biological material and production of triploids

Naturally diploid and tetraploid loaches (*M. anguillicaudatus*) were collected from waters near Zhijiang and Wuhan city, Hubei province, China, respectively. During May 2006, triploid loaches were produced by hybridization between diploid male and tetraploid female loach, following Zhang and Arai (1996). Through cross, $2n \times 2n$ and $4n \times 4n$, we got the diploid and tetraploid progeny, respectively. The ploidy level of diploid and tetraploid loach was determined by flow cytometry using the methods adopted by Zhang and Arai (1996) prior to breeding experiments. Briefly, red blood cells taken by caudal artery puncture were stained with propidium iodide (Sigma, USA), and DNA contents of erythrocytic nuclei were measured using a flow cytometer (Becton Dickinson FACS Calibur). Then the progeny with different ploidy level were reared in 9 m^3 tanks using similar water conditions. Water temperature was kept from 20 to 26 °C and mean dissolved oxygen concentration was close to saturation level (mean: 8.5±0.5 mg/l). The fish were fed with rotifers during the early stage and later with the moistly pelleted feed which was made with powdery feed. Specimens were periodically sampled and their ploidy was determined by the flow cytometric assessment of the nuclear DNA content in erythrocytes.

2.2. Sampling

Sampling was conducted during October 2006. Specimens of 6 months age with an average body weight of 6.29 g (ranged from 4.37 to 8.63 g) and body length of 9.56 cm (ranged from 9.9 to 12.4 cm) were used in the experiments. Fish were left undisturbed and fasting for 2 days prior to sampling. The specimens were anaesthetized with MS222 (Sandoz) (100 mg/L), and the peripheral blood was collected under sterile conditions by puncture of the caudal vein with a heparin-coated 23-gauge needle attached to a 2.5 ml syringe.

2.3. Haematological indices and light microscopy

Red blood cell counts were estimated immediately after sampling using standard haematological techniques (Dacie and Lewis, 2001). In particular, erythrocyte counts were performed on diluted blood samples (1:200 dilution in Dacie's fluid) using a Neubauer haemocytometer. Blood smears, 2 for each fish, were prepared using a pinpoint amount of blood. Air-dried smears were fixed in absolute methanol and stained with Wright–Giemsa (WG) fluid. The stained smears were observed and photographed under light microscopy with a video camera linked to computer image analysis software (Motic Images Advanced 3.2, USA). Different leucocytes were counted simultaneously and 200 total cells (RBC+WBC) per slide were counted twice. One hundred cells of each type were measured with computer image analyses software, including the length (a) and width (b) of the cell and nucleus. The volume (V) of both the cell and its nucleus were computed using the following formula for ellipsoids or oblate spheroids (Benfey and Sutterlin, 1984a,b): $V = 4/3 \times \pi\ (a/2) \times (b/2)^2$.

Two 50 μL haematocrit tubes per blood sample were filled with blood and kept refrigerated (4 °C) in an upright position until centrifuged (5 min at 12,000 ×g), then haematocrit measured directly from the tubes. One blood sample was refrigerated for later haemoglobin (Hb) evaluation. Total blood haemoglobin content was determined according to the method

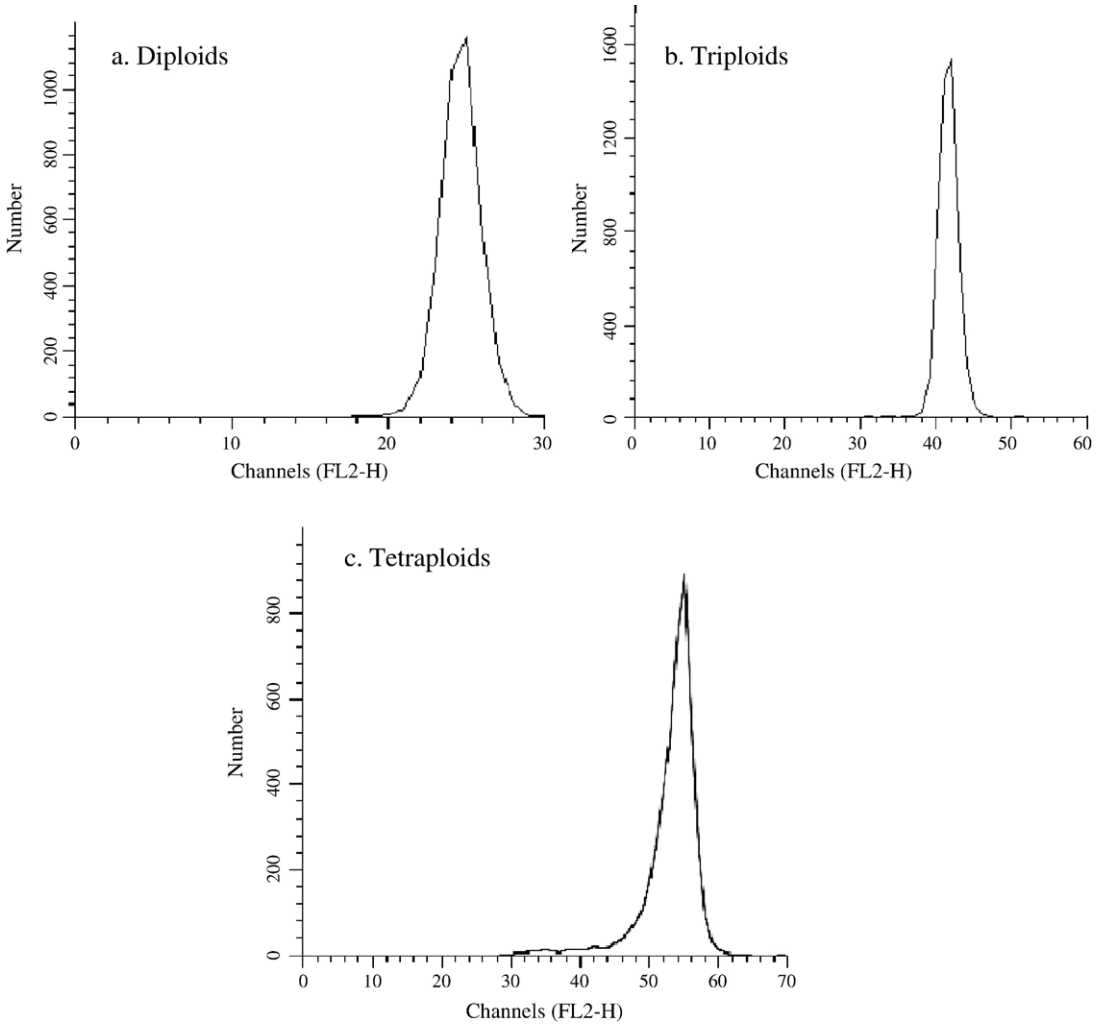

Fig. 1. Flow cytometry histograms for ploidy determination of loach (*M. anguillicaudatus*) progeny on the basis of DNA content in (a) normal diploid progeny with 2C DNA from the natural diploid female × diploid male cross; (b) normal triploid progeny with 3C DNA from the natural diploid male × tetraploid female hybridization; (c) normal tetraploid progeny with 4C DNA from the natural tetraploid female × tetraploid male cross.

of Drabkin and Austin (1935) based on oxidation of haemoglobin to cyanmethaemoglobin in the presence of potassium ferricyanide and a subsequent absorbance reading at 540 nm (Kit 525, Sigma). For erythrocyte osmotic brittleness (EOB), one drop blood was added to a number of 1 ml NaCl solutions, containing concentrations from 0.20–0.40% at 0.02% intervals (Xu, 2004). After 2 h, these solutions were centrifuged at 500 ×*g* for 5 min, and the concentration of complete hemolysate was taken as the value of maximum erythrocyte resistance.

After measurement of total red blood cell count (RBCC), haemoglobin concentration (Hb), and haematocrit (Hct), other haematological parameters such as mean cell volume (MCV), mean cellular haemoglobin content (MCH), and mean cell haemoglobin concentration (MCHC) were calculated using the following formulas (Dacie and Lewis, 2001):

$$\mathrm{MCV(fl)} = \mathrm{Hct/RBCC}(10^6 \mu L^{-1})$$
$$\mathrm{MCH(pg)} = [\mathrm{Hb(g\ dL^{-1}} \times 10]/\mathrm{RBCC}(10^6 \mathrm{mm^{-1}})$$
$$\mathrm{MCHC}(g^{-1}) = [\mathrm{Hb(g\ dL^{-1})} \times 10]/\mathrm{Hct}.$$

2.4. Statistical analyses

The data was expressed as mean ± SD and compared using single-factor STATISTICA 6.0 (ploidy). The differences were accepted as significant if $P < 0.05$.

3. Results

The analyzed diploid fish included 10 males and 10 females, while the triploids included 8 females and 2 males and the tetraploids 10 males and 10 females.

3.1. Ploidy in the examined animals

Polyploid status of all specimens was determined by flow cytometric assessment of the nuclear DNA content in erythrocytes. All the putative triploid and tetraploid loaches were characterised by about 1.5 and 2.0 fold the nuclear DNA amount of the diploid fish, respectively (Fig. 1), thus confirming their polyploidy status and the success of triploidization.

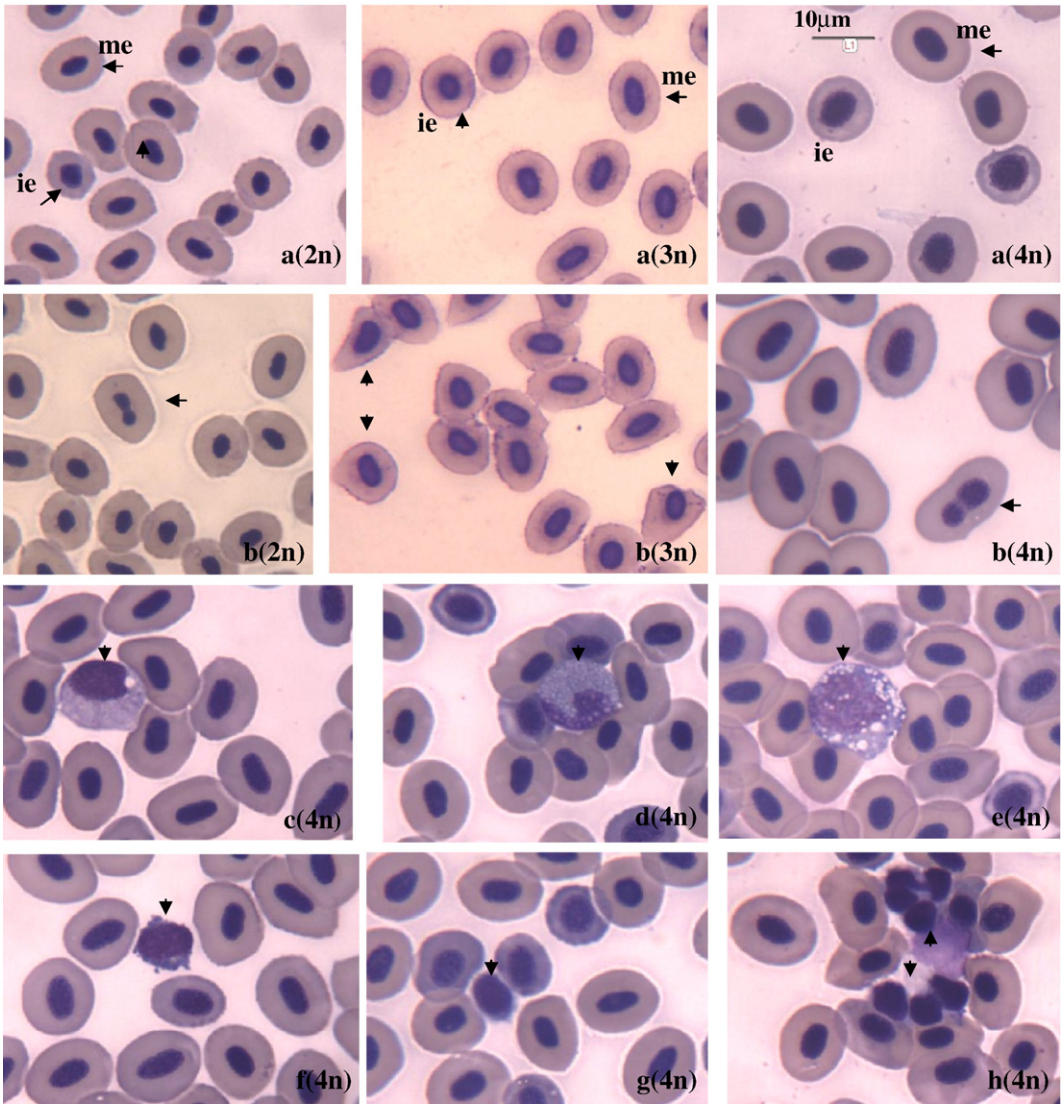

Fig. 2. Microstructure of cells in loach (*M. anguillicaudatus*) blood smears stained with Wright–Giemsa (×500. Scale bar=10 μm). 4*n*, tetraploids; 3*n*, triploids; 2*n*, diploids. (a(2*n*)) immature erythrocytes and mature erythrocytes in diploids; (a(3*n*)) immature erythrocytes and mature erythrocytes in triploids; (a(4*n*)) immature erythrocytes (ie) and mature erythrocytes (me) in tetraploids; (b(2*n*)) dividing erythrocyte in diploids; (b(3*n*)) atypical erythrocytes in triploids; (b(4*n*)) dividing erythrocyte in tetraploids; (c(4*n*)) neutrophil; (d(4*n*)) eosinophil; (e(4*n*)) monocyte; (f(4*n*)) lymphocyte; (g(4*n*)) single thrombocyte; (h(4*n*)) cluster thrombocytes. c–h all in tetraploids. The cells indicated in each legend are shown by arrows.

3.2. Morphology of the peripheral blood cells

Erythrocytes were the most main cells in peripheral blood smears of loach. Small numbers of blood leucocytes (thrombocytes, lymphocytes, granulocytes including neutrophils and eosinophils, and monocytes) were distributed diffusely among the dense erythrocytes, single or clump. There was a nonsignificant difference among the morphology of the cells in different ploidy loach. Therefore, we used tetraploid loach to describe the morphology of peripheral blood cells (Fig. 2). These cells are detailed below.

3.2.1. Erythrocyte

There were two kinds of erythrocytes found in loach blood. The predominant shape of erythrocyte was found as elliptical cell with an oval, central or nearly central, dark purple-stained nucleus with a blue-gray-stained cytoplasm (Fig. 2: a). Surface of these cells was smooth. Another kind of erythrocyte was immature erythrocyte, bigger or smaller than the former (Fig. 2: a). They were round in shape, fewer in number and called as reticulocytes or immature erythrocytes. They had magenta-stained chromatin of reticular appearance and blue-stained cytoplasm, darker than the mature erythrocyte. Cell membranes and nuclear membranes were not clear as in mature cells. The division of nucleus and cytoplasm of erythrocytes could be observed occasionally in tetraploids (Fig. 2: b(4*n*)) and diploids (Fig. 2: b(2*n*)). Many atypical cells were observed in triploids that were not generally seen in diploids or tetraploids. These included a high prevalence of dumbbell-shaped (bilobed), teardrop-shaped, and tailed cells (Fig. 2: b(3*n*)).

Table 1
Differential leucocyte cell counts (DLC) of diploid, triploid and tetraploid loach (*M. anguillicaudatus*), mean±SD

	Cells	Thrombocyte	Monocyte	Lymphocyte	Neutrophil	Eosinophil
Percent (%)	Diploids	35.56±8.69	5.02±1.78	20.37±5.26	37.13±7.62	1.92±0.23
	Triploids	42.78±9.58	8.06±2.36	19.62±3.24	29.54±5.22	
	Tetraploids	49.35±10.98	5.88±2.31	22.58±6.23	20.32±4.26	1.87±0.34

3.2.2. Granulocyte

There were two kinds of granulocytes in peripheral blood of loach: neutrophils and eosinophils. The neutrophils were of several sizes, but generally larger than erythrocytes in volume and were of several shapes, including spherical, pear shaped or an irregular ellipse. The nucleus stained purple was usually ovoid and eccentric, and may extend into a blunt ended pseudopodium. Occasionally, the nucleus should be observed as a ribbon-like structure across the diameter of the cell, horseshoe-shaped, band, two segmented shape and centrically or eccentrically located (Fig. 2: *c*(4*n*)). The nucleus to cytoplasm ratio of granulocytes was smaller than that of monocyte and lymphocyte. The cytoplasm was stained light blue and the nucleus was also stained lighter than that of the monocytes and lymphocytes. The eosinophils were observed rarely in the peripheral blood, being round or nearly round in shape. The cytoplasm was light blue and full of large, spherical, stained light blue granules which almost covered the nucleus (Fig. 2: d(4*n*)). We were not able to find any eosinophil in the blood of triploids.

3.2.3. Monocyte

In this cell, the reticulated and eccentric nucleus stained dark blue and was usually ovoid, kidney-shaped or horseshoe-shaped but could be more irregularly shaped or lobed. It also had vacuolated cytoplasm (Fig. 2: e(4*n*)) which may extend into pseudopodia. The nucleus–cytoplasm ratio of monocyte was intermediate between lymphocytes and the granulocytes. The cytoplasm of the cell stained slightly darker than that of the neutrophils.

3.2.4. Lymphocyte

Lymphocyte were spherical, irregularly spherical, round, or horseshoe shaped, while the slightly irregular nucleus heavily stained in purple. The size of the cell was variable. In the smaller lymphocytes the nucleus occupied the major space in the cells while large lymphocytes had a greater quantity of cytoplasm which, sometimes, was seen extended into pseudopodia or apophysis on its surface (Fig. 2: f(4*n*)).

3.2.5. Thrombocyte

Thrombocytes appeared as round, oval or fusiform shaped cells, with oval and fusiform thrombocytes being most frequent, the nucleus followed the shape of the cell. They appeared as single cells (Fig. 2: g(4*n*)) or in clusters (Fig. 2: h(4*n*)) and had a large central nucleus that stained dark purple. Chromatin clusters were more abundant in fusiform thrombocytes. There was a small amounted, nearly transparent cytoplasm that surrounded the nucleus.

3.3. Differential blood cell counts and blood cell sizes

There was a significant difference in differential leucocyte cell counts among different ploidy types of loach. The results of differential leucocytes cell counts (DLC) are shown in Table 1. There were more thrombocytes in tetraploid loach while more neutrophils in diploids. Also, we did not find eosinophils in the blood of triploid loach.

Tables 2 and 3 summarize the results obtained from different blood cell measurements. Differences of different ploidy types were observed in structural dimensions (volume, width, and

Table 2
Comparison of red blood cells measurements of diploid, triploid and tetraploid loach (*M. anguillicaudatus*), mean±SD

Cell features	Diploids (2*n*)	Triploids (3*n*)	Tetraploids (4*n*)
Cytoplasm width (μm)	8.87±0.50	10.18±0.75	11.12±0.72
Cytoplasm length (μm)	12.02±0.72	13.55±0.63	14.25±0.85
Cytoplasm volume (μm^3)	494.91±5.71	735.76±5.22	922.15±9.71
Ratio of cytoplasm volumes		3*n*/2*n*=1.48*	4*n*/2*n*=1.86*
Nuclear width (μm)	3.01±0.11	3.50±0.31	3.69±0.26
Nuclear length (μm)	4.66±0.34	5.47±0.46	6.50±0.50
Nuclear volume (μm^3)	10.99±2.80	35.21±5.88	46.16±7.52
Ratio of nuclear volumes		3*n*/2*n*=1.59*	4*n*/2*n*=2.09*

An asterisk marks significant differences ($p<0.05$) in tetraploid cells with respect to diploid cells and triploid cells with respect to diploid cells, but designates no statistical significance ($p>0.05$) between the test value and expect value (4*n*/2*n*=2, 3*n*/2*n*=1.5).

Table 3
Comparison of blood leucocytes measurements of diploid, triploid and tetraploid loach (*M. anguillicaudatus*), mean±SD

Items		Diploids (2*n*)	Triploids (3*n*)	Tetraploids (4*n*)
Neutrophil	Length (μm)	12.60±1.46	13.54±1.56	14.88±1.46
	Width (μm)	11.29±1.16	12.83±1.06	14.08±1.11
	Ratio of volumes		3*n*/2*n*=1.38*	4*n*/2*n*=1.84*
Thrombocyte	Length (μm)	6.81±0.87	7.65±1.02	8.89±0.99
	Width (μm)	5.85±0.86	6.82±0.93	7.69±1.14
	Ratio of volumes		3*n*/2*n*=1.52*	4*n*/2*n*=2.16*
Lymphocyte	Length (μm)	10.78±2.23	11.56±1.56	12.20±1.38
	Width (μm)	10.21±2.05	10.52±1.33	10.97±1.15
	Ratio of volumes		3*n*/2*n*=1.13**	4*n*/2*n*=1.31**
Monocyte	Length (μm)	14.09±1.45	15.82±1.87	17.01±1.60
	Width (μm)	12.60±1.56	13.16±1.23	14.95±1.04
	Ratio of volumes		3*n*/2*n*=1.22**	4*n*/2*n*=1.70**

An asterisk marks significant differences ($p<0.05$) in tetraploid cells with respect to diploid ones and triploid cells with respect to diploid ones, but designates no statistical significance ($p>0.05$) between the test value and the expect value (4*n*/2*n*=2, 3*n*/2*n*=1.5); Two asterisk designate statistical significance ($p<0.05$) between the test value and the expect value.

Table 4
Comparison of some haematological parameters among diploid, triploid and tetraploid loach (*M. anguillicaudatus*), mean±SD

Primary haematological parameters	Diploids (2*n*)	Triploids (3*n*)	Tetraploids (4*n*)
RBCC (10^6 mm^{-3})	2.23±0.24*	1.61±0.14**	1.09±0.18
Hct (%)	0.41±0.03	0.38±0.09	0.36±0.05
Hb (g dL^{-1})	6.23±0.72*	8.48±0.47**	11.24±0.97
MCV (fl)	186.14±21.47*	237.61±23.12**	337.30±54.14
MCH (pg)	28.35±5.13*	52.89±4.37**	105.05±17.01
MCHC (g L^{-1})	152.07±17.56*	223.16±12.39**	312.22±26.92
Erythrocytes osmotic brittleness (g l^{-1} NaCl)	3.12±0.06*	2.76±0.13**	2.33±0.07

An asterisk marks significant differences ($p<0.05$) between diploids and triploids for that parameter. Two asterisks mark significant differences ($p<0.05$) between triploids and tetraploids for that parameter.

length) of the cells and nuclei. The width and length of all kinds of blood cells in different ploidy loaches decreased along with the decreasing ploidy level. The mean volume of erythrocytes and leucocytes (thrombocyte and neutrophil) in tetrapoids were significantly larger than that in diploids, with a proportional relation being nearly 2:1. The mean volume of leucocytes (thrombocyte and neutrophil) in tripoids was also significantly larger than that of diploids, with a proportional relation being nearly 3:2. The volumetric ratios of erythrocytes and leucocytes (thrombocyte and neutrophil) in tetraploid, triploid and diploid were consistent with the ploidy level ratio of 4:3:2. There was no expected proportional value ($4n/2n=2$, $3n/2n=1.5$) in monocytes and lymphocytes, but in tetraploids, it was also significantly larger than those in triploids and diploids, while in triploids, larger than those in diploids. Possible size of eosinophil among the ploidy groups was not evaluated, due to failure of eosinophil observation in the blood of triploid loach.

3.4. Haematological measurements

Haematological measurements in diploid, triploid and tetraploid loach are given in Table 4. Haematocrit was similar among ploidies. Significant differences were observed in the total haemoglobin concentration and the mean cellular haemoglobin concentrations increased significantly along with the increasing ploidy level. The erythrocyte count decreased significantly with increasing ploidy level. Tetraploid fish displayed significantly lowest RBCC ($p<0.001$), but highest mean cell volume than triploids and diploids. There was significant difference in erythrocyte osmotic brittleness among the cytotypes, which descended with the increase of ploidy level. The tetraploids erythrocytes were more resistant to osmotic stress than diploid and triploid ones.

4. Discussion

The erythrocyte of loach (*M. anguillicaudatus*) is very large compared to blood corpuscles in other teleostean fish. This reflects a physiological acclimatization to habitat in deeper water or soft muddy bottom, as well as its sedentary life style. This confirms the general inverse relationship between erythrocyte size and aerobic swimming ability of teleosts (Lay and Baldwin, 1999). The value of Hb in the diploid loach was higher than values for most fishes, such as sea bass *Dicentrarchus labrax* (Peruzzi et al., 2005), shortnose sturgeon *Acipenser brevirostrum* (Beyea et al., 2005), and other sturgeons *Acipenser persicus*, *Huso huso* (Mahmoud et al., 2001). As inference, we may correlate it to the life style of the fish. It inhabits the bottom of muddy stagnant swamps or rice fields and these environments provide good dissolved oxygen supplies during part of year, but when they become stagnant and even dry up at other times, dissolved oxygen becomes low to non existent. In such poor hypoxic conditions, which require relatively higher ability of being able to endure hypoxic environment, loach has its physiological base of high Hb value and RBCC value (Xu, 2004).

The blood of loach contained a larger number of mature erythrocytes than immature erythrocytes or reticulocytes. The reticulocyte count may be an indicator of a regenerative response that can be modulated by anaemic processes and environmental factors such as temperature and dissolved oxygen availability. Multiple atypical cells were observed in the triploids that were not commonly seen in diploids and tetraploids. This included a high prevalence of dumbbell-shaped (bilobed), teardrop-shaped and tailed cells. Such cells have previously been suggested to be mitotically dividing cells either during late stages of mitosis (dumbbell-shaped with split nucleus indicating telophase/cytokinesis) or post-mitosis (teardrop and tailed cells), when juvenile cells restructure their plasma membrane and eventually form elliptical-shaped mature erythrocytes (Houston, 1997). Triploid salmonids also exhibited a high incidence of such cells (Benfey, 1999). Erythrocytes which are undergoing mitosis (nucleus or cytoplasm) can occasionally be found in loach, but not all the individuals posed that kind of blood cell. Fan et al. (2000) reported no erythrocytes splitting in *Sciaenops ocellatus* in fresh water or in sea water. Splitting of erythrocytes was found in Mandarin fish *Siniperca chuatsi* and European eel *Anguilla anguilla* (Yuan et al., 1998; Zhou et al., 2002). The difference may be specific to the species or biogeography of the fish. Among the leucocytes of loach blood, there were a large number of lymphocytes and neutrophils with less monocytes, few eosinophils and no basophils which were similar to most fish; but there were relatively more thrombocytes compared to other species. This may be related to its habit of inhabiting deeper water sites or soft muddy bottoms which need strong cruor enginery because of its vulnerability and bleeding. Maximum number of thrombocytes was observed in tetraploids, which may provide tetraploid loach with stronger ability of blood clotting. We did not find eosinophil in triploids. Possibly because we had too few to detect these cells.

As expected, erythrocyte dimensions were significantly different among ploidies (Benfey, 1999). The results revealed an increase in erythrocyte size and a decrease in erythrocyte number with the increasing ploidy level, in agreement with previously reported increase in cell volume of polyploid animals (Purdom, 1993; Guo et al., 1996; Benfey, 1999). This haematological profile of fewer and larger erythrocytes is consistent with findings for other species (Benfey, 1999). In teleost fish,

association of increase in erythrocyte size with polyploidy has been reported and measurement of red blood cell dimensions has been proposed as a rapid and inexpensive assay for triploidy determination (Krasznai et al., 1984; Ueno, 1984; Sezaki et al., 1988, 1991; Yamamoto and Iida, 1994; Benfey, 1999). In our study, the volumetric ratio of erythrocytes in tetraploids, triploids and diploids was consistent with the ploidy level ratio of 4:3:2. Therefore, variable sizes of erythrocytes in diploids, triploids and tetraploids could be used for determination of ploidy level in loach species. In spite of these variations in blood cell size, no differences in the haematocrit value was observed among diploids, triploids and tetraploids, as the increase in volume of polyploidy cells was counterbalanced by the decrease in cell counts. Although this phenomenon is poorly understood, it has been shown for most tissues and cells and allows maintenance of normal size of triploid organs and individuals (Benfey, 1999).

We found that total blood haemoglobin concentration increased in the blood of polyploids containing significantly fewer erythrocytes. The average volume of red blood cells was greater in polyploids, such that the mean cellular haemoglobin content in erythrocytes of polyploids was significantly greater than that in diploids, and the mean cellular haemoglobin concentrations was also significant greater than that in diploids. Reported values for total Hb and MCHC concentrations in diploid and polyploid fish are not consistent, whereas the mean cellular haemoglobin content is commonly reported to be higher in polyploids than in diploids (Benfey, 1999). Ballarin et al. (2004) reported no differences in total Hb but higher MCH values in triploid shi drum *U. cirrosa* as compared to their diploid counterparts. Peruzzi et al. (2005) reported that total Hb concentration was reduced in the blood of triploids, while the average volume of red blood cells and the mean cellular haemoglobin content in erythrocytes was significant greater in triploids, and the mean cellular haemoglobin concentrations were equivalent in sea bass *D. labrax*. These results were similar to values for silver crucian carp *Carassius auratus* (Vetešník et al., 2006). Beyea et al. (2005) reported total blood haemoglobin concentration and cellular haemoglobin concentration did not differ significantly between diploids and triploids but cellular haemoglobin content was elevated in triploids as a result of the significantly greater size of triploid erythrocytes in shortnose sturgeon *A. brevirostrum*. In salmonids, despite some contrasting results on total blood haemoglobin levels and blood-oxygen carrying capacity, triploid fish were found to be similar to diploids in their overall oxygen-consumption rates and swimming performances under normal or stress conditions (Stillwell and Benfey, 1995). We did not find any report in line with our results that total Hb concentration was increased in the blood along with the increase in ploidy level, although the MCH is commonly reported to be higher in polyploids. The differences may be specific to the species or biogeography of the fish due to the different living habit. Tetraploid loach is a natural existent species. Natural surviving condition makes tetraploids more resistant to hypoxic conditions with high Hb value and they grow more quickly (Yin et al., 2005).

Erythrocyte osmotic brittleness is the physiological index of showing the characteristic of erythrocytes, related to membranous fluidity. Polyploid erythrocytes were more resistant to osmotic stress than diploid ones in our study, which was consistent with findings for other species (Ballarin et al., 2004). The hypothesis of a reduced exchange of polyploid cells with the external environment fits the observation of a lower osmotic fragility of tetraploid erythrocytes with respect to triploid and diploid ones.

This is the first study to report baseline haematological status of diploid, triploid and tetraploid loach *M. anguillicaudatus*. Based on the results, it can be concluded that the higher ploidy level loach may have some advantage with respect to maximum oxygen carrying capacity of the blood and more thrombocytes inducing better ability for blood clotting. Further studies considering performance of different ploidy loach related experimental challenges should provide a better understanding of the comparative adaptability of diploid, triploid and tetraploid loach.

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