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Genomic Evolution of Growth and Reproduction Related Genes in Shrimp

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Abstract As globally important economic aquaculture species, the growth and reproductive capacities of shrimp directly determine the efficiency and sustainability of the industry. In recent years, the rapid advancement of genomics and multi-omics technologies has provided critical support for elucidating the functions and evolutionary trajectories of genes related to shrimp growth and reproduction. This study systematically reviews the progress in shrimp genomics, focusing on the functional classification, expression characteristics, phylogenetic relationships, and selection pressure analyses of growth- and reproduction-related genes. Through case studies of three representative species-*Litopenaeus vannamei* (Pacific white shrimp), *Penaeus monodon* (black tiger shrimp), and *Macrobrachium nipponense* (oriental river prawn)-the evolutionary features of key genes in terms of function and regulation are analyzed. Furthermore, the roles of gene family expansion, gene duplication and pseudogenes, and transcriptional regulatory elements in genomic functional evolution are summarized. By integrating transcriptomic, proteomic, and epigenetic data, the study reveals adaptive evolutionary mechanisms of shrimp under environmental stressors such as salinity changes, pollution, and pathogens. The findings provide a theoretical foundation for molecular breeding and biological mechanism studies in shrimp.

Keywords Genome evolution; Reproductive regulation; Adaptive evolution; Epigenetics; *Litopenaeus vannamei*

1 Introduction

Shrimps (especially the Shrimp Family) are the pillar species of the global aquaculture industry and occupy an important position in international trade. Taking Pacific white shrimp (*Litopenaeus vannamei*) as an example, China introduced farming in the late 1980s, and its output increased from 1.429 9 million tons in 2013 to 2.098 6 million tons in 2022, an increase of 46.8%. Currently, Pacific white shrimp is the highest-yield crustacean farmed animal in China, with significant breeding scale and economic value. Globally, with the increasing demand for aquatic proteins, shrimp farming industry has expanded rapidly in Asia, America and other regions. The shrimp farming industry has created huge economic benefits: the shrimp farming output value in the two northwestern Mexico states alone is close to US$676 million in 2020. It can be seen that shrimp farming plays an important role in the fishery economy and food supply, both in China and around the world.

The growth rate and reproductive ability of shrimps are key traits that determine the benefits of farming. Rapidly grown and larger individual varieties tend to have higher market value and yield. Therefore, increasing the growth rate of shrimps has always been an important goal of breeding. However, during the process of high-intensity breeding and introduction and domestication, many shrimp strains have problems such as growth stagnation and individual size. The reduction in genetic diversity is considered to be one of the reasons: microsatellite analysis shows that some commercial populations of Pacific white shrimp have low genetic diversity, which may lead to deterioration in growth and stress resistance (Ren et al., 2020). In terms of reproduction, the fertility of shrimp determines the supply and breeding cycle of seedlings. Highly fertile shrimps can provide sufficient quality larvae, which is crucial for the expansion of the breeding scale. In actual production, eye shank removal technology is often used to promote the maturity of shrimp, but this operation will cause stress on the shrimp. The lack of reproductive capacity is also reflected in some imported varieties: due to long-term closure breeding, the neatness and survival rate of shrimp seedlings have decreased, and parent shrimps need to be repurchased from abroad every year to maintain production. Therefore, improving the growth and reproductive traits of shrimps is of great significance to solving the current industrial bottlenecks and achieving sustainable and efficient breeding.

With the development of genomics, scientists began to examine shrimp growth and reproductive traits from the perspective of genomic evolution. Genome evolution research can reveal key genetic variation and evolutionary drivers that affect traits, providing a theoretical basis for genetic breeding. In recent years, the whole genome sequences of various shrimp species have been deciphered successively, and the acquisition of high-quality genome maps has made comparative genome and evolutionary analysis possible. Studies have shown that there are a large number of species-specific genes and tandem repeat genes in the shrimp genome, which are the characteristics of the formation of shrimp during the long-term evolution (Tan et al., 2019). Some progress has been made in the evolutionary research on growth and reproduction-related genes: comparing the genomes of different species, it was found that the significant expansion of the visual and motor-related genes of shrimp may be related to the evolutionary adaptation of their benthic lifestyle; for example, multiple genes on the ecdysterone pathway in the Pacific white shrimp genome have undergone positive selection, explaining the genetic basis of their frequent molting and rapid growth (Zhao et al., 2021). In addition, simple sequence repeats (SSRs) are present in large quantities in the shrimp genome and have been found to drive the plasticity and adaptive evolution of the shrimp genome.

2 Progress in Genomics o Shrimp

2.1 Milestones and technological evolution of genome sequencing of shrimp species

Over the past decade, genome sequencing of shrimp species has achieved a leap from scratch. In 2014, Chinese scientists took the lead in deciphering the genome sketch of Chinese prawns (*Fenneropenaeus chinensis*), opening the prelude to crustacean genome research. Subsequently, the genotype composition of the Pacific white shrimp released in 2019 was the first high-quality reference map for shrimp genomes. The genome is about 1.66 Gb in size, encodes about 25 596 genes, and Scaffold N50 reaches 605 Kb (Pérez-Enríquez et al., 2024). The study found that a major feature of the shrimp genome is its high repetition: simple tandem repeats account for more than 23.93% of the genome, which is the highest content among the measured species (Yuan et al., 2021). Huge repeat sequences and complex genomic structures once hindered the assembly of shrimp genomes, but with the evolution of high-throughput sequencing technology, this problem has gradually been overcome. In the early stage, the hybrid strategy of second-generation sequencing (Illumina) combined with BAC library was adopted to barely construct the shrimp genome framework. Entering the era of third-generation sequencing, the high-yield PacBio/ONT long-read and Hi-C assistive technologies have been applied to shrimp genome assembly. In terms of freshwater large shrimp, the high-quality reference genome of *Macrobrachium rosenbergii* has also been released, providing a basis for studying crustacean gender decisions, etc.

2.2 Genome annotation and functional annotation methods

Obtaining genomic sequences is only the first step, and it is more critical to accurately annotate genes and functional elements in the sequence. Annotation of shrimp genomes usually adopts a multi-strategic approach: on the one hand, alignment of homologous sequences can predict coding regions and conservative genes (e.g., aligning known crustacean gene sequences with newly assembled genomes); on the other hand, evidence-based support is supported by transcriptome data, exon-intron structures and transcripts are determined by aligning mRNA/cDNA sequences (Li et al., 2012; Wei et al., 2014). For large genomes such as Pacific white shrimp, researchers comprehensively used Ab initio prediction, homologous alignment and transcriptome assembly to annotate genes. The results showed that the genome of Pacific white shrimp contains about 25,000 protein-encoding genes. In addition to coding genes, non-coding RNAs, repeat sequences and regulatory elements in the genome are also annotated and classified through specialized software. In terms of functional annotation, using public databases (such as NCBI Nr and Swiss-Prot) to perform homologous search of predicted proteins can be conferred on preliminary functional annotation of genes. In addition, the distribution of gene roles in biological processes, molecular function and cellular components can be understood through Gene Ontology (GO) classification and KEGG pathway analysis.

2.3 Genomic databases and information resources (such as NCBI, ShrimpBase, etc.)

As multiple shrimp genomes were measured one after another, a large amount of data was stored in public databases and opened to scientific researchers. The NCBI genome database contains the assembly sequence and gene annotation information of species such as Vannebane prawns, squids, and biosparagus Rohman, which can be obtained through Bioproject or Genome numbers. In addition to comprehensive databases, professional databases for specific groups have also been established one after another. For example, the "PenBase" database specifically includes sequence and functional information of the shrimp antibacterial peptide (penaedin) family, providing a convenient tool for the study of shrimp immune genes (Senapin et al., 2012). Some scholars in China have established a shrimp breeding database to collect family breeding pedigrees, phenotypic traits and gene marker information to facilitate genetic evaluation and seed selection decisions.

3 Functional Classification and Expression Characteristics of Growth-Related genes

3.1 The main gene classes and mechanisms of action that regulate growth (such as GH, IGF, mTOR pathways)

The growth regulation of shrimp involves multi-level gene networks such as endocrine hormones, nutritional metabolism, and cell proliferation. Among them, the insulin/insulin-like growth factor signaling pathway (IIS pathway) is believed to play a central role in crustacean growth and metabolism. The IIS pathway includes key signal nodes such as insulin-like growth factor (IGF), insulin receptor (IR), downstream PI3K-AKT and mTOR. Studies have shown that the shrimp IIS pathway gene has unique evolutionary characteristics: three insulin-like growth factor binding protein (IGFBP) genes were identified in the Pacific white shrimp genome, and their structures were different. Among them, IGFBP1 contains three functional domains IB, Kazal and IgC2, while IGFBP2 and IGFBP3 only contain the IB domain. RNA interference experiments found that knockdown of IGFBP1 significantly reduced the weight gain rate in shrimp, indicating that IGFBP1 plays a key role in promoting growth (Pang et al., 2021). In addition, shrimp insulin receptor genes also show evolutionary innovation. Su et al. (2024) cloned a novel insulin-like receptor LvRTK2 in vannerbine shrimp, which belongs to the unreported subtype of the insulin receptor tyrosine kinase family. Expression and functional analysis showed that LvRTK2 was mainly highly expressed in digestive organs and reproductive tissues. After administration of exogenous glucose and starvation, LvRTK2 mediates the regulation of sugar metabolism in shrimps; long-term and short-term interference with the *LvRTK2* gene both lead to a decrease in shrimp growth rate (Su et al., 2024). This suggests that the receptor is not only involved in growth but also closely related to energy balance. In addition to the IIS pathway, the ecdysterone (20E) signaling pathway also affects shrimp growth, because crustaceans can only continue to grow after molting.

3.2 Analysis of expression profiles in tissue and development stages

To understand the functions of growth-related genes, researchers often analyze gene expression profiles at different tissues and developmental stages through the transcriptome. Overall, shrimp growth regulation genes are highly expressed in energy-metabolic organs (such as hepatopancreas, muscles) and endocrine organs. The newly discovered LvRTK2 receptor is expressed in both the ovaries of female shrimp and the testes of male shrimp, and is expressed in digestive tissues such as the hepatopancreas. This distribution pattern suggests that the receptor not only mediates growth metabolic signals, but may also be involved in the regulation of gonad development. In terms of developmental stage, the expression of many growth-related genes changes dynamically with the development of shrimp body (Figure 1). Taking the example of muscular development-related genes, transcriptome studies show that during the transition from larval to shrimp, structural myoproteins such as myosin subunit genes are gradually upregulated to meet higher exercise needs (Xia et al., 2023). Correspondingly, genes that affect the cell growth cycle are expressed higher in the juvenile period and tend to decline in the adult stage, which is consistent with the phenomenon that individual growth rate slows down with development. In addition, some genes related to peeling and regeneration (such as chitinase, chitinase) are significantly upregulated in the early stage of molting and rapidly decline after molting, reflecting the cyclical changes in the developmental stage (Gao et al., 2017).

3.3 Effects of exogenous factors such as nutrition, temperature, and density on gene expression

Environmental factors often act on shrimp growth performance by affecting gene expression. Nutritional levels are the primary factor: protein and fat content in the feed regulates the expression of digestive enzymes and growth factor genes. For example, under the conditions of providing high-quality baits (live sand silkworms, etc.), the genes related to energy supply in the female shrimp's ovaries are significantly upregulated, and the yolk protein and ecdysterone synthesis pathways are more active, which helps accelerate reproductive and growth processes (Awad et al., 2025). On the contrary, when malnutrition is present, shrimp may reduce metabolic rate by downregulating the IIS pathway gene and temporarily stop growing to maintain basic survival. Temperature is another important variable. Shrimps are warm-warming animals, and changes in water temperature will trigger a series of changes in gene expression of stress responses. When the ambient temperature is below the optimal growth temperature, the heat shock protein (*HSP*) gene is significantly upregulated in shrimp tissues to prevent protein denaturation damage. A study of the HSP gene family of Pacific white shrimp identified up to 76 *HSP* genes that showed diverse response patterns to different stressors. Among them, under low temperature stress, some *HSP70* and *HSP90* genes are strongly induced in tissues such as gills, hepatopancreas, and help the body restore homeostasis (Peng et al., 2016). Factors such as farming density and water quality will also affect shrimp growth through gene expression pathways. High-density farming can easily cause the accumulation of metabolic waste in water (such as ammonia nitrogen and nitrite). These stresses will trigger the expression changes of detoxification enzymes and immune factor-related genes, thereby allocating some energy for stress resistance rather than growth. In actual breeding, optimizing feed nutrition, controlling water temperature and reducing stress density are essentially creating environmental conditions conducive to the optimal expression of growth genes.

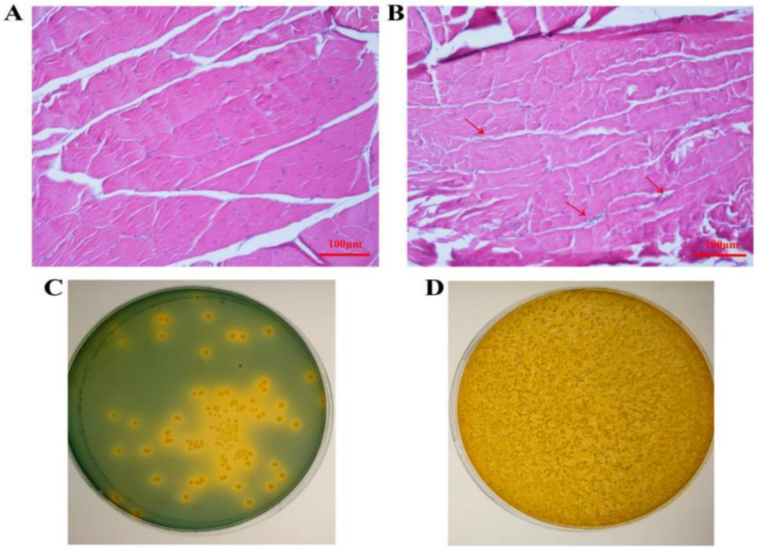

Figure 1 Histological sections of muscles and total viable bacteria count after *LvMEF2* interference. (A,C) control group(PBS); (B,D) *dsMEF2* group. The arrows in panel (B) refers to the region of nuclear agglutination (Adopted from Xia et al., 2023)

4 Functional Analysis of Reproduction-Related Genes

4.1 Ovarian/testicle development regulation genes and their expression dynamics

The reproduction process of shrimps includes gonad development and maturation, gamete formation, and egg laying and hatching, and is finely regulated by a series of genes. In female shrimp ovaries, the most important nutrient supply protein is yolk protein (Vg), which is synthesized by the hepatopancreas and transported to the ovaries through blood and is ingested and utilized. The genes that regulate yolk protein synthesis and accumulation directly determine the quality and fertility of the egg. During ovarian maturation, the *Vg* gene expression increased significantly, while the expression of the yolk protein receptor (VgR) gene on the oocyte membrane was synchronously enhanced to promote yolk uptake (Li et al., 2022). As the ovaries enter a period of decline, the expression of these genes is rapidly downregulated, achieving renewal of the reproductive cycle. Related to egg formation are hormone synthetase genes such as ecdysterone (20E): Studies have found that during the oocyte development period, the 20E level increases, and the expression of its synthetic rate-limiting enzyme genes also increases, suggesting that ecdysterone may play a promoting role in ovarian development. In male shrimp, testicular development involves spermatogenesis and spermatopod formation. Supporting cells in the semen secrete a variety of factors that affect spermocyte meiosis, with some of the key regulatory genes derived from the gender-determining pathway (Alfaro-Montoya, 2010). By comparing gonad transcriptomes at different developmental stages, dynamic patterns of reproductive genes can be observed. For example, when the gonads of the larval shrimp are not differentiated, the expression of genes related to gametegenesis is very low; during the gonad differentiation and maturation stage, these genes (such as Cyclin related to meiosis, etc.) are rapidly activated, and their expression peaks correspond to the rapid gamete proliferation stage.

4.2 Research on the mechanism of differentiation between male and female and gender determination (such as Dmrt, Foxl2)

Crustaceans have complex and diverse gender-determining mechanisms. Most economic shrimps are dioecious and have male and female dimorphs. Unlike mammals that are dominated by sex chromosomal genes (*SRYs*), crustaceans often control gender differentiation by hormones secreted by male islets (also known as male glands, endocrine organs unique to males). The insulin-like androgen hormone (IAG) produced by male islets is considered a switch: as long as the male shrimp larvae produces IAG, the male differentiation pathway will be initiated; if several IAG signals are disturbed, the individual will develop into a female or a male-female interferometer (Yang et al., 2020). In recent years, some conservative regulators involving gender differentiation have also been found in freshwater shrimp. The Dmrt gene family and the *Foxl2* gene are thought to play roles similar to the determinants of vertebrate gonads in invertebrates. Xu et al. (2022) cloned and analyzed the *Foxl2* gene of *M. rohbrofa* and found that the transcription factor it encodes is expressed in sperm cells in the testicles and oocytes in the ovary, and the expression is highest in the early stage of ovarian development (Stage I). This bisexual expression pattern suggests that *Foxl2* may play a role in gonad development in both male and female, rather than just promoting ovarian differentiation (Xu et al., 2022). In some crustaceans (such as crayfish), genes such as *Dmrt1* were also identified, which are highly expressed in male embryos and are inhibited in females. These findings suggest that crustaceans are likely to have a conserved gender-regulating gene network, including the IAG-led male pathway and the Foxl2/Dmrt-mediated gonad differentiation pathway, which interact together to determine the final gender phenotype. It should be noted that the gender determination patterns of different shrimp species vary.

4.3 Reproductive hormone signaling pathway and neuro-endocrine regulatory genes

The reproduction process of shrimp is finely controlled by the endocrine system and involves multi-level regulation of the brain-stem-godal axis. Similar to the pituitary-godal axis of vertebrates, crustaceans have endocrine organs and hormones that correspond to their functional functions. The X-organ-sinus complex in the stalk of shrimp eye is equivalent to the endocrine center and secretes a variety of peptide hormones, one of which is the inhibitory hormone (GIH, also known as the gonadal inhibitory hormone). GIH belongs to the crustacean hyperglycemia hormone (CHH) family, which inhibits the development and maturation of female shrimp's ovaries. When the eye stalk removal (GIH source removal) is used in breeding production, the shrimp ovaries will mature quickly, which has long been applied to shrimp reproduction control. It can be seen that GIH and other eye stalk hormone genes play a negative switch role in reproductive regulation. At present, multiple member genes of the CHH superfamily of the Pacific white shrimp have been identified (such as *CHH, MIH, VIH*, etc.) and have demonstrated their function in stress metabolism and reproductive regulation (Wang et al., 2019). In addition to central hormones, the gonad itself secretes hormones that affect the reproductive process. The ovaries of female shrimps will produce steroid hormones such as Eksorbenzodione, which can promote ovarian development. In terms of neuromodulation, neurotransmitters such as somatostatin-like peptides and dopamine distributed in the brain and ganglion are believed to be involved in the regulation of reproductive signals. They indirectly affect the expression of gonad genes by regulating the secretion of upstream endocrine hormones. Molecular studies in recent years have also revealed the existence and role of reproductive-related hormone receptor genes. For example, receptor proteins and downstream signaling pathway genes of CHH family hormones have been identified in shrimp, which will trigger a series of transcriptional responses once activated.

5 Analysis of the Evolutionary Trajectory and Selection Pressure of Key Genes

5.1 Construction and comparison of phylogenetic tree of growth/reproduction genes

To explore the evolutionary history of growth and reproduction-related genes, researchers often compare homologous gene relationships in different species by constructing phylogenetic trees. Such analysis can reveal gene origin and differentiation and species specificity. Taking the insulin-like growth factor binding protein (IGFBP) as an example, Pang et al. (2021) compared the phylogenetic relationship between three *IGFBP* genes in vannerbine shrimp and other invertebrates and vertebrates IGFBP. The results show that shrimp IGFBP and insects and other invertebrates are clustered into one branch, which is obviously different from the IGFBP subfamily of mammals. This suggests that shrimp IGFBP is evolutionarily unique to invertebrates and may also differ in function. For males to decide to switch the *IAG* gene, the phylogenetic tree constructed by Lü et al. (2023) shows that the IAG of small freshwater shrimps (such as the genus Cypress) gathers into one branch, while the IAG of marine shrimps gathers into another branch, and the two separate in the tree. This suggests that *IAG* genes may have lineage-specific differentiation in different ecological groups, and their functional regulation may be different. Through phylogenetic tree comparisons, we can also speculate on gene replication and loss events. For example, if a shrimp species has two sequences on a tree that corresponds to one gene of another species, it means that a gene duplication has occurred; conversely, if it is missing, it may be a gene loss or pseudogeneization.

5.2 Analysis of positive selection, negative selection and neutral evolutionary events

The selection pressures that genes are subject to during evolution can be inferred by molecular evolution rate analysis. Researchers have discovered some interesting selection patterns for growth and reproduction-related genes in the shrimp genome. Positive selection (adaptive evolution) often acts on genes that are functionally important and require rapid adaptation to the environment. At the same time, artificial breeding is also driving selective changes in some genes. By resequencing the genome of 6 Vannebine shrimp breeding lines, approximately 370 000 significantly differentiated SNP sites and a batch of selected genes were identified. Some of these genes are related to growth and immune function and are considered to be artificially selected during breeding and domestication. These genes exhibit high frequency enrichment of specific alleles in breeding line populations, reflecting the directed selection pressure of breeding on fast growth and disease-resistant traits (Ma et al., 2024). In contrast to positive selection, negative selection (purification selection) keeps functionally critical reproductive genes highly evolutionarily conserved. In addition, a large number of structural and metabolic-related genes are also maintained by purification selection, and their amino acid sequences are almost unchanged in shrimp evolution. As for neutral evolution, it mainly involves those non-functional regions or redundant genes in the shrimp genome. A high degree of polymorphism in simple repeat sequences (SSRs) is an example: many of the newly expanded SSRs in the shrimp genome vary in length between different strains, but do not affect survival adaptation. This suggests that these SSR variants may be neutral evolutionary products and are not subject to obvious choice constraints. After some duplicate genes are functionally replaced or redundant, their mutations accumulate in a neutral way, gradually turning into pseudogenes present in the genome.

5.3 Convergence and differentiation and evolutionary characteristics of functional genes among different shrimps

Shrimps differentiate into diverse ecological groups during the long evolution process, and different species show evolutionary characteristics of convergence or divergence in functional genes. Convergent evolution refers to the fact that species without kinship evolve similar genetic characteristics when facing similar environmental pressures. Among shrimps, low-salt environmental adaptation is an example. Vannebane prawns and Chinese prawns live in low salinity waters of the estuary. Although their phylogenetic distance is far away, their genomes have experienced a large expansion of simple repeat sequences (SSRs), which is believed to confer adaptability to low salt stress (Zhang et al., 2023). Multiomic analysis showed that these SSR enrichment near osmotic regulation-related genes, affecting gene expression, thereby improving survival under low-salt conditions. This suggests that the two shrimps achieve convergent environmental adaptation through similar genomic mechanisms in independent evolution.

On the other hand, differentiation evolution (divergent evolution) is also evident among different shrimps. Differences in living environment and life history have led to different evolutionary paths in various species. Compared with shallow and nearshore shrimp, their heat shock protein gene family may experience different selection pressures: deep-sea species may lose some unnecessary HSP subtypes, while shallow and sea species expand the *HSP* gene to cope with frequent temperature fluctuations. Take gender determination as an example: Some freshwater shrimps (such as crayfish) have evolved the WZ-type sex chromosome determination mechanism, while most marine shrimps still rely on both gonad hormone regulation and have no obvious sex chromosomes (Rogl et al., 2018). This reflects the divergence evolution of reproductive genes across lineages.

6 Gene Family Expansion and Regulatory Mechanism Evolution

6.1 Expansion/shrinkage modes of growth and reproduction-related gene families (such as HSP, trypsin)

An important source of genome size and functional diversity is the expansion or contraction of gene families. Shrimps have experienced multiple gene repetition events during their evolution, forming some unique gene families. The expanded *HSP* genes have functional differentiation between each other, which can respond to various stresses such as heat stress, hypoxia, pathogenic infection, etc., thereby improving the environmental tolerance of shrimps. For example, digestive enzyme genes such as trypsin and amylase also form a multi-copy family in omnivorous shrimp. There are studies that have reported that Pacific white shrimp have at least 10 copies of trypsin genes, which are arranged in tandem in the genome. The presence of these repeated genes allows shrimp to efficiently digest various baits consumed, improving nutritional utilization and growth rate. This family expansion of digestive enzyme genes may be the result of long-term selection of high-density aquaculture and artificial compound feeds (Chen et al., 2023). In addition, immune-related gene families (such as antimicrobial peptide Penaeidin, multiple pattern recognition receptors, etc.) have also expanded significantly in the shrimp. This gives shrimp a huge arsenal of immune weapons to resist a variety of pathogens. In contrast to expansion, certain gene families specifically contract and even lose in shrimp. Nuclear hormone receptor families, which are present in many insects, have fewer species in the shrimp genome, which may reflect the loss of genes caused by differences in the developmental patterns of crustaceans and insects.

6.2 Contributions of repeat genes and pseudogenes in evolution

Gene repetition is one of the important driving forces for biological evolutionary innovation. Many new functions in the shrimp genome come from the differentiation and evolution of repeated genes. When one gene is repeated, one of the two copies often maintains the original function, and the other accumulates mutations under looser selection pressure, thereby obtaining new functions (i.e. secondary functionalization) or becoming a pseudogene that loses function. In shrimp, some duplicate genes have shown signs of functional differentiation. For example, the expression of different trypsin gene copies in the digestive tract of shrimp is different, and the enzyme activity of different protein substrates is also slightly different (presumably). This suggests that they may perform their own functions for diverse food sources and are examples of repeated evolution producing functional innovations (Xiong et al., 2024). Some duplicate genes gradually accumulate a large number of mutations into pseudogenes, which are "forgotten" by the genome. Although pseudogenes do not encode functional proteins, their presence provides an evolutionary "molecular fossil" that records the history of species' genome changes. In addition, repeated new genes may sometimes share the function of the original gene, i.e. the dose effect. For example, shrimps have multiple copies of microsporidium resistance genes, which may make them more resistant to pathogens (Santos et al., 2021). At the genomic scale, repetitive events are mostly caused by mechanisms such as unequal crossing and transposon-mediated replication. In the shrimp ancestral genome, the expansion of a large number of simple repeats creates opportunities for chromosomal fragment translocation. Repeated DNA fragments may carry adjacent genes to replicate together during rearrangement, resulting in large fragment replication of the genome. Some of these repeated genes remain in full function, while others are silent as pseudogenes due to lack of regulatory elements or mutations.

6.3 Co-evolution of regulatory elements (such as promoters, enhancers) and transcription factors

In addition to coding sequences, the evolution of a large number of non-coding regulatory elements in the genome also profoundly affects the changes in gene function. Sequence changes in cis-regulatory elements such as promoters and enhancers often change the spatiotemporal expression pattern of genes, thereby causing phenotype differences. In shrimp evolution, we observed that some regulatory elements and the transcription factors they regulated have a coordinated evolution. A typical case is the role of simple repeat sequences (SSRs) in regulatory regions: researchers used multiomics to find that some SSRs in the genome of Pacific white shrimp were inserted into gene promoters or introns, resulting in differences in expression of nearby genes under different environments. For example, when the promoter of an osmotic regulatory gene contains variable length AT repeats, its expression level under low salt stress is significantly higher than that of alleles without duplicates. This indicates that slight changes in regulatory elements (such as changes in repeat length) can affect transcription intensity. At the same time, transcription factors involved in binding these elements also responded in evolution. Either the DNA binding domain of the transcription factor has a mutation that matches the regulatory sequence, or the expression profile changes to adapt to the presence of new enhancers. This "co-evolution" of regulatory factors and target sequences ensures the homeostasis of the gene expression regulatory network. Foxl2, a transcription factor associated with estrogen receptor in shrimp, has slightly different DNA binding sites in different species. Correspondingly, the regulatory elements retained on the Foxl2 promoter in each species also differ, possibly reflecting the remodeling of the Foxl2-target gene axis in different evolutionary contexts.

7 Case Analysis

7.1 Evolution and functional analysis of the IGF signaling pathway in Pacific white shrimp

As an important breeding species, the growth regulation mechanism of Pacific white shrimp has always attracted much attention. In recent years, a series of results have been achieved in the study of insulin-like growth factor (IGF) signaling pathways. Three IGF-binding protein (IGFBP) genes in the IGF signaling pathway were identified in vannerbine shrimp and their structural and evolutionary relationships were compared. The results show that LvIGFBP1 has a Kazal-type serine protease inhibitory domain that is not found in mammalian IGFBP, showing structural innovations unique to crustaceans. This structural evolution may confer new regulatory functions to IGFBP1, such as binding to IGF and participating in trypsin inhibition, thereby connecting growth signals to digestive metabolic pathways (speculative mechanisms). Functionally, IGFBP1 was expressed at high levels in most tissues, and RNAi knockdown experiments resulted in hindering growth of shrimp, and their weight gain was significantly lower than that of the control group. This proves that IGFBP1 is a key positive regulator of growth promoting in the IGF pathway. While IGFBP2 and IGFBP3 are mainly expressed in low quantities in blood cells, the impact of knockdown on overall growth is not obvious, suggesting that they may play more roles in local environments or are partially redundant in function (Pang et al., 2021). Evolutionary analysis speculates that the other differentiates into the RTK2 type. LvRTK2 is highly conserved in shrimps, but there is no corresponding homologue in hexapods such as insects, indicating that its function may be unique to crustaceans. Functional experiments show that interfering with LvRTK2 will reduce the blood sugar removal efficiency and growth rate of shrimp after feeding. Combined with the fact that this receptor is mainly expressed in the hepatopancreas and reproductive organs, it is speculated that LvRTK2 is not only involved in general growth and metabolism, but may also connect nutritional status with reproductive energy distribution - promoting growth and reproductive development through the IIS pathway when nutrients are sufficient, and inhibiting reproductive investment when nutrients are scarce to preserve one's own survival.

7.2 Comparative evolutionary analysis of genes related to ovarian development in black tiger shrimp

Black tiger shrimp (*Penaeus monodon*) is one of the important breeding shrimps, and its ovarian maturation is a key step in achieving reproduction. Researchers conducted multi-level comparative analysis on the development of ovarian ovaries of Black tiger shrimp. On the one hand, comparison of ovarian gene expression under different treatment conditions within the same species reveals the molecular mechanisms that regulate ovarian maturity. Experts used transcriptome technology to compare the similarities and differences in ovarian gene expression in black tiger shrimp under ocular stalk removal and feeding live bait. The results showed that ovaries with only the stalk removal (simulated to remove the inhibitor GIH) and ovaries with only the extra fertile live bait were not able to reach full maturity; while in the treatment group that performed stalk removal and live bait enhancement, genes related to lipid synthesis, energy metabolism and oocyte maturation in the ovaries were synchronized upregulated, and the expression levels of key genes such as yolk protein and ecdyketone synthetase were significantly higher than those of the single treatment group (Figure 2) (Li et al., 2022). This finding shows that the maturation of black tiger shrimps requires the joint effect of endocrine releasing (removing inhibitory signals) and nutritional enhancement, and the two have synergistic effects.

On the other hand, the comparison of ovarian development genes among different species also reveals some convergences and differences. By comparing the multiomic data of ovarian shrimps and Pacific white shrimps, it was found that although the two belong to different genera, the changes in gene expression on paths such as energy metabolism and yolk formation during ovarian maturation have similar patterns, both manifested as the stages of accumulation of nutrients and then activation of oocyte maturation; this reflects a convergent evolutionary model of crustacean reproduction. However, there are also differences in the ovarian responses of the two shrimps in terms of immune and stress-related genes.

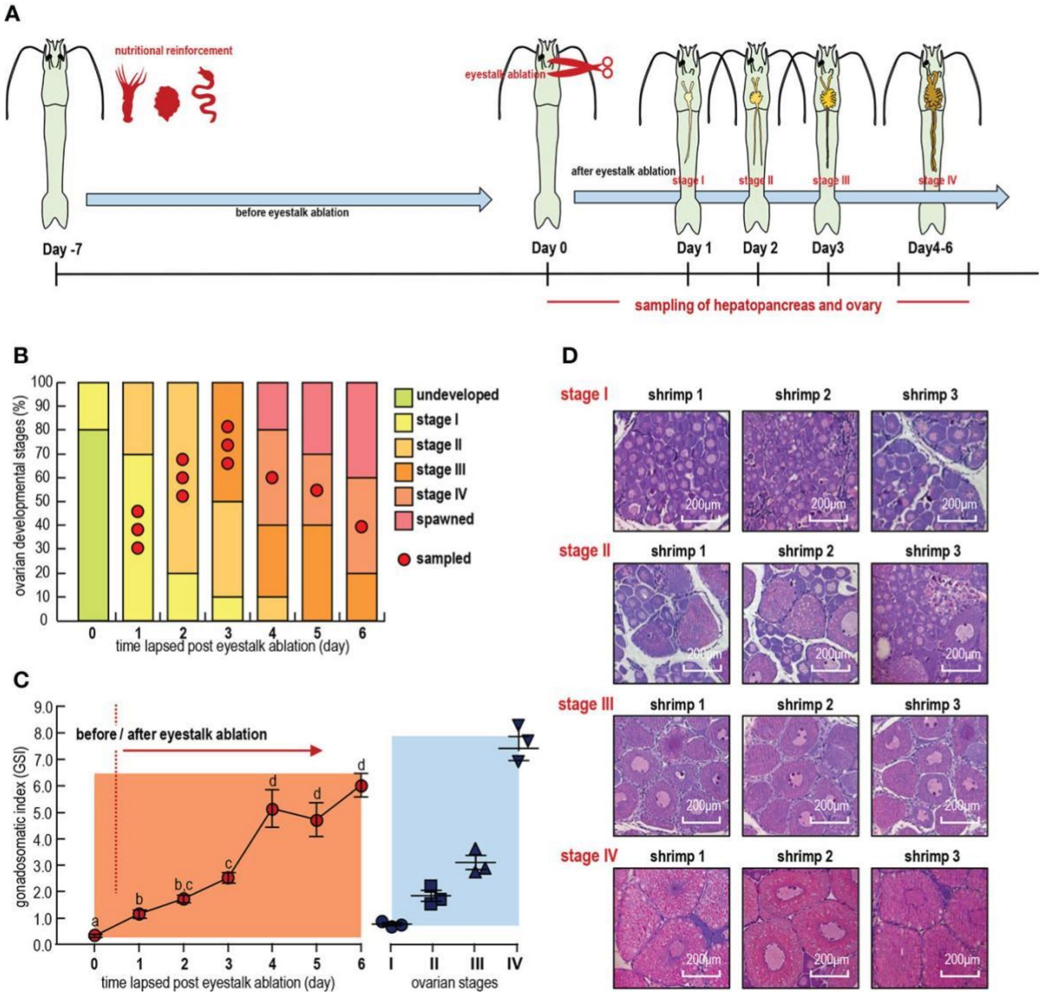

Figure 2 (A) Representative ovaries at different time points. (B) Distribution of samples over ovarian developmental stages. (C) Changes in gonadosomatic index (GSI) with days and ovarian developmental stages after eyestalk removal. Error bars represent standard derivations. Different letters indicate significant differences between time points of the same feed group (n = 3, mean ± sem, $P < 0.05$, ANOVA Duncan test). (D) HE staining of the ovaries and liver and pancreas. Stage: Four periods of ovarian development (Adopted from Li et al., 2022)

7.3 Research on the evolution of gender-determined genes of Japanese swamp shrimp

Japanese swamp shrimp (*Macrobrachium nipponense*) is an important freshwater shrimp in East Asia, and its gender determination mechanism is of great research value. Unlike marine prawns that rely mainly on the male gonad hormone IAG, swamps may have the dual characteristics of genetic gender determination and endocrine gender differentiation. In recent years, research on Japanese marsh shrimp has identified a batch of key genes related to gender differentiation, providing clues to explore the evolutionary mechanism of its gender decision. First, the male islet hormone (IAG) gene was also found in Japanese worm shrimp and showed a sequence feature that was highly homologous to worm shrimp Rohman. It is speculated that IAG also plays a male switch role in this species (Cai et al., 2023). Second, the researchers cloned the *Mn-Foxl2* gene of Japanese marshmallows and found that it was expressed at high levels in female embryos while significantly inhibited in male embryos. This is different from the co-expression of Foxl2, a male or female, in *M. Rohbac*. Again, the presence of the *Dmrt* gene was also detected in Japanese marshmallows and it was confirmed that it was expressed in males than females. Dmrt is a conservative sex determinant and is responsible for activating male traits in many species. The male biased expression of Japanese marshmallow Dmrt indicates that its function may be similar to that of vertebrate Dmrt1, responsible for testicular differentiation.

8 Multiomics Integration Revealed Adaptive Evolution Mechanisms

8.1 Synergistic application of transcriptome and proteome analysis in expression regulation

In functional genomics studies, single collective data are sometimes insufficient to explain complex biological phenomena. Transcriptomics provides information on mRNA levels, but only in conjunction with proteomics can we understand the status of the final product of gene expression. Integrating the two omics data can reveal the gene regulation mechanism more comprehensively. In shrimp research, the combination of transcriptome-proteome applications are gradually emerging. A typical case is a multiomic study of environmental stress responses. A 3-month salinity stress experiment was conducted using Japanese marshmallows as a model, and the transcriptome and proteome changes in the hepatopancreatic and gill tissue were monitored. The results showed that under high salt exposure, there were significant changes in mRNA levels of thousands of genes in the gills and hepatopancreas (DEGs), but only hundreds of corresponding proteins showed significant differences (DEPs). By correlating transcription and protein data, researchers found that only a small number of pathways showed consistent significant changes at both levels, including protein secretion pathways, cGMP-PKG signaling pathways, aminoglycopy, etc. (Fan et al., 2023). On the other hand, the transcriptional levels of many genes are not consistent with protein levels: for example, about 2,000 differential genes were observed in the hepatopancreas of the low-salt (7‰) group, but there was almost no significant change in the corresponding protein. This may be due to factors such as post-transcriptional regulation, protein stability or translation efficiency. This is the value of multiomic integration: it helps us filter out real significant changes in function. When there is only transcriptome data, a large number of differentially expressed genes may not have functional effects, and after binding to the proteome, it can focus on those "core genes" whose transcripts and proteins have changed.

8.2 Epigenetic mechanism: regulation of expression by DNA methylation and miRNA

In addition to changes in the DNA sequence itself, epigenetic regulation plays an important role in the adaptive evolution of shrimps. Epigenetic markers such as DNA methylation and non-coding RNA (such as microRNAs, miRNAs) can regulate gene expression without changing the gene sequence, thereby achieving a rapid response to environmental or developmental signals. In recent years, breakthroughs have been made in the study of DNA methylation in shrimp. Some people used whole-genome sulfite sequencing (WGBS) to compare muscle methylation levels in individuals in the same line as Pacific white shrimp. The results showed that the 5-methylcytosine level in the entire genome of fast-growing individuals was about 2.00%, significantly lower than that of slow-growing individuals. When analyzed in conjunction with the transcriptome, it was found that elevated methylation levels were often accompanied by downregulation of expression of certain metabolic and growth-related genes. A key gene associated with muscle development is highly methylated in the promoter region in slow-growing shrimp, resulting in a significant decrease in its mRNA expression. This reveals part of the reason for growth differences at the epigenetic level: DNA methylation promotes genes by silencing growth, which inhibits the growth rate of shrimp. This study is the first experimental evidence to directly prove that DNA methylation affects crustacean growth traits, and is of milestone significance. Similarly, DNA methylation is also involved in environmental adaptation in shrimp. In addition to DNA methylation, non-coding RNAs such as miRNA are also an important link in regulating gene expression. The shrimp genome encodes numerous miRNAs that inhibit translation or promote mRNA degradation by binding to the 3’UTR of the target mRNA. During reproduction and growth, the expression profiles of different miRNAs are dynamically changed, thereby achieving fine control of regulatory networks.

8.3 Gene response and adaptation driven by environmental pressure (salinity, pollution, pathogens)

The water environment in which shrimps live is complex and changeable, and environmental pressures such as salinity, temperature, pollutants and pathogens have jointly shaped the adaptive changes in their genome. A large number of studies have shown that different environmental factors induce specific gene responses in shrimps, thereby improving adaptability. Salinity is one of the determinants of aquatic biodistribution. Under high salt conditions, gill tissues upregulate sodium/potassium ATPase, inorganic ion transporters and other genes, improving the salt discharge ability; while at moderate salinity (7‰ and 14‰), metabolic enzyme activity decreased, implying that the salinity range is close to the osmotic pressure isotensin point of the shrimp, which minimizes its energy metabolic load. In long-term evolution, the genomes of prawns and marsh shrimps that live in salt-changing environments such as estuaries and salt marsh are often rich in variable regulatory elements, allowing the above-mentioned osmotic pressure regulatory genes to quickly adjust their expression.

Stress of pollutants (such as heavy metals, pesticides) can also induce stress responses at the genomic level. Prawns live along shallow coasts and are inevitably exposed to industrial and agricultural pollution. In order to detoxify and tolerate, these shrimps evolved expanded detoxifying enzyme genes (such as glutathione transferase GST, multifunctional oxidase CYP, etc.), which quickly upregulate expression in the presence of toxic substances, converting the poison into a non-toxic form. Pathogenic principle is another important pressure in shrimp evolution. Shrimps are prone to infection with Vibrio, WSSV, etc., and the periodic outbreaks over the years have caused heavy losses to breeding. Under evolutionary selection, surviving shrimp species often have stronger immune gene reserves and rapid immune activation capabilities. Genome sequencing shows that Pacific white shrimp amplifies many immune receptors and effector molecular genes, such as pattern recognition receptors (PRRs), antimicrobial peptide PEN, phenol oxidase progen, etc. Amplification of these genes improves shrimp's ability to recognize and remove multiple pathogens. Transcriptome studies in recent years have also confirmed that when encountering pathogen infection, the immune genes in shrimps quickly and significantly change their expression.

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Conflict of Interest Disclosure

The authors confirm that the study was conducted without any commercial or financial relationships and could be interpreted as a potential conflict of interest.

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