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Interactions between mitochondrial and nuclear genomes and co-regulation of mitochondrial and nuclear gene expression in reciprocal intergeneric hybrids between *Carassius auratus* red var. × *Cyprinus carpio* L.

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ABSTRACT

Genetic interactions between nuclear and mitochondrial genomes always play an important role in growth and development. However, it is not feasible to directly perform these studies in some animals. The reciprocal hybrid fishes obtained from intergeneric hybridization are an effective research model for the same nuclear genome and different mitochondrial genomes. The transcriptomes of embryonic development (Blastula Period, Gastrula Period, Segmentation Period and Hatching Period) were obtained from the two reciprocal hybrids of *Carassius auratus* red var. (RCC) × *Cyprinus carpio* L. (CC) and their parents. Most of the reads (average > 99.90%) in the transcriptome of F_1-RC (*C. auratus* red var. (♀) × *C. carpio* L. (♂)) and F_1-CR (*C. carpio* L. (♀) × *C. auratus* red var. (♂)) were optimally mapped to the maternal mitochondrial genome (MMG), respectively. Then, the other reads with optimally mapped to paternal mitochondrial genome exhibited that the partial coverages and low-level (<0.4%) expressions of paternal mitochondrial genes were in the four developmental stages of them, especially hatching period, in which paternal mitochondrial DNA (mtDNA) is always eliminated in animals. In the four stages, some changes of 13 mitochondrial gene expressions were occurred in comparisons of the female parents and their hybrid progenies (F_1-RC vs. RCC and F_1-CR vs. CC), although no significantly differential expression (DE) was detected in them. Moreover, in each embryonic development stages, the positive correlation between the number of differential expression of nuclear-encoded mitochondrial genes and the *p-value* of differential expression of mitochondrial genes help us understanding how the detail of mitochondrial-nuclear genetic interactions operate in hybrids. The detection of paternal mitochondrial genome fragments in hatching period provides us insight into potential escape mechanisms which may disrupt or inactivate PME in hybrids. These differential expression of nuclear-encoded mitochondrial genes between reciprocal hybrids may help us understanding of the genetic basis of growth in hybrids and used in their breeding project.

1. Introduction

Mitochondria are important organelles in eukaryotic cells, more than 90% of the energy required for cell survival is provided by ATP synthesized in mitochondria [1]. In fish, mitochondrial DNA (mtDNA) contains a single non-coding region responsible for initiation of genome replication, as well as coding sequences for tRNAs, rRNAs, and proteins involved in oxidative phosphorylation [2,3]. Although sperm mitochondria can enter the egg during fertilization, the mechanisms of selective paternal mitochondrial elimination (PME) are unknown. One possible hypothesis is that PME is mediated by mitochondrial endonuclease G and usually occurs during embryonic development [4,5]. As a result, mitochondria were considered as maternal inheritance for a long time due to the lack of research or reports on the mechanism of PME.

Heteroplasmy of mtDNA is the presence of multiple mitochondrial genomes in an individual or population. Some reports have described this

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phenomenon as more widespread in naturel population of some species, such as North-East Atlantic cod populations [6]. Meanwhile, the coexistence of biparental mtDNA sequences was observed in chum salmon and Japanese flounder [7–9]. Furthermore, a low level of paternal mtDNA (0.01%–0.1%) is routinely detected in interspecific hybrid mice derived from backcrosses between *Mus musculus* and *M. spretus* [10]. A small quantity of recombinant mtDNA has been observed in some individuals derived from the intergeneric hybrid lineage of *C. auratus* red var. (♀) × *C. carpio* L. (♂) [11]. Although elimination of paternal mtDNA has been observed during embryonic development in both reciprocal hybrids between *C. auratus* red var. and *Megalobrama amblycephala* [12], escape mechanisms may exist and inactivate PME in hybrids because of different nuclear genetic backgrounds [10].

MtDNA recombination has been reported in freshwater mussels [13], and could be considered an evidence for biparental mtDNA inheritance. The coexistence of multiple mitochondrial genomes, as well as mtDNA recombination, can be investigated easily in the hybrid progenies of different species based on specific SNPs and variable number tandem repeats [10,14]. The potential mechanism of mtDNA recombination has been reported to be related to a mitochondrial Rad52-type recombination system of bacteriophage origin [15]. In addition, increasing evidences support the notion that mtDNA recombination is a closely coordinated and synchronized process that functions to repair and replicate multiple mitochondrial genomes [15].

Although mitochondrial respiratory complexes are predominantly composed of polypeptides that are encoded by nuclear genes and imported into the mitochondria post-translation, their assembly and function are dependent on the expression of the mitochondrially-encoded polypeptides. Furthermore, the mitochondrial genome is dependent on nuclear-encoded proteins for replication, repair, transcription, and translation [16]. Therefore, biparental mtDNA inheritance and mtDNA recombination may affect oxidative phosphorylation and further shape the diversity of energy demands in different tissues [17]. The order of magnitude mutation rate differences between the mtDNA and nuclear genome requires tight co-evolution of the two genomes [18–20]. Changes in the mitochondrial genome could lead to variations in many traits such as hair color and height [17]. To investigate potential genetic changes and mitochondrial-nuclear interactions, our study focused on expression of paternal mtDNA during embryonic development and changes in the expression of nuclear-encoded mitochondrial genes in hybrid fish strains. Our results provide a new viewpoint of mitochondrial evolution in hybrid vertebrates and a comprehensive description of the co-regulation of mitochondrial and nuclear genomes.

2. Materials and methods

2.1. Origin of reciprocal hybrids

C. auratus red var. and *C. carpio* L. belong to different genera of the family Cyprinidae. Allotetraploid progenies (F_3–F_{28}, 4n = 200) were obtained by the selfcorssing of allodiploid hybrids of *C. auratus* red var. and *C. carpio* L. (F_1–F_2, 2n = 100) [21,22] (Fig. 1). We specifically focused on potential genetic changes in mitochondrial gene expression during development to investigate differences in mitochondrial effects between the two reciprocal F_1 hybrids (F_1-RC and F_1-CR) (Fig. 1).

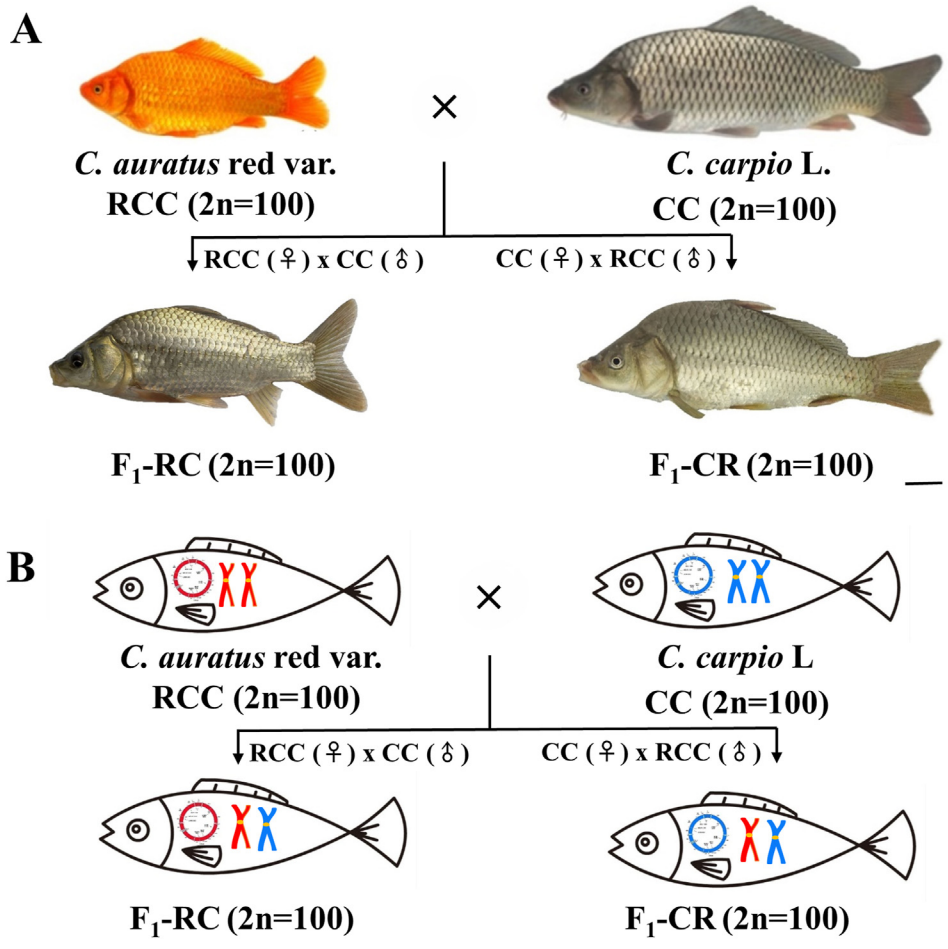

Fig. 1. Procedure for generating first-generation reciprocal hybrids by interbreeding *C. auratus* red var. and *C. carpio* L. A. The physical appearance of *C. auratus* red var. (2n = 100), *C. carpio* L. (2n = 100), and their two hybrids F_1-RC (2n = 100) and F_1-CR (2n = 100) B. The genotypes and mitochondrial genome of *C. auratus* red var., *C. carpio* L., and their two hybrids F_1-RC and F_1-CR. Both F_1-CR and F_1-RC chromosomes of diploid progenies are composed of one set of chromosomes from female parent and one set from male parent, and the mitochondrial genome follows the maternal inheritance. Scale bar: 6.0 cm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.2. Experimental animals

Fertile *C. auratus* red var. and *C. carpio* L. were obtained from the Engineering Center of Polyploidy Fish Breeding of the National Education Ministry, Hunan Normal University, Hunan, China. Embryos at all four developmental stages (blastula, gastrula, segmentation, and hatching) were collected from both purebred *C. auratus* red var. and purebred *C. carpio* L. Next, female *C. auratus* red var. were crossed with male *C. carpio* L. (F_1-RC), and female *C. carpio* L. were crossed with male *C. auratus* red var. (F_1-CR), and the resulting first-generation allodiploid hybrid embryos were collected at all four developmental stages. All embryo samples were treated with DEPC-treated water (Sangon Biotech Co., Ltd., Shanghai, China) to eliminate contamination. These experiments were conducted at the Engineering Center of Polyploidy Fish Breeding of the National Education Ministry, Hunan Normal University, Hunan, China, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.3. mRNA-seq

Total RNA (~2 μg) was extracted from mixed samples of 20 embryos that were collected from the same cross at the same developmental stage. After the RNA quality was assessed, paired-end libraries were constructed using a TruSeq® RNA library prep kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. For each mixed sample, an mRNA-seq library was sequenced with paired ends (2 × 150 bp) using a NovaSeq 6000 Sequencing System (Illumina, Sad Diego, CA, USA). Raw reads produced from Illumina sequencing were filtered by removing the adapter sequences and low-quality reads using the Trimmomatic software [23]. The quality of the pretreated data was assessed using FastQC [24] (v0.11.9) software. Illumina sequencing platform produced 441.54 G original data. After trimming the raw data, 1.44 billion clean reads were obtained, with the clean reads/raw reads rate ranging from 96.65% to 97.87% in all 48 samples.

2.4. Detection of paternal mitochondrial reads in the transcriptome data

The mRNA-seq reads from the intergeneric hybrids (F_1-RC and F_1-CR) were mapped to the combined mitochondrial DNA sequences of *C. auratus* red var. (NCBI accession No.: AY714387.1) and *C. carpio* L. (NCBI accession No.: AP009047.1) using bowtie2 software (v 2.3.4.3) [25] with defult parameter. F_1-RC reads that mapped to the *C. carpio* L. mitochondrial genome and F_1-CR reads that mapped to the *C. auratus* red var. mitochondrial genome were identified using perl script (extract.fasta.from.sam.using.list.pl) (downloaded from https://github.com/MadsAlbertsen/multi-metagenome/blob/master/reassembly). Next, the potential paternal reads identified as described above from three biological replicates were merged into a single file, and duplicated reads were deleted. Finally, these potential paternal reads from the same development stage were remapped to paternal and maternal mitochondrial DNA sequences using bowtie2 (v2.3.4.3) software [25] with defult parameter. A further visual inspection step was performed to check the potential paternal mitochondrial reads. Only reads with higher similarity to the paternal mitochondrial genome (PMG) than to the maternal mitochondrial genome were considered to be authentic paternal reads.

2.5. Mitochondrial gene expression profiles

After quality checking, the *C. auratus* red var., *C. carpio* L., and intergeneric hybrid (F_1-RC and F_1-CR) mRNA-seq reads were mapped to 13 maternal mitochondrial genes (*nd1, nd2, cox1, cox2, atp8, atp6, co3, nd3, nd4l, nd4, nd5, nd6,* and *cytb*) using Salmon (v1.1.0) software with default options [26]. The mitochondrial gene annotations were obtained from their annotated files (NCBI accession No.: AY714387.1 and AP009047.1). The number of mapped reads and the transcripts per million (TPM) values were obtained from the output results. Differential expression analyses were performed using the DESeq2 package based on a threshold of $|\log_2 \text{fold change}| > 1$ and a false discovery rate of <0.01 for three biological replicates. Paired *t*-tests were used to assess differences in gene expression in three pairwise comparisons (RCC vs. F_1-RC, F_1-RC vs. F_1-CR, and CC vs. F_1-CR).

2.6. Nuclear-encoded mitochondrial gene expression profiles

The mRNA-seq reads of *C. auratus* red var. and *C. carpio* L. were mapped to the predicted coding sequences in the *C. auratus* red var. genome (Genome Warehouse in BIG Data Center BioProject No.: PRJCA001234) [27] and the *C. carpio* L. genome (NCBI accession No.: PRJNA510861) [28]. The mRNA-seq reads of the intergeneric hybrids (F_1-RC and F_1-CR) were also mapped to the genome sequences of *C. auratus* red var. and *C. carpio* L.. These read-mapping analyses were conducted using Salmon software with default options [26]. Genes were annotated by performing BLASTX searches in NCBI, Gene Ontology, and Swiss-Prot. Gene expression values for *C. auratus* red var. and *C. carpio* L. were determined by quantifying the reads that mapped to their respective mitogenmes, while gene expression values for the two hybrids were assessed by determining the average number of reads that mapped to each of the two reference genomes for each gene [29]. Expression values were determined and a DE analysis was performed for nuclear genes as described above.

3. Results

3.1. Detection of paternal mitochondrial genes

Using the mRNA-seq data, we investigated whether intergeneric hybridization altered the expression of mitochondrial genes. In total, 441.54 Gb of raw sequencing data was obtained from 48 transcriptomes (Table S1 in Supporting Information). After quality checking, 1.44 billion clean reads (432.13 Gb) were retained for subsequent analyses. To investigate whether paternal mitochondrial genes were expressed during embryo development, all the clean reads from the F_1-RC and F_1-CR embryos were mapped to the *C. auratus* red var. and *C. carpio* L. mitochondrial DNA genomes. Overall, 55–1633 paternal mitochondrial reads were detected during the four developmental stages in F_1-RC, while 181–1203 paternal mitochondrial reads were detected in F_1-CR, the paternal DNA fragments are usually retained more in gastrula period, but it is always eliminated in hatching period (Table 1). However, most of the reads (average > 99.90%) from both F_1-RC and F_1-CR mapped to the maternal mitochondrial genome (MMG) (Table 1). The reads mapped to the paternal mitochondrial genome (PMG) are shown in Fig. 2. Interestingly, the results showed that the reads from F_1-RC and F_1-CR covered the entire MMG, whereas only partial coverage of the PMG was observed (Fig. 2). Furthermore, the same regions of the PMG were expressed in both the four developmental stages of F_1-RC and F_1-CR (Fig. 2). Overall, partial coverage and low-level (<0.4%) expression of paternal mtDNA were detected in transcript level.

3.2. Changes in mitochondrial gene expression associated with hybridization

Because of the difference of nuclear and mitochondrial inheritance, the source of mitochondrial DNA of the two hybrids is different, but the source of nuclear DNA is the same. Although a small number of paternal mitochondrial genes appeared to be expressed in the hybrid progeny, the mitochondrial inheritance in the hybrids was dominated by the female parents. Therefore, we decided to investigate the expression of 13 maternal mitochondrial genes to identify potential genetic changes in mtDNA and the nuclear genome that occurred after hybridization.

No significant differences in the expression of these 13 mitochondrial genes were observed between RCC and F_1-RC. However, the levels of gene expression between RCC and F_1-RC differed among the four

Table 1
Number of parental mitochondrial reads detected in the two reciprocal cross hybrids based on transcriptome data.

			Blastula Period	Gastrula Period	Segmentation Period	Hatching Period
F_1-RC	Paternal CC mitochondrion	Sample 1	974 (0.40%)	862 (0.03%)	197 (0.01%)	251 (0.02%)
		Sample 2	218 (0.10%)	1633 (0.06%)	145 (0.01%)	98 (0.01%)
		Sample 3	241 (0.08%)	993 (0.04%)	119 (0.01%)	55 (<0.01%)
	Maternal RCC mitochondrion	Sample 1	239,969 (99.60%)	2,615,511 (99.97%)	1,409,275 (99.99%)	1,309,155 (99.98%)
		Sample 2	214,177 (99.90%)	2,951,028 (99.94%)	1,467,846 (99.99%)	1,126,872 (99.99%)
		Sample 3	300,686 (99.92%)	2,790,486 (99.96%)	1,327,883 (99.99%)	1,367,968 (>99.99%)
F_1-CR	Maternal CC mitochondrion	Sample 1	441,905 (99.90%)	618,927 (99.85%)	1,079,805 (99.93%)	1,298,366 (99.93%)
		Sample 2	463,383 (99.90%)	755,762 (99.91%)	1,250,508 (99.92%)	980,285 (99.98%)
		Sample 3	360,017 (99.86%)	742,393 (99.89%)	1,628,323 (99.93%)	1,118,493 (99.98%)
	Paternal RCC mitochondrion	Sample 1	447 (0.10%)	944 (0.15%)	759 (0.07%)	230 (0.02%)
		Sample 2	480 (0.10%)	701 (0.09%)	1009 (0.08%)	219 (0.02%)
		Sample 3	508 (0.14%)	849 (0.11%)	1203 (0.07%)	181 (0.02%)

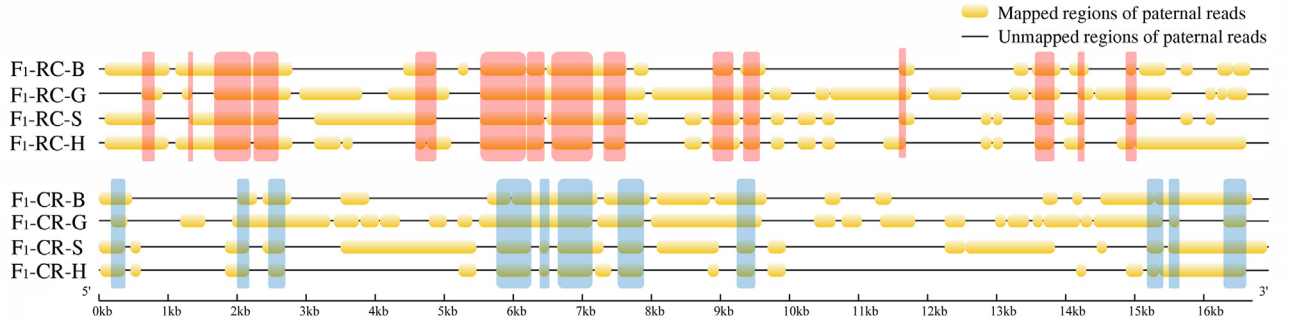

Fig. 2. Regions of the paternal mitochondrial DNA sequences detected in the transcriptomes of the two reciprocal hybrids. B, blastula; G, gastrula; S, segmentation; H, hatching. Areas shaded in red were expressed in F_1-RC, while areas shaded in blue were expressed in F_1-CR. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

developmental stages. The maximum difference in gene expression occurred in the gastrula period ($p = 0.0898$), while the minimum difference was detected during the blastula period ($p = 0.6956$) (Fig. 3 and Table 2). Similarly, there was no significant DE between CC and F_1-CR during the four development stages. However, the maximum difference in gene expression was observed during the segmentation period ($p = 0.0223$), while the minimum difference was detected during the hatching period ($p = 0.7046$) (Fig. 3 and Table 2). Interestingly, the differences between the two reciprocal hybrids changed depending on the developmental stage. Furthermore, the maximum difference in expression of the 13 homologous RCC and CC mitochondrial genes between F_1-RC and F_1-CR was detected during the segmentation period ($p = 0.6878$), while the minimum difference was observed during the hatching period ($p = 0.9471$) (Fig. 3 and Table 2). Overall, the degree of difference in gene expression between F_1-RC and F_1-CR (comparison 2) was smaller than those seen for comparisons 1 (RCC vs. F_1-RC) and 3 (CC vs. F_1-CR) (Fig. 3 and Table 2).

No obvious changes in expression of the 13 mitochondrial genes during the four developmental stages were observed (Fig. S1 in Supporting Information). However, similar expression patterns were observed for some of the mitochondrial genes, including *nd1*, *nd2*, *cox2*, *atp8*, and *nd3* (Fig. S1 in Supporting Information). One of these similar expression patterns included RCC and F_1-RC, reflecting similar expression of some genes in the RCC mitochondrial genome, while the other similar expression patterns included CC and F_1-CR and was related to the expression of genes in the CC mitochondrial genome.

3.3. Expression of nuclear-encoded mitochondrial genes

More differences in mitochondrial gene expression were observed in comparisons 1 and 3 than in comparison 2, prompting us to investigate the expression of nuclear genes that regulate mitochondrial gene expression in the two reciprocal hybrids. We assessed the expression of 1013 nuclear-encoded genes implicated in mitochondrial function [30], and found that only 417 were detectable in the all transcriptome data (Table S2 in Supporting Information). DE analysis identified the greatest number of differentially expressed genes (DEGs) between the two hybrids was detected in the segmentation period (67 genes up-regulated in F_1-CR and 78 genes up-regulated in F_1-RC) (Fig. 4). In contrast, the fewest number of DEGs were detected during the hatching period (13 genes up-regulated in F_1-CR, and 16 genes up-regulated in F_1-RC) (Fig. 4). Interesting, more up-regulated genes were found in F_1-RC than ones in F_1-CR (Fig. 4).

3.4. Expression of nuclear-encoded mitochondrial genes during ontogenesis

To investigate nuclear-encoded mitochondrial gene expression during ontogenesis, we analyzed DEGs that were shared by both hybrids during the four development stages. The five expression patterns are shown in Fig. 5. In total, 73 genes belonged to the same differential expression trend (DET) during two development stages. Only two genes (*vdac2*, *ndufa7*) exhibited the opposite DET. Interestingly, no shared DEGs were detected in more than two developmental stages. Most of the shared DEGs (70 genes) were expressed during the gastrula and segmentation periods, while only six of the shared DEGs were expressed during the blastula and hatching periods (Fig. 5). Furthermore, more shared up-regulated genes were detected in F_1-RC compared with F_1-CR (F_1-RC vs. F_1-CR: 5 vs. 1 and 40 vs. 30) (Fig. 5).

4. Discussion

Reciprocal hybridization is a useful model for investigating identical nuclear genomes and different mitochondrial genomes. In our study, we found that differences in mtDNA between reciprocal hybrid progeny of *C. auratus* red var. and *C. carpio* L. may affect the growth of these progeny. Differences in energy metabolism and growth traits, including individual body size and growth rate, were observed in the two reciprocals intergeneric F_1 hybrids [31,32] (Fig. 1). Comparing these hybrids

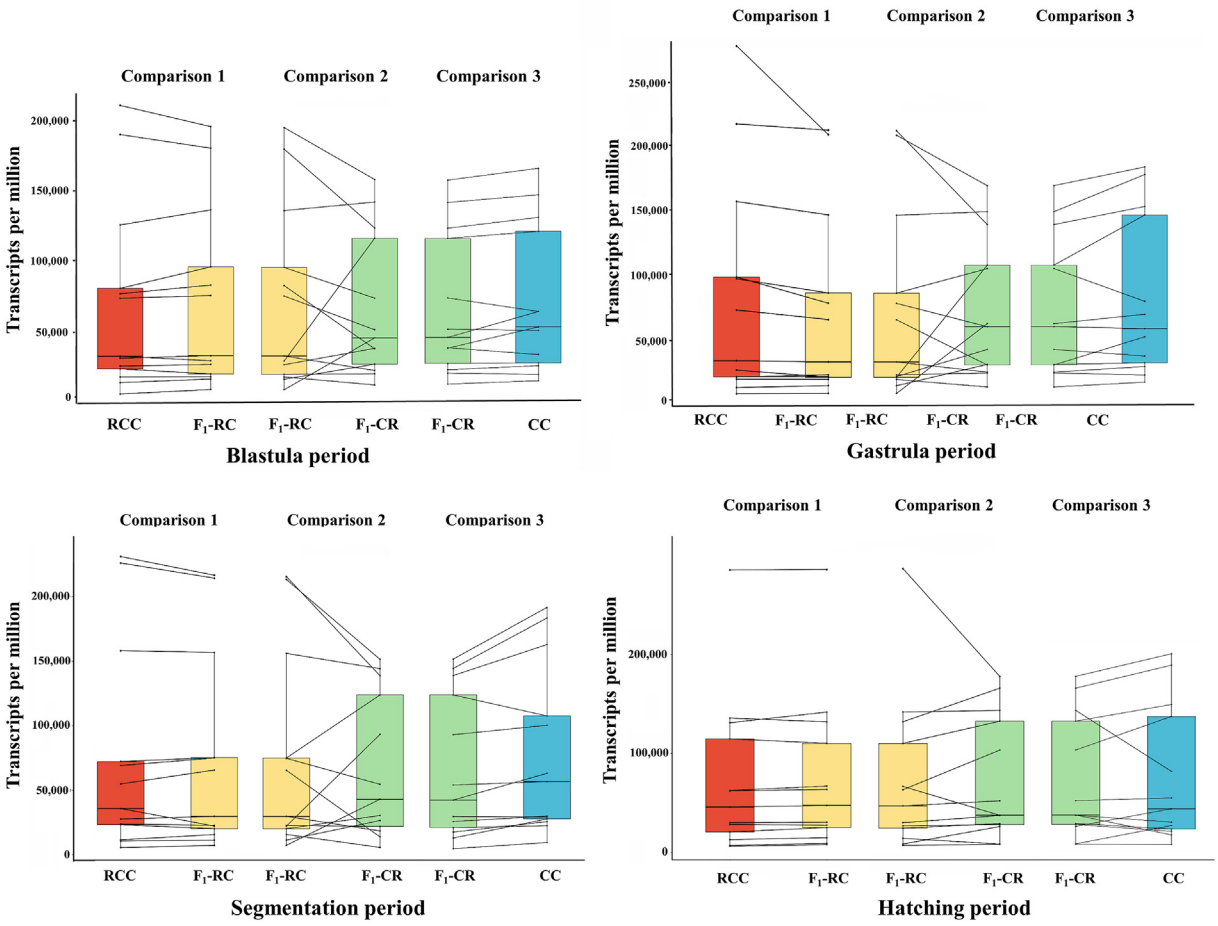

Fig. 3. Differential expression (DE) analysis of maternal mitochondrial genes during the four developmental stages, the lines show the difference of expression of 13 mitochondrial genes in the three species. Comparison 1: *C. auratus* red var. vs. F_1-RC; comparison 2: F_1-RC vs. F_1-CR; comparison 3: *C. carpio* L. vs. F_1-CR. No genes with significant DE were observed in any of the comparisons. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Distribution of *p-value* obtained from the paired *t*-test analyses.

		Blastula Period	Gastrula Period	Segmentation Period	Hatching Period
Comparison 1	RCC vs. F_1-RC	0.6956	0.0898	0.5549	0.1576
Comparison 2	F_1-RC vs. F_1-CR	0.7340	0.8778	0.6878	0.9471
Comparison 3	CC vs. F_1-CR	0.0955	0.1114	0.0223	0.7046

allowed us to distinguish not only mitochondrial effects but also interactions between the mitochondrial and nuclear genomes, and further explore their potential effect on growth traits.

MtDNA is inherited maternally in most animals, and a variety of mechanisms operate to eliminate paternal mtDNA in zygotes [31,32]. However, our results show that mitochondrial heteroplasmy existed in the two reciprocal F_1 hybrids during the hatching period (Fig. 2), during which period the paternal mitochondria was eliminated in self-crossing of *C. auratus* red var [12]. We speculated that an escape mechanism may exist that disrupts or inactivates PME when different nuclear and mitochondrial genomes are merged in hybrids, especially intergeneric hybrids [10]. In addition, we detected the existence of non-maternal mitochondrial fragments in all samples, which were extracted from 20 embryos collected during the same developmental period, and these fragments have high similarity with the paternal mitochondria. (Fig. 2, Table 1). These results suggest that escape mechanisms may be a universal feature of intergeneric hybridization.

Only a portion of the PMG was covered by the mitochondrial reads, whereas complete coverage of the MMG was observed (Fig. 2, Table 1), suggesting that recombination may have occurred between MMG and PMG, or between nuclear DNA and paternal mitochondria DNA [33]. In addition, both F_1-RC and F_1-CR exhibited coverage of the same regions of PMG, suggesting that similar portions of the paternal mtDNA were retained in the MMG or in the nuclear genome (Fig. 2). Unfortunately, we were unable to assess this, as we did not collect genomic DNA data as part of this study. Overall, although the detection of paternal mitochondrial sequences was based on transcripts, the partial coverage and low-level (<0.4%) expression of paternal mtDNA still could shed us insight into biparental inheritance in interspecific hybridization (Fig. 2) [7–9].

In comparisons 1 and 3, different expression patterns were observed between female parents and their hybrid progeny, although no significant DE were detected among the 13 mitochondrial genes during the four developmental stages (Fig. 3). These results suggest that the differences in mitochondrial gene expression may be due to the hybrids' nuclear genomes [16]. Interesting, the expression of five mitochondrial genes (*nd1*, *nd2*, *cox2*, *atp8*, and *nd3*) in the hybrid progeny formed two clusters consistent with their female parents, suggesting that the expression of these five genes was only slightly affected by changes in the nuclear genome (Fig. S1 in Supporting Information). In contrast, the lack of obvious clustering of expression of the other eight mitochondrial genes suggests that they may be regulated by complex mechanisms, leading to diverse expression patterns (Fig. S1 in Supporting Information).

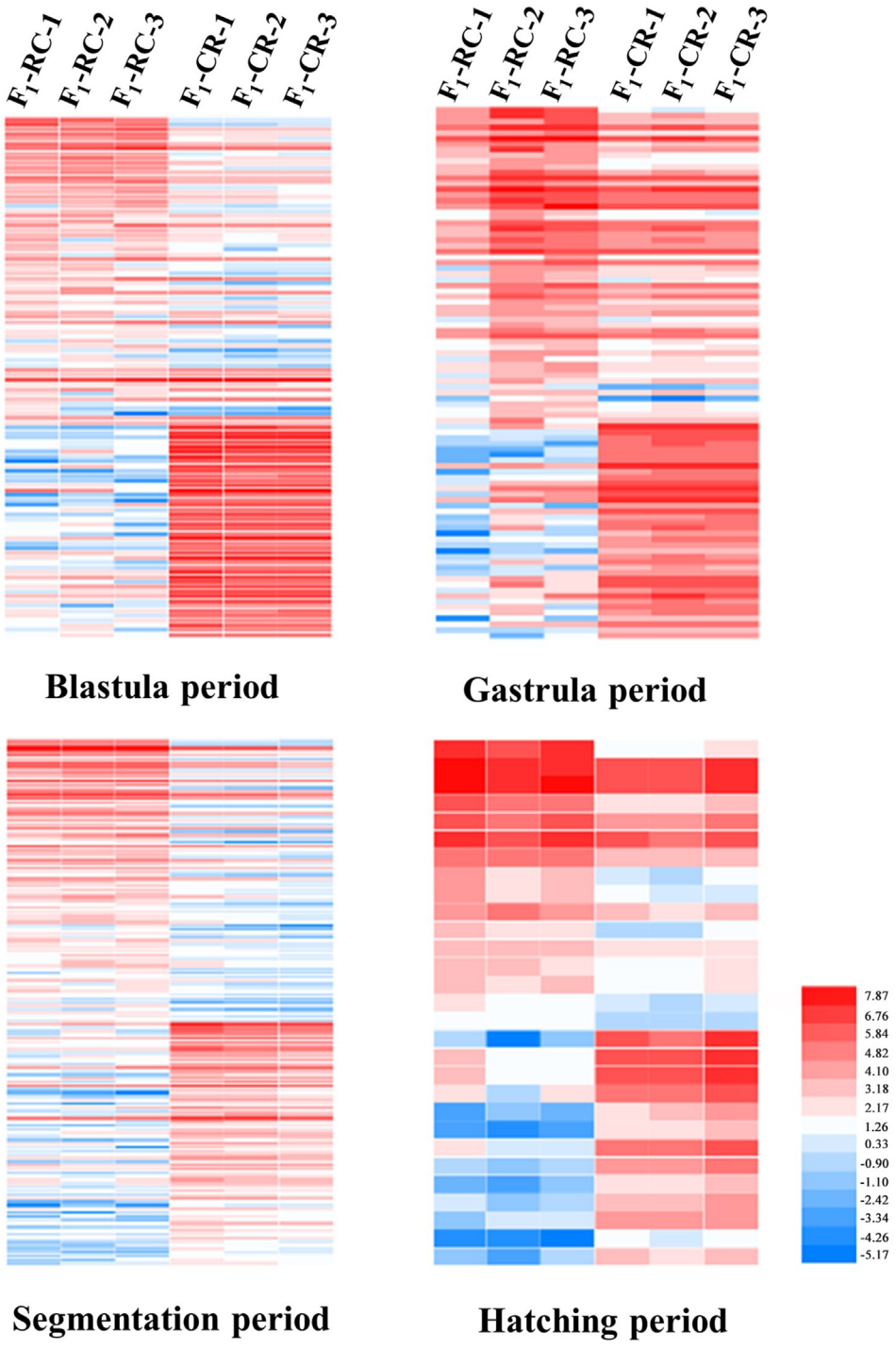

Fig. 4. Differential expression analysis of nuclear-encoded mitochondrial genes in the two reciprocal hybrids. One hundred and eight differentially expressed genes (DEGs) (44 genes up-regulated in F_1-CR and 64 genes up-regulated in F_1-RC) were detected during the blastula period. Ninety-four DEGs (38 up-regulated in F_1-CR and 56 up-regulated in F_1-RC) were detected during the gastrula period. One hundred and forty-five DEGs (67 up-regulated in F_1-CR and 78 up-regulated in F_1-RC) were detected during the segmentation period. Twenty-nine DEGs (13 up-regulated in F_1-CR and 16 up-regulated in F_1-RC) were detected during the hatching period. Red represents up-regulated expression, while blue represents down-regulated expression. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

To determine how mitochondrial gene expression is affected by changes in nuclear gene expression during embryonic development in intergeneric hybrids, we analyzed the expression of nuclear-encoded mitochondrial genes [34] (Fig. 3) and found that the number of DEGs between the two reciprocal intergeneric hybrids was positively correlated with the *p*-value of the difference in gene expression (108 genes vs. $p = 0.7340$ during the blastula period, 94 genes vs. $p = 0.8778$ during the gastrula period, 145 genes vs. $p = 0.6878$ during the segmentation period, and 29 genes vs. $p = 0.9471$ during the hatching period) (Fig. 4, Table 2). Changes in the nuclear genome disrupt intracellular coadapted gene complexes, which can have detrimental effects on individuals within populations by changing growth rates, development, and reproduction because of decreased levels of ATP production [29,35]. The 70 DEGs that were identified during both the gastrula and segmentation periods indicate that major regulatory differences occur during these two embryonic developmental stages, and that nuclear genes substantially affect the expression of mitochondrial genes, leading to differences in energy production between the two reciprocal hybrids (Fig. 5).

5. Conclusions

Our work provides a new insight into complex mitochondrial inheritance and mitochondrial-nuclear interactions in intergeneric hybrids. We are currently exploring the potential effects of mitochondrial-nuclear

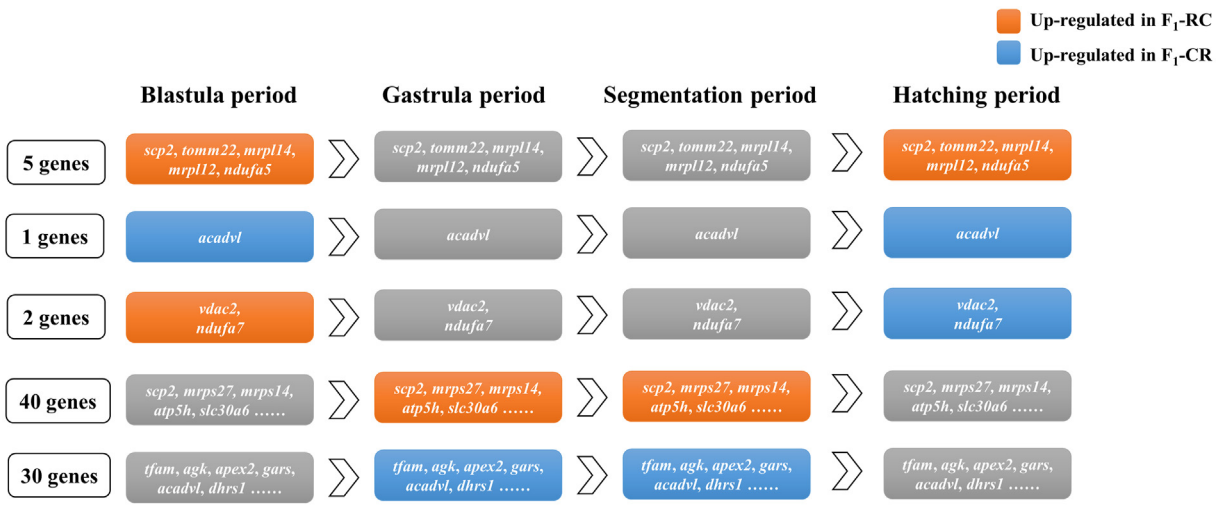

Fig. 5. Five expression patterns during the four developmental periods that differed between the two reciprocal hybrids. Differential expression analysis of nuclear-encoded mitochondrial genes identified 78 genes that were differentially expressed in both hybrids during multiple developmental periods. Most of the changes in expression of the shared differentially expressed genes (70 genes) were observed during the gastrula and segmentation periods.

interactions on hybrid incompatibility and altered metabolism, which may affect individual body size and growth.

Availability of data and material

All short-read RNA-seq data have been deposited in the database (NGDC) under the following accession number: PRJCA003625.

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Authors' contributions

SJL and LR and XG wrote the manuscript. SJL, LR, JLC and XG modified the manuscript and designed the study. LR, HZ and XG carried out bioinformatics analyses. XG, JLC, XYZ, XJY and CCT provided assistance in extracting the raw material. All authors read and approved the final manuscript.

Declaration of competing interest

The authors identity no competing or conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.repbre.2021.12.001.

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