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Cytogenetic Analysis of Reciprocal Hybrids Reveals a Robertsonian Translocation between Mud Loach (*Misgurnus mizolepis*) and Cyprinid Loach (*M. anguillicaudatus*)

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ABSTRACT Reciprocal hybrids between the mud loach (*Misgurnus mizolepis*) and cyprinid loach (*M. anguillicaudatus*) were produced by artificial fertilization. The chromosome number of mud loach was 2n=48, consisting of 12M+4SM+32A chromosomes. The cyprinid loach has 2n=50, consisting of 10M+4SM+36A chromosomes. The chromosome numbers of the diploid reciprocal hybrids were 2n=49, consisting of 11M+4SM+34A chromosomes. All the karyotypes documented in this study had the same arm number of 64. There was no evidence of chromosomal polymorphisms or sex-related heteromorphism. The cytogenetic traits of the hybrid genotypes were intermediate between those of the parent species. In all genotypes, the chromosomal NORs localized to the terminal short arms of the same metacentric chromosome pair. These results suggest that Robertsonian translocation occurred between metacentric chromosome 1 of mud loach and acrocentric chromosome of cyprinid loach.

Key words : *Misgurnus mizolepis, Misgurnus anguillicaudatus*, reciprocal hybrids, karyotype, genome size, Ag-NOR

INTRODUCTION

Fishes of the family Cobitidae comprise two subfamilies, Cobitinae and Botinae, which include about 26 genera and around 177 species (Nelson, 2006). Cytogenetic studies of 46 species of Cobitidae have been performed (Animal Genome Size Database, 2011). Chromosomal information of this family has demonstrated important phenomena, including chromosomal rearrangements and polyploidization events in the evolution of the early vertebrates. Two species of the Cobitinae subfamily, the mud loach (*Misgurnus mizolepis* Günter, 1888) and cyprinid loach (*M. anguillicaudatus* Cantor, 1842) are both found in the freshwater of Korea and China.

The hybridization of fish species may be beneficial in combining the advantageous attributes of the parental species. The results of reciprocal hybridizations between the mud loach and cyprinid loach suggested that the hybrids showed excellent hatching success and early viability (Kim *et al.*, 1995). Interspecies hybridization also provides information on the genetic, evolutionary, and behavioral relationships between the parental species. However, most fish cytogenetic studies have focused on either the chromosomal arm numbers of the hybrids or the heteromorphic chromosomes. Recently, a few studies (Cioffi *et al.*, 2010) have focused on the molecular and cytotaxonomic aspects of karyotypic differentiation in fishes, but the contribution of karyology to fish phylogenetics has so far been minimal. The comparative karyology of *M. mizolepis* and *M. anguillicaudatus*, particularly the differential distribution of NORs (nucleolar organizing regions) in their metaphase chromosomes, has not yet been analyzed.

The objective of this study was to identify the origin of the haploid complement of chromosomes found in these hybrids and to evaluate the chromosomal NOR phenotypes of the mud loach, the cyprinid loach, and their reciprocal hybrids.

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MATERIALS AND METHODS

Abbreviations

The abbreviations of the four genotypes examined in this study are as follows: MM, mud loach genotype; AA, cyprinid loach genotype; MA, diploid hybrid produced from female mud loach and male cyprinid loach; AM, diploid hybrid produced from female cyprinid loach and male mud loach.

Production of hybrids

The mud loach and cyprinid loach were obtained from a fish farm located in Jeollabuk-do, Korea. The fish were temporarily maintained in laboratory aquariums at 28°C. Eggs were obtained from 20 mature females (10 of each species) after a single intraperitoneal injection of human chorionic gonadotropin (HCG) at a dose of 6～8 IU HCG g^{-1} bodyweight. Sperm were obtained from 12 mature males (six of each species) after an injection of HCG (2 IU HCG g^{-1}/bodyweight). The eggs from each parental species were fertilized with sperm from either mud loach or cyprinid loach to produce reciprocal hybrids.

Erythrocyte nuclear size

The blood from each genotype was collected from the caudal vein of each specimen. The extracted blood was smeared on a glass slide, then fixed with 95% ethanol and stained with a 10% phosphate-buffered Giemsa solution for 20 min. The major (a) and minor (b) axes of 200 erythrocyte nuclei were measured using a micrometer under 100 × magnifications. The surface area (s) and volume (v) were calculated as "$s=ab\pi/4$ and $v=4[a/2][b/2]^2\pi/3$".

Chromosome analysis

Fish with a body length of 7～15 cm were injected intramuscularly with 0.2 mL of 0.3% colchicine and maintained for 4 h. Their kidneys were removed for chromosome preparation. To release the single cells, the kidneys were minced and subjected to hypotonic treatment in 0.075 M KCl for 8 min at room temperature. After the kidney cells were harvested and the hypotonic solution removed, the cells were fixed for 8 min at 4°C with standard acetic acid : methanol (1 : 3) fixation. The fixed material was washed and resuspended in fresh fixative, dropped onto pre-cleaned slides, and air dried. The slides were stained in a 10% phosphate-buffered Giemsa solution. The chromosomal indices were measured from photographs of the metaphase chromosomes. These measurements were used to compute the relative chromosomal lengths and to determine centromeric position of each chromosome (Levan *et al.*, 1964).

Ag-NOR analysis of erythrocytes and chromosomes

The procedure used to identify NORs was originally described by Howell and Black (1980). Briefly, the prepared slides were treated with a gelatin-silver nitrate solution, covered with a coverslip, incubated at 68°C for at least 2 min, washed in deionized water, and air dried.

Flow-cytometric analysis

A flow-cytometric analysis was performed to estimate the average cellular DNA content. Blood cells, at a concentration of more than 10^6 cells mL^{-1}, were collected from the caudal veins of 20 individuals per genotype. The cells were fixed in cold 70% ethanol and stained with Cystain DNA 2 steps kit (Partec GmbH, Münster, Germany) for 15～30 min. The stained cell suspension was analyzed with a Partec PA-II flow cytometer (Partec GmbH).

Statistical analysis

Differences in the genome contents and erythrocyte sizes of the genotypes were tested by one-way analysis of variance (AVOVA) and comparisons between means were made with the Tukey test. Differences were considered significant at *P* levels of 0.05 or 0.01.

RESULTS

The erythrocyte surface areas of the mud loach and cyprinid loach were 10.63 ± 0.21 and 11.15 ± 0.22 μm^2, respectively, and their volumes were 19.10 ± 0.27 and 20.93 ± 1.23 μm^3, respectively. The erythrocyte surface areas and volumes of the reciprocal hybrids were intermediate between those of their parents (Table 1). The hy-

Table 1. Comparison of erythrocyte nuclear sizes of mud loach (MM), cyprinid loach (AA) and their hybrids (MA & AM)

Item	MM	MA	AM	AA
Major axis (μm)	5.12±0.27	5.16±0.18	5.17±0.27	5.21±0.28
Minor axis (μm)	2.67±0.36	2.71±0.21	2.71±0.21	2.75±0.27
Surface area (μm^2)	10.63 ± 0.21^{a}	10.89 ± 0.28^{ab}	10.91 ± 0.19^{ab}	11.15 ± 0.22^{b}
Volume (μm^3)	19.10 ± 0.27^{a}	19.92 ± 0.22^{ab}	20.14 ± 0.26^{ab}	20.93 ± 1.23^{b}

Values indicate means±SD of five independent experiments. Means with different superscript letter in a same low are significantly different ($P < 0.05$).
M: haploid from mud loach A: haploid from cyprinid loach
MA: mud loach (♀) × cyprinid loach (♂), AM: cyprinid loach (♀) × mud loach (♂)

Table 2. Genome size measurement of mud loach (MM), cyprinid loach (AA) and their hybrids (MA & AM) determined by flow-cytometry

Genotypes	Genome size (pg/cell)	Related value to mud loach sperm DNA (%)
Mud loach (MM)	2.798 ± 0.03	199.86
MM (♀) × AA (♂)	2.902 ± 0.05	207.29
AA (♀) × MM (♂)	2.913 ± 0.06	208.07
Cyprinid loach (AA)	3.035 ± 0.03	216.79
Mud loach sperm (M)*	1.40	100

*From Hardie and Hebert (2004)
Values indicate means ± SD of five independent experiments.
M: haploid from mud loach A: haploid from cyprinid loach
MA: mud loach (♀) × cyprinid loach (♂), AM: cyprinid loach (♀) × mud loach (♂)

Table 3. Frequency distribution of erythrocyte nucleolar organizer regions of mud loach (MM), cyprinid loach (AA) and their hybrids (MA & AM)

Experimental group	No. of fish examined	No. of Ag-stained erythrocytes	No. of Ag-NORs/cell				
			0	1	2	3	4
MM	10	100	4	24	67	5	0
MA	10	100	3	27	65	4	1
AM	10	100	3	31	64	2	0
AA	10	100	3	27	62	7	1

M: haploid from mud loach A: haploid from cyprinid loach
MA: mud loach (♀) × cyprinid loach (♂), AM: cyprinid loach (♀) × mud loach (♂)

Fig. 1. Representative metaphases and idiograms of female (a) and male (b) mud loach. Bars are 10 μm.

brids, MA and AM, showed average cellular DNA contents that were intermediate between those of the mud loach and the cyprinid loach. No difference in DNA content was found between the sexes of each genotype (Table 2).

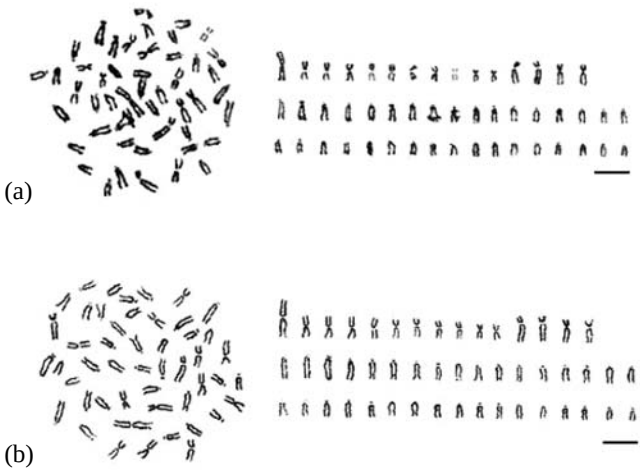

Fig. 2. Representative metaphases and idiograms of hybrid between mud loach and cyprinid loach (MA) female (a) and male (b). Bars are 10 μm.

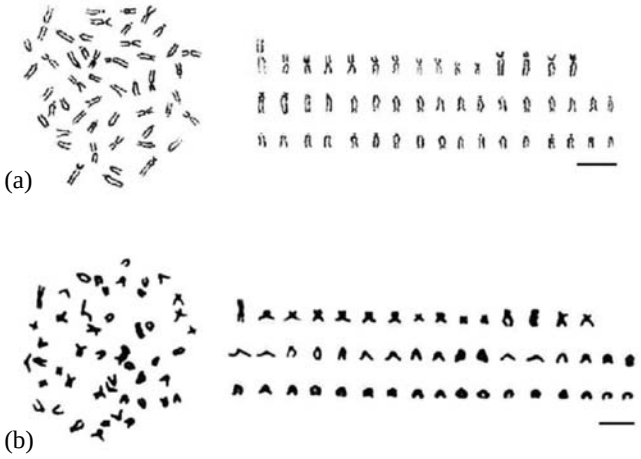

Fig. 3. Representative metaphases and idiograms of hybrid between cyprinid loach and mud loach (AM) female (a) and male (b). Bars are 10 μm.

The numbers of erythrocytic Ag-NORs counted indicated one and two nucleoli/cell for all genotypes (Table 3). Site and intensities of erythrocytic Ag-NORs did not allow the reliable identification in the hybrids and parental genotypes. The chromosome number of mud loach was 2n=48, consisting of 12M+4SM+32A chromosomes (Fig. 1). The cyprinid loach has 2n=50, consisting of 10M+4SM+36A chromosomes (Fig. 4). The hybrids between the two species had 2n=49 chromosomes. Both hybrid chromosome complements included 11M, 4SM, and 34A chromosomes (Figs. 2, 3). All genotypes used in this study had the same arm number of 64. There was

Table 4. Chromosome counts of mud loach (MM), cyprinid loach (AA) and their hybrids (MA & AM)

Genotype	No. of fish examined	Sex	No. of metaphase counted	Frequency of chromosome number						
				46	47	48	49	50	51	52
MM	15	F	122	1	9	109	3	0	0	0
		M	153	4	10	137	2	0	0	0
MA	15	F	187	0	2	9	174	2	0	0
		M	155	0	5	12	136	2	0	0
AM	15	F	189	0	1	15	173	0	0	0
		M	152	0	2	8	142	0	0	0
AA	15	F	138	0	0	2	8	125	3	0
		M	171	0	0	1	14	155	1	0

M: haploid from mud loach A: haploid from cyprinid loach
MA: mud loach (♀) × cyprinid loach (♂), AM: cyprinid loach (♀) × mud loach (♂)
F: female, M: male

Table 5. Frequency distribution of chromosomal Ag-NORs (nucleolar organizer regions) of mud loach (MM), cyprinid loach (AA) and their hybrids (MA & AM)

Genotype	No. of fish examined	Sex	No. of active Ag-NOR counted	Frequency of chromosomal Ag-NORs				
				0	1	2	3	4
MM	15	F	120	3	6	107	3	1
		M	120	5	3	110	2	0
MA	15	F	120	9	13	93	5	0
		M	120	2	5	109	3	1
AM	15	F	120	7	9	102	1	1
		M	120	5	13	98	3	1
AA	15	F	120	3	7	105	4	1
		M	120	2	6	109	2	1

M: haploid from mud loach A: haploid from cyprinid loach
MA: mud loach (♀) × cyprinid loach (♂), AM: cyprinid loach (♀) × mud loach (♂)
F: female, M: male

Table 6. Numeral characteristics of the karyotype of mud loach (MM)

Chromosome pair no.	Long arm (μm)	Short arm (μm)	Total length (μm)	Relative length (%)	Centromeric index	Classification
1	5.700	5.700	11.400	0.086	50.000	Metacentric
2	3.581	3.508	7.088	0.053	49.485	Metacentric
3	3.508	3.471	6.979	0.053	49.738	Metacentric
4	3.508	3.420	6.928	0.052	49.367	Metacentric
5	2.558	1.827	4.385	0.033	41.667	Metacentric
6	2.448	1.681	4.129	0.031	40.708	Metacentric
7	5.298	2.010	7.308	0.055	27.500	Submetacentric
8	4.750	1.535	6.285	0.047	24.419	Submetacentric
9	6.431	0	6.431	0.049	0	Acrocentric
10	6.212	0	6.212	0.047	0	Acrocentric
11	6.138	0	6.138	0.046	0	Acrocentric
12	5.883	0	5.883	0.044	0	Acrocentric
13	5.262	0	5.262	0.040	0	Acrocentric
14	5.152	0	5.152	0.039	0	Acrocentric
15	5.115	0	5.115	0.039	0	Acrocentric
16	5.079	0	5.079	0.038	0	Acrocentric
17	4.750	0	4.750	0.036	0	Acrocentric
18	4.604	0	4.604	0.035	0	Acrocentric
19	4.458	0	4.458	0.034	0	Acrocentric
20	4.165	0	4.165	0.031	0	Acrocentric
21	4.129	0	4.129	0.031	0	Acrocentric
22	4.019	0	4.019	0.030	0	Acrocentric
23	3.508	0	3.508	0.026	0	Acrocentric
24	3.142	0	3.142	0.024	0	Acrocentric

Table 7. Numeral characteristics of the karyotype of hybrid between female mud loach and male cyprinid loach (MA)

Chromosome pair no.	Long arm (μm)	Short arm (μm)	Total length (μm)	Relative length (%)	Centromeric index	Classification
1	5.500	5.500	11.000	0.084	50.000	Metacentric
2	3.420	3.363	6.782	0.052	49.580	Metacentric
3	3.341	3.320	6.661	0.051	49.840	Metacentric
4	3.341	3.263	6.604	0.050	49.407	Metacentric
5	2.415	1.703	4.118	0.031	41.349	Metacentric
6	2.315	1.553	3.869	0.030	40.147	Metacentric
7	5.094	1.888	6.982	0.053	27.041	Submetacentric
8	4.560	1.432	5.992	0.046	23.900	Submetacentric
9	6.198	0	6.198	0.047	0	Acrocentric
10	5.984	0	5.984	0.046	0	Acrocentric
11	5.920	0	5.920	0.045	0	Acrocentric
12	5.664	0	5.664	0.043	0	Acrocentric
13	5.058	0	5.058	0.039	0	Acrocentric
14	4.973	0	4.973	0.038	0	Acrocentric
15	4.916	0	4.916	0.037	0	Acrocentric
16	4.880	0	4.880	0.037	0	Acrocentric
17	4.574	0	4.574	0.035	0	Acrocentric
18	4.431	0	4.431	0.034	0	Acrocentric
19	4.282	0	4.282	0.033	0	Acrocentric
20	4.004	0	4.004	0.031	0	Acrocentric
21	3.990	0	3.990	0.030	0	Acrocentric
22	3.954	0	3.954	0.030	0	Acrocentric
23	3.861	0	3.861	0.029	0	Acrocentric
24	3.348	0	3.348	0.026	0	Acrocentric
25	2.992	0	2.992	0.023	0	Acrocentric

M: haploid from mud loach A: haploid from cyprinid loach

Table 8. Numeral characteristics of the karyotype of hybrid between female cyprinid loach and male mud loach (AM)

Chromosome pair no.	Long arm (μm)	Short arm (μm)	Total length (μm)	Relative length (%)	Centromeric index	Classification
1	5.500	5.500	11.000	0.084	50.000	Metacentric
2	3.390	3.325	6.714	0.052	49.516	Metacentric
3	3.296	3.274	6.570	0.050	49.835	Metacentric
4	3.296	3.223	6.519	0.050	49.446	Metacentric
5	2.371	1.641	4.011	0.031	40.901	Metacentric
6	2.255	1.482	3.737	0.029	39.652	Metacentric
7	5.081	1.836	6.917	0.053	26.541	Submetacentric
8	4.553	1.373	5.926	0.045	23.171	Submetacentric
9	6.216	0	6.216	0.048	0	Acrocentric
10	5.999	0	5.999	0.046	0	Acrocentric
11	5.934	0	5.934	0.046	0	Acrocentric
12	5.673	0	5.673	0.044	0	Acrocentric
13	5.066	0	5.066	0.039	0	Acrocentric
14	4.965	0	4.965	0.038	0	Acrocentric
15	4.900	0	4.900	0.038	0	Acrocentric
16	4.871	0	4.871	0.037	0	Acrocentric
17	4.575	0	4.575	0.035	0	Acrocentric
18	4.423	0	4.423	0.034	0	Acrocentric
19	4.264	0	4.264	0.033	0	Acrocentric
20	3.975	0	3.975	0.031	0	Acrocentric
21	3.961	0	3.961	0.030	0	Acrocentric
22	3.932	0	3.932	0.030	0	Acrocentric
23	3.838	0	3.838	0.029	0	Acrocentric
24	3.310	0	3.310	0.025	0	Acrocentric
25	2.963	0	2.963	0.023	0	Acrocentric

M: haploid from mud loach A: haploid from cyprinid loach

Table 9. Numeral characteristics of the karyotype of cyprinid loach (AA)

Chromosome pair no.	Long arm (μm)	Short arm (μm)	Total length (μm)	Relative length (%)	Centromeric index	Classification
1	3.380	3.308	6.688	0.054	49.459	Metacentric
2	3.279	3.264	6.543	0.052	49.889	Metacentric
3	3.279	3.206	6.485	0.052	49.442	Metacentric
4	2.345	1.628	3.973	0.032	40.984	Metacentric
5	2.244	1.462	3.706	0.030	39.453	Metacentric
6	5.001	1.766	6.767	0.054	26.096	Submetacentric
7	4.538	1.361	5.899	0.047	23.067	Submetacentric
8	6.152	0	6.152	0.049	0	Acrocentric
9	6.007	0	6.007	0.048	0	Acrocentric
10	5.942	0	5.942	0.048	0	Acrocentric
11	5.899	0	5.899	0.047	0	Acrocentric
12	5.682	0	5.682	0.046	0	Acrocentric
13	5.088	0	5.088	0.041	0	Acrocentric
14	4.972	0	4.972	0.040	0	Acrocentric
15	4.885	0	4.885	0.039	0	Acrocentric
16	4.878	0	4.878	0.039	0	Acrocentric
17	4.567	0	4.567	0.037	0	Acrocentric
18	4.437	0	4.437	0.036	0	Acrocentric
19	4.256	0	4.256	0.034	0	Acrocentric
20	3.981	0	3.981	0.032	0	Acrocentric
21	3.959	0	3.959	0.032	0	Acrocentric
22	3.937	0	3.937	0.032	0	Acrocentric
23	3.843	0	3.843	0.031	0	Acrocentric
24	3.293	0	3.293	0.026	0	Acrocentric
25	2.967	0	2.967	0.024	0	Acrocentric

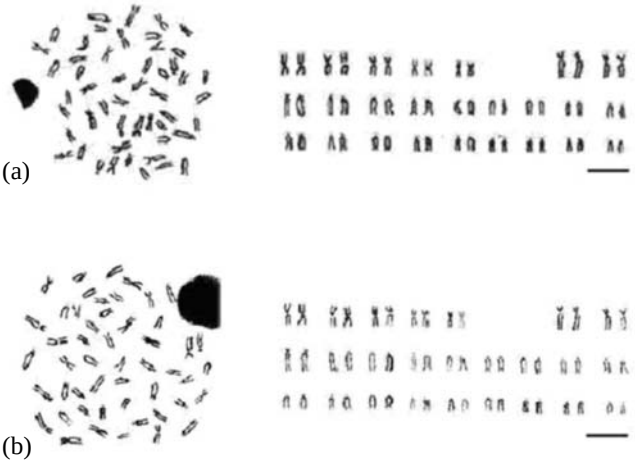

Fig. 4. Representative metaphases and idiograms of female (a) and male (b) cyprinid loach. Bars are 10 μm.

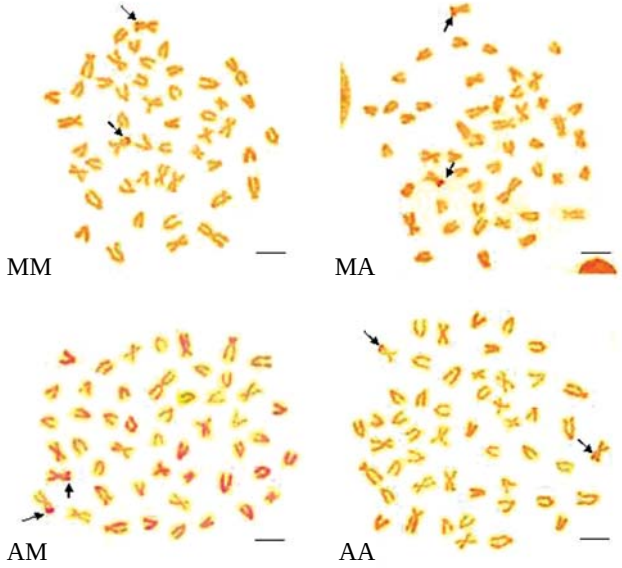

Fig. 5. Ag-NORs stained metaphases of mud loach (MM), cyprinid loach (AA) and their hybrids (MA & AM). Bars are 10 μm. The arrows indicate active Ag-NORs signal.

no evidence of chromosomal polymorphisms, such as aneuploidy or sex-related heteromorphism. The haploid complement of the mud loach contains one fewer chromosome than that of the cyprinid loach. The largest metacentric chromosome (relative length of 0.086%) in the haploid complement of the mud loach spread was equivalent to two acrocentric chromosomes in the haploid complement of the cyprinid loach (Tables 6, 9). The extra chromosome (relative length of 0.084%) in the haploid complements of the reciprocal hybrids derived from the largest metacentric chromosome of the mud loach (Tables 7, 8).

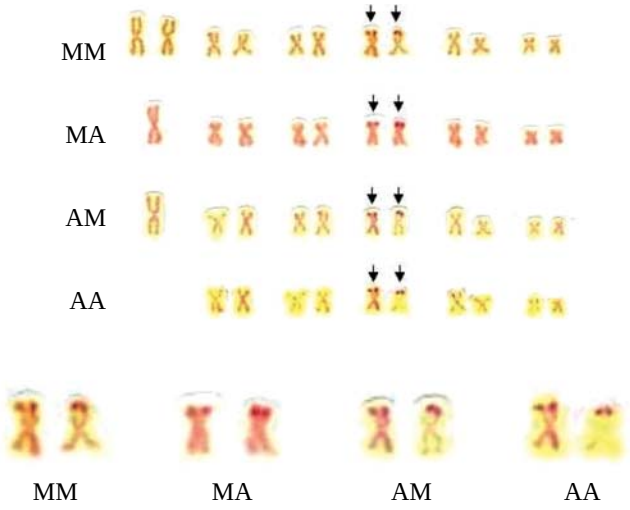

Fig. 6. Partial karyotypes of mud loach (MM), cyprinid loach (AA) and their hybrids (MA & AM) to show a pair of chromosomes displaying active Ag-NORs.

The numbers and location(s) of active chromosomal NORs were identical between and within the four genotypes examined. All these active NORs were located telomerically and occurred as a single pair (Figs. 5, 6) (Table 5). Numerical analysis showed that the active NORs were on the same chromosomes in all the genotypes examined. There was no evidence of chromosomal NOR polymorphisms between the genotypes or within the genotypes.

DISCUSSION

Interspecific hybridization is used commercially in aquaculture to produce desired changes in the attributes of fish strains. Moreover, interspecific or intergeneric hybridization can contribute data about the inheritance of chromosomal variations caused by Robertsonian rearrangements (Boron, 2003), such as the fusion of two acrocentric chromosomes into one metacentric chromosome. The haploid chromosome sets of the parental species in the chromosome complements of interspecific hybrids have been identified in some species (Martin *et al.*, 2008; Hashimoto *et al.*, 2009).

Measuring of hematological parameters is a quick method of determining polyploidy, because increases in the erythrocyte cell and nuclear volumes are associated with results from an increase in their DNA contents. However, this method appears to be unreliable for hybrids between species with similar genome sizes, such as those examined in this study.

Flow cytometry allows the precise determination of the amount of DNA in the tissue cells of fish embryos and the blood cells of juvenile and adult specimens. This method has allowed the detection of mosaicism in genetically manipulated kokanee salmon (*Oncorhynchus nerka*) (Tanaka *et al.*, 2003). The hybrids, MA and AM, were intermediate cellular DNA contents between those of the mud loach and the cyprinid loach. Therefore, the C-values observed in this study may indicate that the possibility of karyoevolution between the mud loach (1.40 pg/cell) and cyprinid loach (1.52 pg/cell) evolved by chromosome rearrangement.

NORs are chromosomal regions involved in the transcription of ribosomal genes. If these regions are active during the interphase that precedes mitosis, they can be detected by silver nitrate staining. Therefore, this technique actually reveals active NORs, but not the rDNA associated with the NORs. Analyzing the number of nucleoli per erythrocyte cell is a simple and quick method for confirming the ploidy or hybrid status of specimens. Analysis of the numbers of erythrocytic NORs has been used effectively to determine ploidy, such as in the triploid rainbow trout (Philips *et al.*, 1986) and hexaploid sturgeon (Flajshans and Vajcova, 2000). In previous studies (Kim *et al.*, 1995; Park *et al.*, 2006), the karyological traits, growth performance, and morphometric traits of these reciprocal hybrids were intermediate between those of the two parents. The reciprocal hybrids also displayed excellent hatching success and early viability. These phenomena indicate the compatibility of the parental genomes and their synchronization in successive cell divisions. However, the haploid sets of the parental species in the chromosomal complements of the reciprocal hybrids were unclear.

In the present study, cytogenetic results may indicate that Robertsonian translocation occurred in the evolution between the mud loach and cyprinid loach. However, further research is required to determine whether Robertsonian fusion occurred between acrocentric chromosomes in the cyprinid loach. Molecular cytogenetic techniques, such as genomic *in situ* hybridization, may allow to a more definitive analysis of these reciprocal hybrids and the origins of the parental fishes, when the total genomic DNA from one parent is labeled and hybridized with the fluorescently labeled chromosomes of the other parental fish. Fluorescent *in situ* hybridization (FISH) analysis of telomeric sequence repeats $(TTAGGG)_n$ may also reveal karyotypic rearrangements attributable to Robertsonian fusion between the parental fish species as the presence of $(TTAGGG)_n$ sequences at nontelomeric sites in addition to the telomeric regions (Phillips *et al.*, 2005; Cioffi *et al.*, 2010).

The numbers and location(s) of active chromosomal NORs were identical between and within the four genotypes examined. All these active NORs were located telomerically and occurred as a single pair. Numerical analysis showed that the active NORs were on the same chromosomes in all the genotypes examined. These re-

sults may indicate that the phylogenetic relationship between the mud loach and cyprinid loach was well-conserved, because they have retained a constant number of active chromosomal NORs throughout evolution. The chromosomal NORs of the ray-fin fishes (Actinopterygii) are generally located in telomeric regions (p or q arm) (Vitturi *et al.*, 2005; Sczepanski *et al.*, 2010). However, it is possible that a NOR can move from one chromosome to another chromosome or from one site to another by mechanisms that do not involve evolutionary chromosomal rearrangements or translocations (Santi-Rampazzo *et al.*, 2008). Several studies have confirmed polymorphisms in the NOR phenotypes of fishes and amphibians (Gold, 1984; Phillips and Rab, 2000).

In the previous studies (Vitturi *et al.*, 2005; Porto-Foresti *et al.*, 2006; Li *et al.*, 2010), the NOR phenotype was detected with chromomycin A_3 or the chromosomal localization of ribosomal genes (rDNAs) by FISH. The Ag-NORs represent the chromosomal regions where the actively transcribed rDNAs (18S, 5.8S and 28S) cluster. FISH of the major or minor rDNAs can detect all rDNA loci on the chromosomes. Consequently, the identification of rDNAs, which include repetitive sequences, on the chromosomes of the parental fishes should lead to the clear molecular characterization of the chromosomes. These data might clarify the various hypotheses about the origin of the extra chromosomes observed in haploid chromosome complements of the hybrids and the process of chromosomal evolution that links the two parental species.

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미꾸라지(*Misgurnus mizolepis*)와 미꾸리(*M. anguillicaudatus*) 및 유도된 종간 잡종의 세포유전학적 연구

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요 약 : 미꾸라지, 미꾸리 및 상반교배를 통해 유도된 종간 잡종의 세포유전학적 분석을 수행하였다. 미꾸라지와 미꾸리의 염색체 수는 각각 2n=48 (12M+4SM+32A), 2n=50 (10M+4SM+36A)이었고, 잡종군들의 염색체 수는 각각 2n=49 (11M+4SM+34A)였다. 모든 그룹의 염색체는 동일한 arm number (NF=64)를 갖고 있었으며, 염색체 다형현상, 암수 간 이형의 염색체는 관찰되지 않았다. 적혈구의 크기, DNA 함량을 분석한 결과 잡종군들은 미꾸라지와 미꾸리의 중간 값을 나타냈다. 염색체의 NORs (nucleolar organizing regions)은 모두 동일한 중부 염색체 단완부에서 Ag-positive signal이 나타났다. 이상의 결과는 미꾸라지의 1번 중부 염색체와 미꾸리의 차단부 염색체가 Robertsonian 형의 염색체 전좌 과정을 거쳤을 것을 시사한다.

찾아보기 낱말 : 미꾸라지, 미꾸리, 잡종, 핵형, genome size, Ag-NOR