Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics 56 (2025) 101605

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics

journal homepage: www.elsevier.com/locate/cbpd

Expression of Hox genes in embryogenesis of crucian carp (*Carassius carassius*) × common carp (*Cyprinus carpio*) hybrid

Rurong Zhao, Yi Zhou, Min Tao, Chun Zhang, Huan Zhong *, Shaojun Liu *

College of Life Sciences, Hunan Normal University, Changsha 410081, Hunan, People's Republic of China

ARTICLE INFO

Edited by Chris Martyniuk

Keywords:
Hox
RNA-seq
Transcriptional regulation
Somite
Hybrid

ABSTRACT

Distant hybridization has been widely applied in fish breeding. Unfortunately, hybrid offspring often exhibit deformities and mortality during embryonic development. It has been demonstrated that the hybrid of the crucian carp (*Carassius carassius*) × common carp (*Cyprinus carpio*) can pass through reproductive isolation and generate fertile offspring. In the present study, we analyzed the transcriptomic changes during the embryonic developmental stages in red crucian carp (HJ), Koi carp (KOC) and the hybrid F_1. In addition, the expression of Hox genes in the three fishes was evaluated. The transcriptome expression analysis revealed that the greatest number of differentially expressed genes during the gastrula stage (GR) period was found among HJ, F_1, and KOC. Specifically, there were 3316 differentially expressed genes between KOC and F_1, 6027 differentially expressed genes between HJ and F_1, and 1232 differentially expressed genes between KOC and HJ. The identification of *cis*- and *trans*-regulatory patterns indicated that most genes subject to *cis*- and/or *trans*- were assigned to *cis-trans* compensating patterns, suggesting a complementary regulatory interaction between the subgenomes. The Hox gene expression comparison results indicated that the genes were low expressed in the GR stage and increased following somite formation. Although gene expression changes were found comparing the F_1 to its parents, the trend of expression changes is consistent in the three fishes. The knowledge about the transcriptomic changes and regulations in hybrids would aid to understand the interaction of subgenomes during embryonic development.

1. Introduction

For a long term, distant hybridization has been considered difficult to occur in nature (Wang et al., 2019). This belief primarily stemmed from researchers' views that reproductive isolation often prevents the formation of fertile offspring from hybrids, thereby inhibiting the establishment of continuous lineages (Barton and De Cara, 2009; Westram et al., 2022). However, recent studies suggest that reproductive isolation is relative. Both in natural reproduction and artificial breeding processes, fertile hybrids have been generated (Glover et al., 2013; Liu et al., 2020). In contrast to intraspecific hybridization, distant hybridization can occur across different species. It enables the combination of biological traits from different species while also breaking the interspecies boundaries. This disruption of boundaries directly leads to significant changes in the genotypes and phenotypes of hybrid progeny, potentially giving rise to new species (Abbott et al., 2013; Shi et al., 2024). Crucian carp (*Carassius auratus*) and common carp (*Cyprinus carpio*) both belong to the family Cyprinidae of the order Cypriniformes (Liu et al., 2001). Morphologically, the common carp is significantly larger than the crucian carp and features distinctive barbels, whereas the crucian carp, in comparison, is smaller and lacks barbels (Liu et al., 2022). Both the two species are popular farmed fish. Hybridization between the crucian carp and common carp can generate F_1 hybrid offspring with poor fertility, yet a small number of both female and male individuals are capable of producing eggs and sperm, respectively (Liu et al., 2001). The progeny of distant hybridization possesses subgenomes derived from different parents, and their expression and regulatory patterns are critical for investigating heterosis and hybrid speciation (Zhong et al., 2019; Zhong et al., 2023). Therefore, conducting research on gene expression and regulation in crucian carp × common carp hybrid will provide a theoretical basis for understanding distant hybridization in fish.

Hox genes play a crucial role in homeotic transformations (Hubert and Wellik, 2023). In *Drosophila*, segmental development changes leading to transformations are defined as homeotic transformations regulating by Hox genes (Morata and Lawrence, 2022). Since Hox genes determine the anterior-posterior morphology during early

* Corresponding authors.
E-mail addresses: zhonghuanzh@126.com (H. Zhong), lsj@hunnu.edu.cn (S. Liu).

https://doi.org/10.1016/j.cbd.2025.101605
Received 15 June 2025; Received in revised form 9 August 2025; Accepted 9 August 2025
Available online 11 August 2025

developmental stages, they have a significant impact on the anterior-posterior morphogenesis of segments (Hombría et al., 2021). Researchers often focus on their early expression and regulatory mechanisms. Studies have shown that histone methylation modifications are significantly associated with Hox gene expression (Liu et al., 2021). For example, Polycomb group (PCG), trithorax groups (TRXG), ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX), and Jumonji domain-containing protein D3 (JMJD3) are key genes regulating histone methylation levels, collectively influencing Hox gene expression and subsequently affecting anterior-posterior axis development (Steens and Klein, 2022). PCG is related to the spatial expression of Hox genes (Gentile and Kmita, 2020); and the disruption of PCG leads to changes in the spatial expression pattern of Hox genes (Gentile and Kmita, 2020). In contrast, TRXG disruption results in decreased Hox gene expression (Afzal and Krumlauf, 2022). Additionally, the nuclear arrangement of Hox gene transcription is associated with their expression (Afzal and Krumlauf, 2022). Hox genes within the same cluster exhibit a close spatial distribution, and their chromosomal binding and distribution with transcriptional regulatory elements, including promoters and enhancers, regulate gene expression (Hajirnis and Mishra, 2021). The genomic arrangement of Hox genes demonstrates their spatiotemporal expression patterns and reflects their morphogenetic processes (Mulhair and Holland, 2024). Future research needs to further investigate whether the expression patterns of Hox genes are conserved across species and the complementary roles among paralogous Hox genes.

Currently, hybrid breeding has been widely applied in the field of fish breeding. Besides selective breeding, distant hybridization is one of the most extensively used methods in fish (Hu et al., 2021). However, the genetic variation patterns of hybrid populations need further study to enrich the theoretical foundation of hybrid breeding. Hox genes regulate the anterior-posterior patterning of the embryo by assigning segmental identities to developing tissues. This study investigates the changes in transcriptomic expression and expression changes of the Hox gene during the embryonic development in the F_1 hybrids of crucian carp and koi carp (*Cyprinus carpio*). The expression characteristics of the Hox gene family provide a basis for understanding the genomic evolution and key regulatory mechanisms of segmental development in the hybrid, offering theoretical support for distant hybridization breeding techniques in fish.

2. Materials and methods

2.1. Fish breeding and sample collection

All experimental materials were obtained from the Hunan Fish Genetics and Breeding Center, Hunan Normal University, Changsha, China. Sexually mature female crucian carp and male koi carp were selected and induced to spawn artificially. Eggs from red crucian carp and sperm from koi carp were collected and fertilized to produce F_1 hybrid. Simultaneously, self-fertilization of both crucian carp and koi carp was performed. The fertilized eggs of the three experimental fish were incubated in culture dishes at a water temperature of 24 °C to 26 °C. The embryos were collected at various developmental stages for each group: gastrula stage (F_1 GR), bud stage (F_1 bud), 6-somite stage (F_1 6S), 14-somite stage (F_1 14S), 22-somite stage (F_1 22S), and long-pec stage (F_1 LP) for F_1 hybrids; gastrula stage (KOC GR), bud stage (KOC bud), 6-somite stage (KOC 6S), 14-somite stage (KOC 14S), 22-somite stage (KOC 22S), and long-pec stage (KOC LP) for koi carp; gastrula stage (HJ GR), bud stage (HJ bud), 6-somite stage (HJ 6S), 14-somite stage (HJ 14S), 22-somite stage (HJ 22S), and long-pec stage (HJ LP) for crucian carp. Embryos were carefully detached from the culture dishes and collected with a clean rubber-tipped pipette, then placed on a clean gauze. For each group, 150 embryos were collected, with every 50 embryos placed into one centrifuge tube. The detached fertilized eggs were washed three times with DEPC-treated water. The rinsed fertilized eggs were placed on DEPC-treated gauze, quickly drained, and fertilized eggs were transferred to centrifuge tubes. The collected embryos were frozen in liquid nitrogen and subsequently stored at −80 °C until RNA extraction.

2.2. RNA-seq

The total RNAs from the different developmental embryos were extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. RNAs were tested by 1.20 % agarose gel and BioPhotometer Plus (Eppendorf, Germany) and the RNA concentration was determined by NanoDrop spectrophotometer (NanoDrop Technologies, USA). The RNA integrity number (RIN) was determined by the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). The RNA with RIN > 7 was used for the following analysis. RNA was extracted separately from the three tubes collected in each group. After extraction and concentration measurement, equal amounts from each tube were pooled into a single tube. mRNA was enriched by magnetic oligo (dT) beads and fragmented by fragmentation buffer. After amplification by random hexamer, the cDNAs were synthesized and purified by AMPure XP beads following PCR to obtain the sequenced cDNA library. A total of 18 cDNA libraries were constructed and sequenced in this study, covering six developmental stages (GR, bud, 6S, 14S, 22S and LP) and three types of fishes (F_1, KOC and HJ). The libraries were sequenced by Illumina NovaSeq 6000.

2.3. Bioinformatic analysis

The raw sequencing data were first subjected to filtering. The data obtained from Illumina NovaSeq sequencing are referred to as raw reads or raw data, which were then subjected to quality control (QC) analysis. Trimmomatic (http://www.usadellab.org/cms/index.php?page=trimmomatic) was used for filtering the raw data based on the following criteria: 1) removing reads containing adaptors; 2) removing reads with more than 10 % N bases; and 3) removing reads with more than 50 % of bases having a quality score below 20. The filtered data are referred to as clean reads. Next, FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) was used to perform QC on the clean reads, and only those clean reads passing QC were used for subsequent bioinformatics analysis. All clean reads were first aligned to the reference genome. The reference genome used in this study was the genome sequence of crucian carp (https://www.ensembl.org/Carassius_auratus/Info/Index). Alignment to the reference genome was performed using HISAT2 with an allowance of 4 mismatches in end-to-end mode and based on the alignment results and the reference genome annotation, the distribution of aligned reads in the reference genome was analyzed. The gene expression was determined using Fragments Per Kilobase of transcript per Million mapped reads (FPKM). The differentially expressed genes were identified using edgeR software. When the fold change ≥ 2 and FDR ≤ 0.05, the genes were determined as differentially expressed genes.

The genomes of crucian carp (https://www.ensembl.org/Carassius_auratus/Info/Index) and common carp (https://www.ensembl.org/Cyprinus_carpio_carpio/Info/Index) were compared to identify the specific (Single Nucleotide Polymorphism, SNP). Subsequently, the mapping clean reads were assigned to maternal and paternal origin using the SNPs. The specific reads from maternal or paternal genomes were identified based on the two standards: first, the reads contained specific SNP genotypes unique to one parent; second, more than 10 reads mapping to the gene. The ratio of maternal to paternal clean reads was used to calculate the allelic gene expression.

The comparative expression values of parental genes are defined as $A = \log2(\text{Maternal/Paternal})$, representing both *cis* and *trans* effects. In contrast, the comparative expression values of alleles in offspring are defined as $B = \log2(F_1\text{-Maternal allele}/F_1\text{-Paternal allele})$, representing *cis* effects. Therefore, *trans* effects can be calculated as A-B. The identification method of the regulatory patterns was according to previous studies in *Drosophila* (Wittkopp et al., 2004) and *Arabidopsis* (Shi et al.,

2012).

2.4. Quantitative PCR (qPCR)

The cDNA was synthesized using 1 μg total RNAs which were extracted by the PrimeScript RT reagent Kit with gDNA Eraser Perfect Real Time kit (Takara, Japan) according to the manufacturer's instructions. The Primers of HoxB1b, HoxA3a, HoxB3a, HoxA5a, HoxC5a, HoxB7a, HoxB9a, HoxC9a, HoxA10b, HoxC10a, HoxA11a, HoxA13a and HoxA13b genes were designed by Primer Express Software Version 3.0 (ABI, USA) (Supplementary Table 1). β-Actin was used as reference genes. qPCR was performed in a StepOnePlus™ Real-Time PCR System (ThermoFisher, USA). Three biological replicates were used for analysis. The PCR reaction mixture had a total volume of 20 μL, consisting of 5 μL ddH_2O, 2 μL cDNA, 1 μL each of 10 μM forward and reverse primers, 1 μL of ROX Reference Dye (50×), and 10 μL of 2× TB Green Premix Ex Taq II (Tli RNase H Plus, Takara, Japan). The reaction was conducted using a two-step method. The first step involved pre-denaturation at 95 °C for 30 s. The second step proceeded to the PCR reaction, which consisted of 40 cycles with the following conditions: 95 °C for 5 s and 60 °C for 30 s. Melting curve analysis was performed after PCR to determine the specific amplification. Relative quantification (RQ) was calculated using the $2^{-\Delta\Delta ct}$ method (Livak and Schmittgen, 2001).

2.5. Statistical analysis

All the statistical analysis was performed using SPSS 22.0. The data was shown as mean ± standard deviation. The significant differences of RQ among the groups were determined by a one-way ANOVA test following Tukey post hoc tests. When $p < 0.05$, the significant differences were confirmed.

3. Results

3.1. Transcriptomic changes during embryogenesis in hybrids

After filtering the low-quality reads and adaptors, a total of 803,467,004 clean reads were retained. On average, each sample obtained 44,637,055.78 reads (Supplementary Table 2). The clean reads of F_1, KOC, and HJ at different embryonic development stages were aligned to the reference genome. The average alignment rate of the clean sequences from 18 libraries was 86.46 %, indicating a high alignment rate (Supplementary Table 3). The differential expressed genes (DEGs) between the fishes in the same stages were performed. For the three comparisons, GR stages had the most DEGs (KOC GR vs F_1 GR: 3316 DEGs; HJ GR vs F_1 GR: 6027 DEGs; KOC GR vs HJ GR: 1232 DEGs), indicating that GR is the stage with significant transcriptomic changes in the hybrid compared to the parents. In contrast, the LP stage had the fewest DEGs (KOC vs and F_1: 180 DEGs, between HJ and F_1: 71 DEGs, and KOC and HJ: 446 DEGs) (Fig. 1) (Supplementary Table 4). In addition, we found more DEGs when comparing F_1 with KOC than when comparing F_1 with HJ almost in all the stages, except the GR stages showing a maternal effect. The results of DEGs were deposited in figshare (https://doi.org/10.6084/m9.figshare.29634575).

3.2. Gene regulation during embryogenesis in hybrids

In the GR stage, the hybrids showed the most genes that were under control by *cis*- and/or *trans*-regulatory effects. A total of 459, 46, 9 and 40 genes were *cis-trans* compensating, *cis-trans* enhancing, only *cis*- and only *trans*-regulation in the hybrid. In other stages, including bud, 6S, 22S, and LP stages, the most genes in the *cis*- and *trans*-regulatory patterns were *cis-trans* compensating and the lowest genes were only *cis*-regulation pattern (Fig. 2a).

Based on the analysis of gene regulation, the KEGG pathway enrichment analysis was conducted on genes under *cis*- and/or *trans*-regulation in various developmental stages of the hybrid. A total of 16 KEGG pathways showed significant enrichment across these 6 stages. Specifically, the GR stage exhibited several unique KEGG pathway enrichments, including 10 pathways. These pathways are primarily involved in adipocytokine signaling, fatty acid degradation, lysosomal function, O-glycan biosynthesis (including mannose type and other types), ribosome biogenesis in eukaryotes, and RNA degradation. In other stages, both the bud and 6S stages consistently enriched pathways such as arginine and proline metabolism, and mitophagy-animal pathway. Overall, during the period of body segment formation, pathways including lipid metabolism, protein metabolism, carbohydrate metabolism, cell cycle, lysosome, peroxisome, and vesicle transport were subject to *cis*- and *trans*-regulation, reflecting their association with unique transcriptional expression regulation in the hybrid (Fig. 2b).

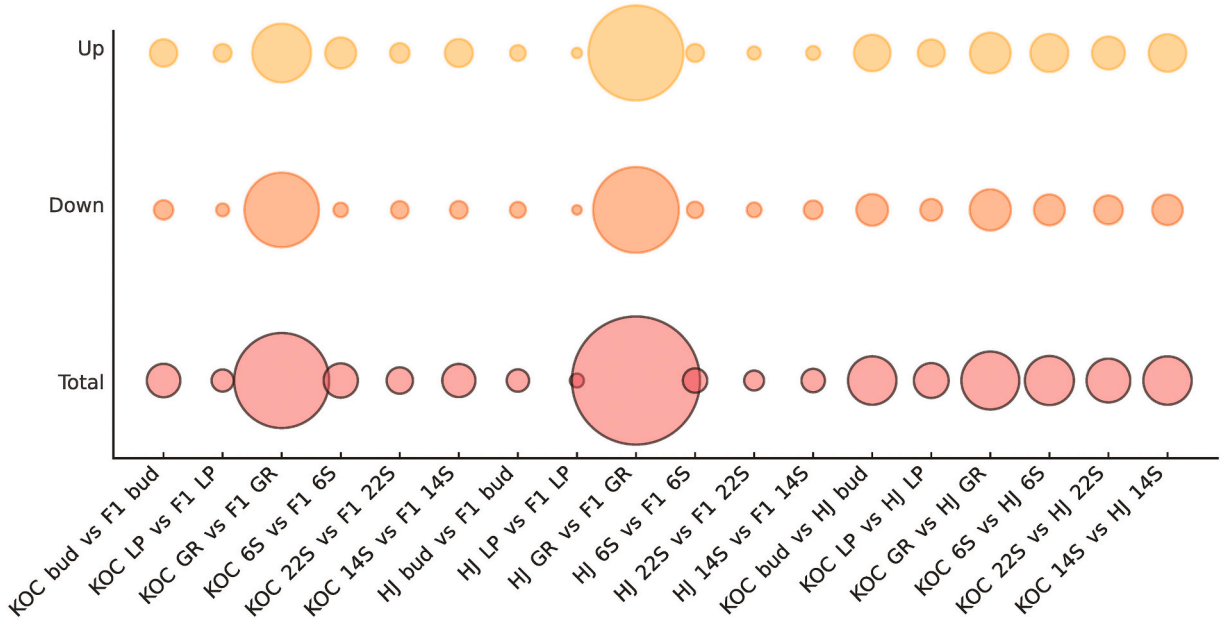

Fig. 1. Differentially expressed gene identified from pairwise comparisons of three fishes in the same stages during the somite formation period.

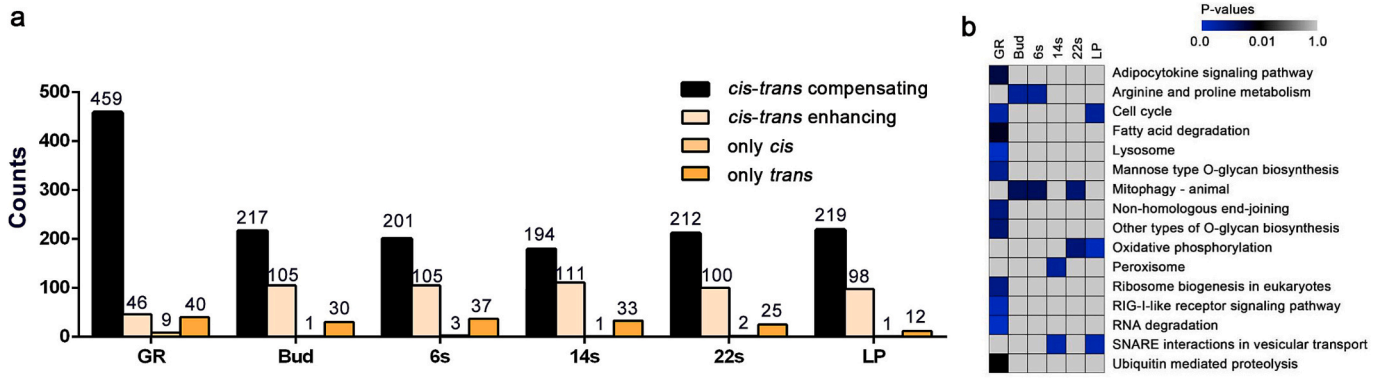

Fig. 2. Identification of *cis*- and *trans*-regulatory effects of genes in the stages. (a) Gene counts of the patterns under *cis*- and/or *trans*-regulatory effects. (b) Enrichment analysis of KEGG terms from the genes under *cis*- and *trans*-regulation by heatmap.

3.3. Hox gene expression in hybrids

In the GR stage, several genes in hybrid exhibited significantly higher expression than in the maternal HJ and paternal KOC, whereas HoxA11, HoxB2, HoxB3, HoxC13, HoxC9 and HoxD13a showed higher expression in KOC than in F_1. Additionally, HJ had 19 Hox genes with higher expression than KOC, while only 12 Hox genes exhibited lower expression compared to KOC. In contrast to the GR stage, Hox gene expression in KOC during the Bud stage was generally higher than in F_1 and HJ. Among the 64 Hox genes examined, 38 genes showed higher expression in KOC compared to F_1, accounting for 59 % of all detected 64 Hox genes. From the 6S to 22S stages and LP stage, gene expression in KOC was significantly higher than that in hybrid F_1 and HJ (Fig. 3). Therefore, except for the GR stage, KOC generally exhibited higher trends in Hox gene expression compared to hybrid F_1 and HJ.

We then used qPCR to analyze expression changes of 12 Hox genes. For HoxA3a, HoxA5a and HoxA13b, no significant changes could be found in the same stage by comparing the F_1 and its parents (Fig. 4a, b, f). For HoxA10b, higher expression was found in the 14S stage from F_1 compared to the HJ and KOC ($p < 0.05$) (Fig. 4c). The HoxA11a showed no significant changes in all the stages except the 14S stage showing a significantly higher expression in HJ and F_1 compared to KOC ($p < 0.05$)

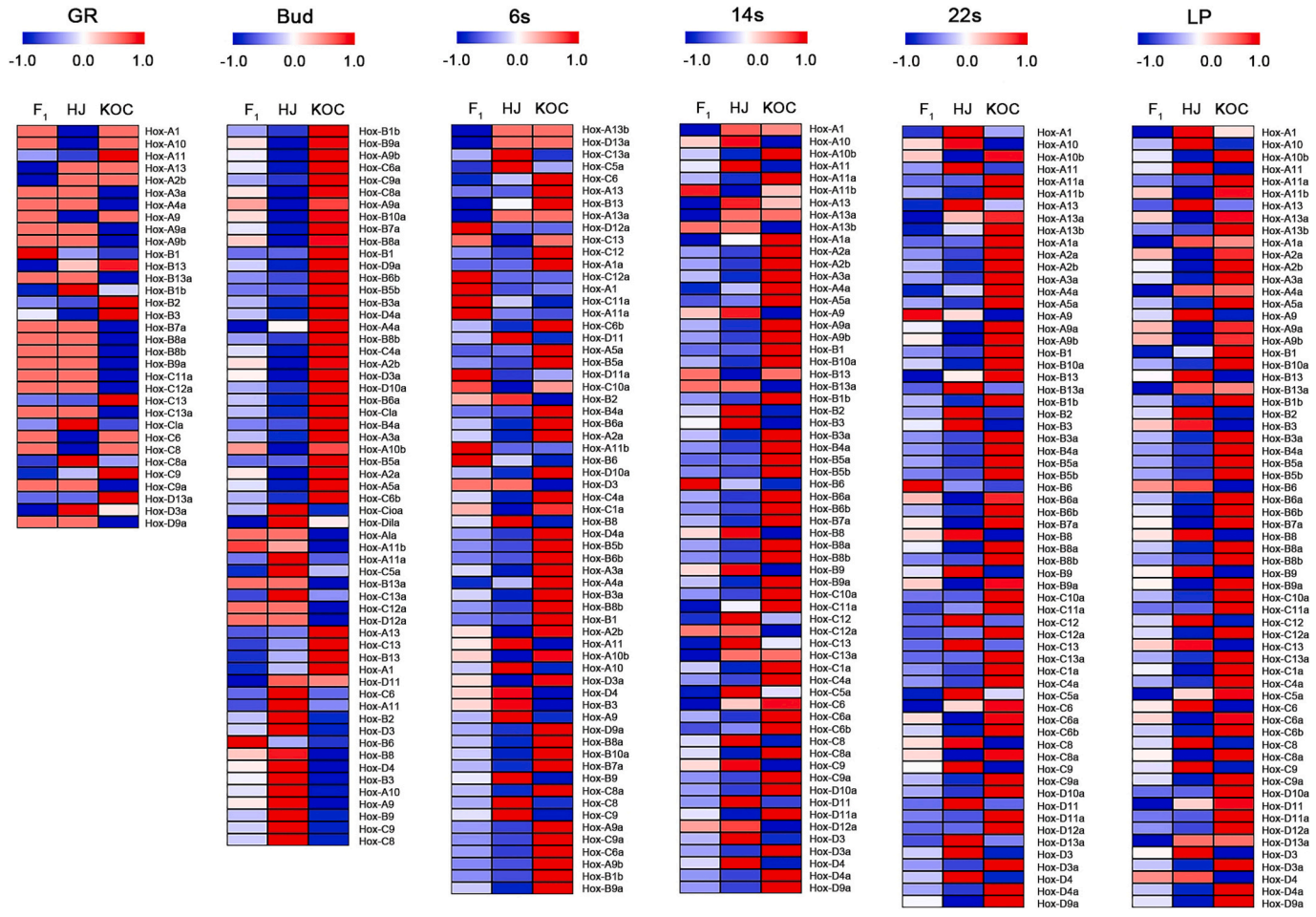

Fig. 3. FPKM comparisons in the three fishes at the same stages of Hox genes by heatmap.

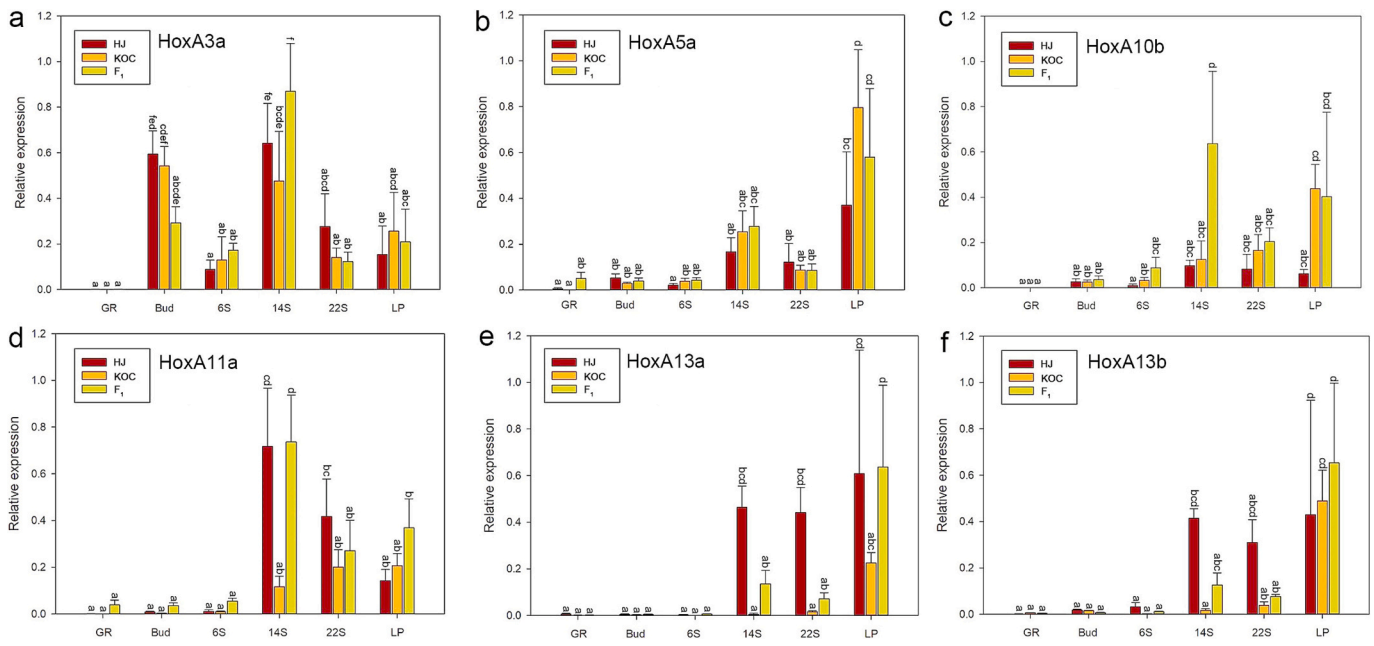

Fig. 4. qPCR analysis of HoxA genes including HoxA3a, HoxA5a, HoxA10b, HoxA11a, HoxA13a, and HoxA13b in different stages from HJ, KOC and F_1. Different lowercase on each bar indicates significant differences ($p < 0.05$).

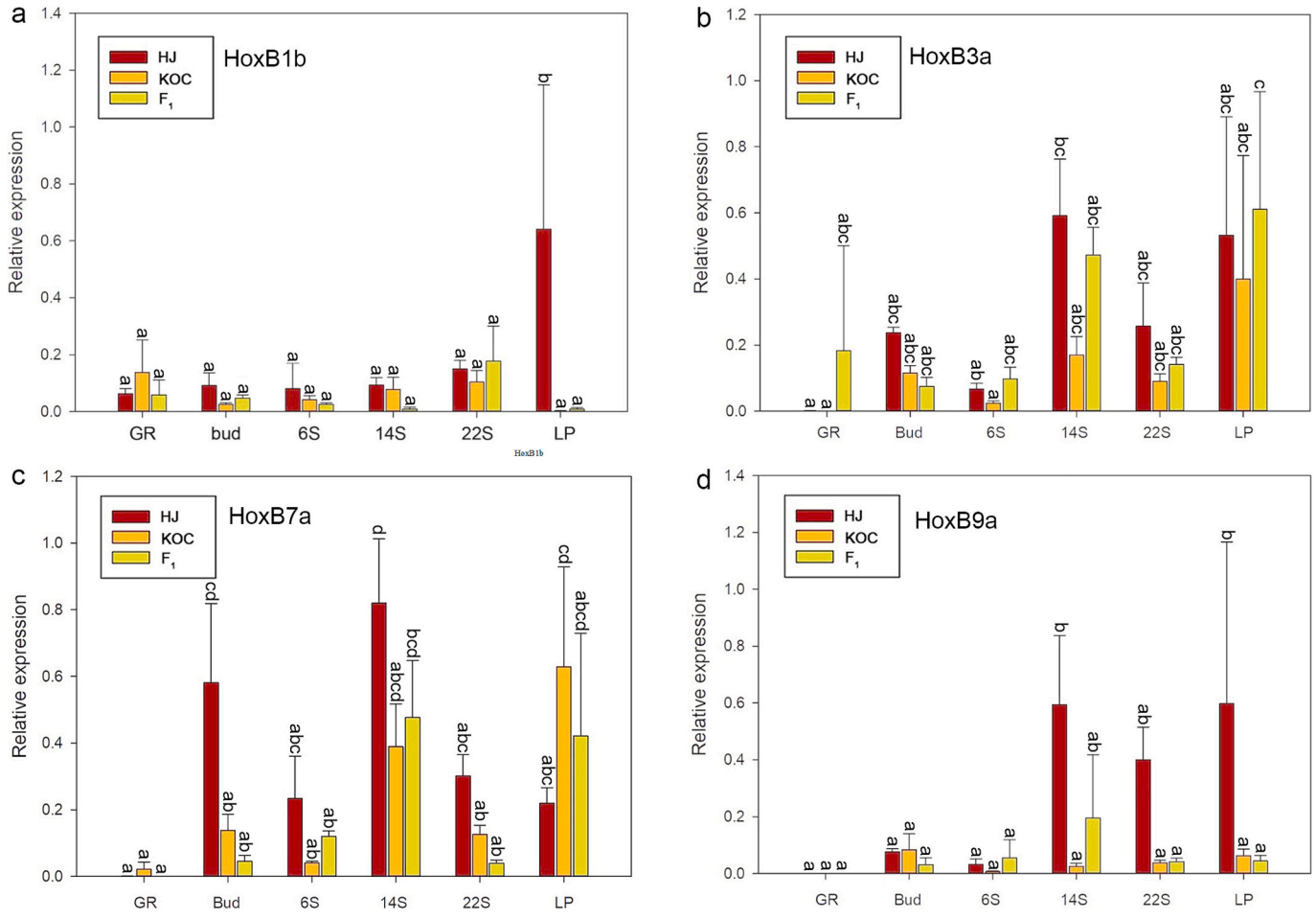

Fig. 5. qPCR analysis of HoxB genes including HoxB1b, HoxB3a, HoxB7a, and HoxB9a in different stages from HJ, KOC and F_1. Different lowercase on each bar indicates significant differences ($p < 0.05$). Three biological replicates were used for analysis.

(Fig. 4d). Higher expression of HoxA13a was found in F_1 than that in HJ from the LP stage ($p < 0.05$) (Fig. 4e). The HoxB1b in HJ was significantly higher compared to KOC and F_1 in the LP stage ($p < 0.05$) (Fig. 5a). No significant changes could be found for HoxB3a among the fishes in the same stages (Fig. 5b). As for HoxB7a, lower expression was found in KOC and F_1 compared to HJ from the bud stage (Fig. 5c). Notably, HJ exhibited markedly elevated HoxB9a expression compared to the KOC and F_1 in LP stage (Fig. 5d). Higher expression of HoxC5a was observed in HJ and F_1 compared to KOC in the 14S stage ($p < 0.05$) (Fig. 6a). As for HoxC9a, higher expression was found in F_1 compared to KOC in the 14S stage ($p < 0.05$) (Fig. 6b). No significant changes could be found in HoxC10a expression among the fishes in the same stages (Fig. 6c).

4. Discussion

In this study, the embryos of GR, bud, 6S, 14S, 22S and LP stages were collected from HJ, KOC, and their hybrid F_1. We constructed cDNA libraries and performed transcriptome sequencing, obtaining a large number of expressed sequences and identifying DEGs. Differential gene screening revealed the highest number of DEGs during the GR stage among the three fishes. As the GR stage is the earliest among all the studied stages, it was found that the highest number of differentially expressed genes between the F_1 and its parents. A previous study on the embryonic transcriptome of a large yellow croaker (*Larimichthys crocea*) revealed significant changes between the blastula and gastrula stages, suggesting that during early embryonic development, there is a transition from maternal transcript expression to embryonic genome activation (Wan et al., 2019). The GR stage, being in the early phase of embryogenesis, might be associated with intense changes in gene expression. Consequently, in the early stages of embryonic development, more aberrantly expressed genes might be observed in the hybrid F_1 compared to its parents (McGirr and Martin, 2020). It has been reported that the hybrid F_1 resulting from distant hybridization has a significantly higher rate of developmental abnormalities compared to the self-crossing of parents, which might be due to gene expression disorders during early embryonic development (Renaut et al., 2009). Notably, due to factors like reproductive isolation and genomic incompatibility, hybrid F_1 resulting from distant hybridization is generally considered sterile or developmentally abnormal (Maheshwari and Barbash, 2011). However, several hybrid progenies have been successfully used in practical production applications in teleost (Liu, 2010). Notably, hybrid red crucian carp × common carp can continue through chromosomal doubling, eventually forming fertile allopolyploid strains (Liu et al., 2001). The present study confirms that specific gene expression patterns during embryonic development might influence the embryonic development of hybrid progenies, especially during the GR stage. The aberrant gene expression during this stage might be related to the high rate of malformations in hybrid progenies.

In the analysis of gene regulation, this study delineates the KEGG signaling pathway enrichment in various developmental stages of the hybrid F_1, influenced by *cis*- and/or *trans*-regulatory genes. Overall, 16 KEGG signaling pathways were significantly enriched across these six stages for the *cis*- and/or *trans*-regulatory genes. Specifically, during the GR stage, multiple unique KEGG signaling pathways were enriched, including 10 pathways. These pathways are primarily involved in fat metabolism, lysosome, carbohydrate metabolism, and nucleic acid processing. This result suggests that during embryonic development, significant regulatory changes predominantly affect metabolism, nucleic acid processing, and key disease resistance signaling pathways. In the early stages of embryonic development, individuals require substantial energy, with carbohydrates and lipids providing high energy levels for fish (Lipavská and Konrádová, 2004; Kawano et al., 2011). During the embryonic stage, energy is mainly supplied by vitellogenin, a high-density glycol-lipoprotein (Augustine et al., 2011). Thus, during early embryonic development, the utilization of carbohydrates and lipids is crucial for energy supply to the developing embryo. Furthermore, extensive replication and synthesis of genetic material are necessary for individual development, particularly during cell division and growth, making the regulation of nucleic acid processing pathways notably prominent during this period (Gillies and Cabernard, 2011). Interestingly, the enrichment of the antibacterial enzyme signaling pathway may indicate a crucial role in the embryo's defense against external factors during embryogenesis. In other developmental stages, the KEGG pathways consistently enriched in the bud and 6S stages were the Arginine and proline metabolism and mitophagy-animal signaling pathways. Overall, during the studied stages, pathways related to fat metabolism, protein metabolism, carbohydrate metabolism, cell cycle, lysosome, peroxisome, and vesicle transport were regulated by both *cis*- and *trans*-acting elements. This correlates with the unique transcriptional regulation of the hybrid genome.

Similar to other animals, after the embryo forms the neural plate, it further develops into the neural groove, neural folds, and neural tube along the dorsal side (Kudoh et al., 2001). On both sides of the neural tube, the paraxial mesoderm gradually develops, and the neural tube and paraxial mesoderm grow towards the tail end of the embryo along the body axis (Mullins, 1998). The paraxial mesoderm aggregates towards the head direction of the embryo (Tani et al., 2020). The separation of two aggregated cell masses forms the first pair of somites (Tani et al., 2020). Subsequently, multiple cell masses aggregate repeatedly to form numerous somites (Pourquié, 2001). In recent years, a substantial amount of research has been published on the molecular biological processes involved in somite formation (Ibarra-Soria et al., 2023; Keseroglu et al., 2023; Uriu and Morelli, 2023). As a crucial gene for somite polarity, Hox determines the function and morphology of each developing somite, playing a vital regulatory role in the development and differentiation of somites (Saito and Suzuki, 2020). Here, we analyzed the gene expression of Hox genes. Overall, during the earliest embryonic development stage analyzed in this study, the GR stage, gene expression was found to be low in all three types of fish. As embryonic development progressed into the somitogenesis stage, Hox gene expression began to upregulate, particularly during the late somitogenesis stage (LP stage), where several genes reached their highest expression levels. Therefore, at the end of the gastrulation stage, somites have not yet formed, resulting in low Hox expression and no necessity for Hox function at this stage. Once somitogenesis begins, Hox gene expression gradually increases to perform its role in regulating the differentiation of somites. This finding is consistent with studies conducted on zebrafish (Yamada et al., 2021) and *Drosophila* (Mallo et al., 2010). The Hox gene expression results showed that the genes in the Hox gene family exhibit relatively stable expression patterns in the hybrid F_1. Since Hox genes play a crucial role in embryonic development, especially in the development of the anteroposterior axis of somites, mutations or significant changes in gene expression levels occur in some members of the Hox gene family results in embryonic deformities, hindering the successful completion of the entire embryonic development process and ultimately leading to embryonic death. Therefore, the relatively stable expression of the Hox gene family in hybrid fish further underscores the conservatism and importance of these genes in their function.

5. Conclusion

The present study focused on the transcriptomic changes, especially showing the gene expression of members of the Hox gene family in the red crucian carp, Koi carp and the hybrid F_1. The results showed that the greatest DEGs were found in the period in GR among HJ, F1 and KOC, and most of the DEGs were assigned to *cis-trans* compensating patterns, which suggested the complementarity regulation of the subgenomes. The Hox gene showed lower expression in the GR stage and higher level in somite formation. The results showed the characteristics of the crucial genes that are associated with somite formation providing clues for the

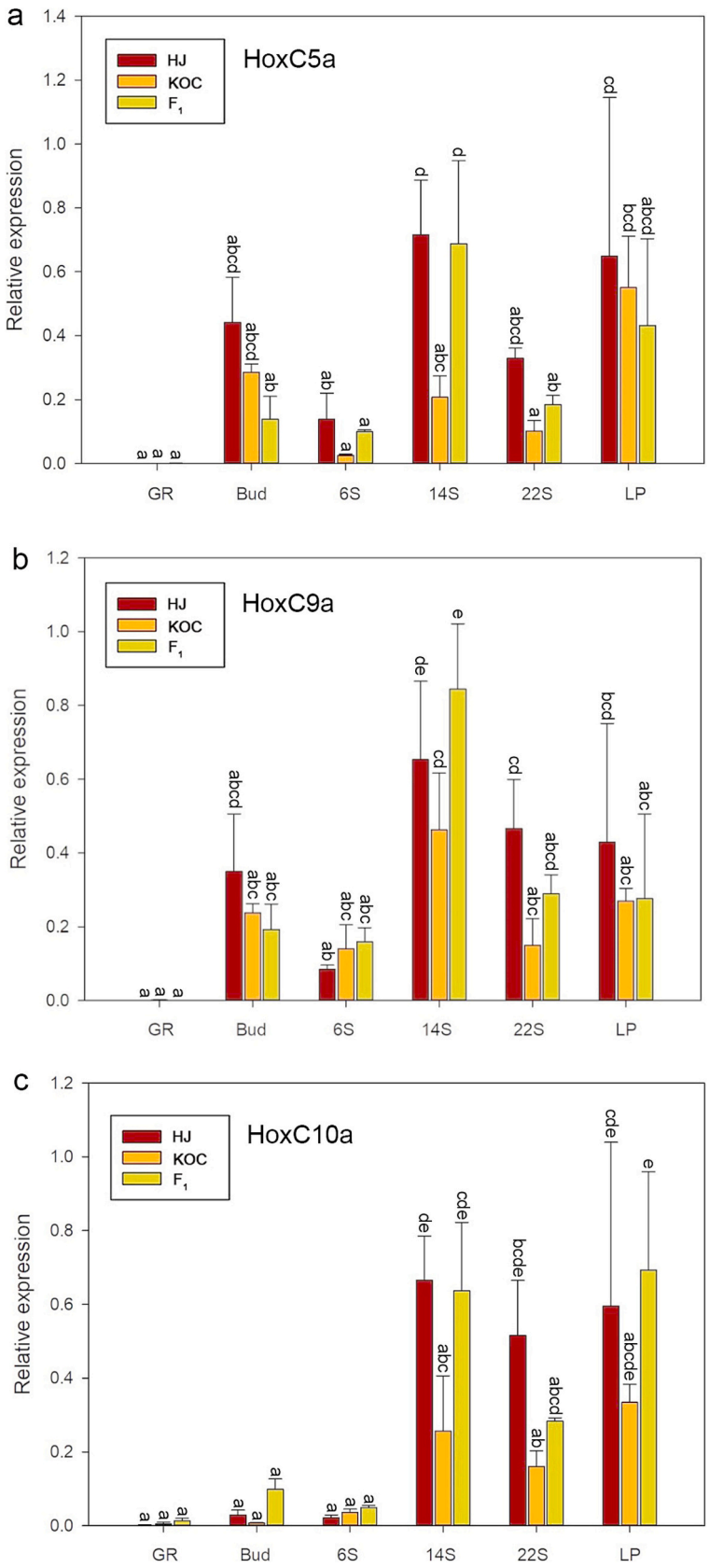

Fig. 6. qPCR analysis of HoxC genes including HoxC5a, HoxC9a, and HoxC10a in different stages from HJ, KOC and F_1. Different lowercase on each bar indicates significant differences ($p < 0.05$). Three biological replicates were used for analysis.

gene expression profile of embryogenesis in hybrid fish, showing a pioneer insight into gene expression and regulation during embryogenesis.

CRediT authorship contribution statement

Rurong Zhao: Writing – original draft, Investigation, Data curation, Conceptualization. **Yi Zhou:** Validation, Software, Formal analysis. **Min Tao:** Visualization, Validation, Software. **Chun Zhang:** Resources, Formal analysis, Data curation. **Huan Zhong:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Shaojun Liu:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was supported by the National Key Research and Development Program of China (2023YFD2400203), the National Natural Science Foundation of China (32473138, 32293254, 32293252), Key Research and Development Program of Hunan Province (2024JK2144), and the Youth Science and Technology Talents Lifting Project of Hunan Province (2023TJ-Z21).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbd.2025.101605.

Data availability

The RNA-seq raw datasets have been deposited in the China National Center for Bioinformation and can be accessed under the BioProject accession number PRJCA044103 (https://ngdc.cncb.ac.cn/).

References

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., et al., 2013. Hybridization and speciation. J. Evol. Biol. 26 (2), 229–246.
Afzal, Z., Krumlauf, R., 2022. Transcriptional regulation and implications for controlling Hox gene expression. J. Dev. Biol. 10 (1), 4.
Augustine, S., Gagnaire, B., Floriani, M., Adam-Guillermin, C., Kooijman, S.A.L.M., 2011. Developmental energetics of zebrafish, *Danio rerio*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 159 (3), 275–283.
Barton, N.H., De Cara, M.A.R., 2009. The evolution of strong reproductive isolation. Evolution 63 (5), 1171–1190.
Gentile, C., Kmita, M., 2020. Polycomb repressive complexes in Hox gene regulation: silencing and beyond. BioEssays 42 (10), 1900249.
Gillies, T.E., Cabernard, C., 2011. Cell division orientation in animals. Curr. Biol. 21 (15), R599–R609.
Glover, K.A., Kanda, N., Haug, T., Pastene, L.A., Øien, N., Seliussen, B.B., Sørvik, A.G.E., Skaug, H.J., 2013. Hybrids between common and Antarctic minke whales are fertile and can back-cross. BMC Genet. 14 (1), 25.
Hajirnis, N., Mishra, R.K., 2021. Homeotic genes: clustering, modularity, and diversity. Front. Cell Dev. Biol. 9, 718308.
Hombría, J.C.-G., García-Ferrés, M., Sánchez-Higueras, C., 2021. Anterior Hox genes and the process of cephalization. Front. Cell Dev. Biol. 9, 718175.
Hu, F.Z., Zhong, H.T., Wu, C., Wang, S., Guo, Z.J., Tao, M., et al., 2021. Development of fisheries in China. Reproduction and Breeding 1 (1), 64–79.
Hubert, K.A., Wellik, D.M., 2023. Hox genes in development and beyond. Development 150 (1), dev192476.
Ibarra-Soria, X., Thierion, E., Mok, G.F., Münsterberg, A.E., Odom, D.T., Marioni, J.C., 2023. A transcriptional and regulatory map of mouse somite maturation. Dev. Cell 58 (19), 1983–1995 e1987.
Kawano, N., Yoshida, K., Miyado, K., Yoshida, M., 2011. Lipid rafts: keys to sperm maturation, fertilization, and early embryogenesis. J. Lipids 2011 (1), 264706.
Keseroglu, K., Zinani, O.Q.H., Keskin, S., Seawall, H., Alpay, E.E., Özbudak, E.M., 2023. Stochastic gene expression and environmental stressors trigger variable somite segmentation phenotypes. Nat. Commun. 14 (1), 6497.
Kudoh, T., Tsang, M., Hukriede, N.A., Chen, X., Dedekian, M., Clarke, C.J., et al., 2001. A gene expression screen in zebrafish embryogenesis. Genome Res. 11 (12), 1979–1987.
Lipavská, H., Konrádová, H., 2004. Somatic embryogenesis in conifers: the role of carbohydrate metabolism. In Vitro Cell. Dev. Biol. - Plant 40 (1), 23–30.
Liu, S.J., 2010. Distant hybridization leads to different ploidy fishes. Sci. China Life Sci. 53 (4), 416–425.
Liu, S.J., Liu, Y., Zhou, G.J., Zhang, X.J., Luo, C., Feng, H., et al., 2001. The formation of tetraploid stocks of red crucian carp × common carp hybrids as an effect of interspecific hybridization. Aquaculture 192 (2), 171–186.
Liu, Q.F., Liu, J.M., Yuan, L.J., Li, L., Tao, M., Zhang, C., et al., 2020. The establishment of the fertile fish lineages derived from distant hybridization by overcoming the reproductive barriers. Reproduction 159 (6), R237–R249.
Liu, P.R., Zhang, J., Du, D., Zhang, D.D., Jin, Z.L., Qiu, W.Q., 2021. Altered DNA methylation pattern reveals epigenetic regulation of Hox genes in thoracic aortic dissection and serves as a biomarker in disease diagnosis. Clin. Epigenetics 13 (1), 124.
Liu, S.J., Zhou, Y., Tao, M., Zhang, C., Qin, Q.B., Zhao, R.R., et al., 2022. The formation of allotetraploids of red crucian carp × common carp. In: Fish Distant Hybridization. Springer Nature Singapore, Singapore, pp. 39–45.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25 (4), 402–408.
Maheshwari, S., Barbash, D.A., 2011. The genetics of hybrid incompatibilities. Annu. Rev. Genet. 45, 331–355.
Mallo, M., Wellik, D.M., Deschamps, J., 2010. Hox genes and regional patterning of the vertebrate body plan. Dev. Biol. 344 (1), 7–15.
McGirr, J.A., Martin, C.H., 2020. Ecological divergence in sympatry causes gene misexpression in hybrids. Mol. Ecol. 29 (14), 2707–2721.
Morata, G., Lawrence, P., 2022. An exciting period of Drosophila developmental biology: of imaginal discs, clones, compartments, parasegments and homeotic genes. Dev. Biol. 484, 12–21.
Mulhair, P.O., Holland, P.W.H., 2024. Evolution of the insect Hox gene cluster: comparative analysis across 243 species. Semin. Cell Dev. Biol. 152-153, 4–15.
Mullins, M.C., 1998. Chapter 10 embryonic axis formation in the zebrafish. In: Methods in Cell Biol, 59. H. Academic Press, pp. 159–178.
Pourquié, O., 2001. Vertebrate Somitogenesis. Annu. Rev. Cell Dev. Biol. 17, 311–350.
Renaut, S., Nolte, A.W., Bernatchez, L., 2009. Gene expression divergence and hybrid misexpression between Lake whitefish species pairs (*Coregonus* spp. Salmonidae). Mol. Biol. Evol. 26 (4), 925–936.
Saito, S., Suzuki, T., 2020. How do signaling and transcription factors regulate both axis elongation and Hox gene expression along the anteroposterior axis? Dev. Growth Differ. 62 (5), 363–375.
Shi, X., Ng, D.W.-K., Zhang, C.Q., Comai, L.C., Ye, W.X., Jeffrey Chen, Z., 2012. *Cis*- and *trans*-regulatory divergence between progenitor species determines gene-expression novelty in Arabidopsis allopolyploids. Nat. Commun. 3 (1), 950.
Shi, Y.J., Huang, J.L., Mi, J.X., Li, J., Meng, F.Y., Zhong, Y., 2024. A model of hybrid speciation process drawn from three new poplar species originating from distant hybridization between sections. Mol. Phylogenet. Evol. 190, 107966.
Steens, J., Klein, D., 2022. HOX genes in stem cells: maintaining cellular identity and regulation of differentiation. Front. Cell Dev. Biol. 10, 1002909.
Tani, S., Chung, U.-i., Ohba, S., Hojo, H., 2020. Understanding paraxial mesoderm development and sclerotome specification for skeletal repair. Exp. Mol. Med. 52 (8), 1166–1177.
Uriu, K., Morelli, L.G., 2023. Orchestration of tissue shape changes and gene expression patterns in development. Semin. Cell Dev. Biol. 147, 24–33.
Wan, H.F., Han, K.H., Jiang, Y.H., Zou, P.F., Zhang, Z.P., Wang, Y.L., 2019. Genome-wide identification and expression profile of the sox gene family during embryo development in large yellow croaker, *Larimichthys crocea*. DNA Cell Biol. 38 (10), 1100–1111.
Wang, S., Tang, C.C., Tao, M., Qin, Q.B., Zhang, C., Luo, K.K., 2019. Establishment and application of distant hybridization technology in fish. Sci. China Life Sci. 62 (1), 22–45.
Westram, A.M., Stankowski, S., Surendranadh, P., Barton, N., 2022. What is reproductive isolation? J. Evol. Biol. 35 (9), 1143–1164.
Wittkopp, P.J., Haerum, B.K., Clark, A.G., 2004. Evolutionary changes in cis and trans gene regulation. Nature 430 (6995), 85–88.
Yamada, K., Maeno, A., Araki, S., Kikuchi, M., Suzuki, M., Ishizaka, M., et al., 2021. An atlas of seven zebrafish hox cluster mutants provides insights into sub/neofunctionalization of vertebrate Hox clusters. Development 148 (11), dev198325.
Zhong, H., Zhang, X.j., Xu, Q., Yan, J.P., Han, Z.J., Zheng, H.F., 2019. Nonadditive and asymmetric allelic expression of growth hormone in hybrid Tilapia. Front. Genet. 10, 961.
Zhong, H., Ren, B.X., Lou, C.Y., Zhou, Y., Luo, Y.J., Xiao, J., 2023. Nonadditive and allele-specific expression of ghrelin in hybrid tilapia. Front. Endocrinol. 14, 1292730.