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Case Study **Open Access**

Genetic Basis and Molecular Mechanisms of Trait Variation in the Domestication of Abalone

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Abstract Abalone is an important seafood shellfish, but it faces bottlenecks such as slow growth, poor stress resistance and limited reproduction efficiency during artificial domestication and breeding. This study reviews the rules of phenotypic trait variation during abalone domestication and deeply analyzes its genetic basis and molecular mechanism. In terms of traits such as growth, stress resistance and reproduction, the domesticated abalone population showed significant variations, and some excellent traits were strengthened by artificial selection. The application of modern molecular breeding technology has promoted the research on genetic improvement of abalone. Multi-omics such as genome sequencing, QTL localization, candidate gene screening, transcriptome and proteome have revealed important genes and signaling pathways that affect the trait of abalone. For example, IGF and mTOR are involved in regulating growth, NF-κB and HSP networks mediate immune resistance, and gonad development is regulated by specific genes. We also discuss the latest attempts and challenges of RNA interference and CRISPR/Cas9 gene editing in abalone functional gene verification. Through cases such as the cultivation of Japanese Ezo abalone (*Haliotis discus hannai*) disease-resistant strains, South African abalone (*Haliotis midae*) multi-generation breeding, and Hainan hybrid abalone multi-omics analysis, the direction of innovation in the abalone seed industry is expected. Research believes that integrating traditional breeding and molecular biology methods is expected to accelerate the genetic improvement of abalone, cultivate new varieties with fast growth and strong resistance to stress, and promote the sustainable development of abalone breeding industry.

Keywords Abalone; Trait variation; Genetic basis; Multiomic analysis; Gene editing

1 Introduction

Abalone (*Haliotis*) has been known as the "crown of sea treasures" since ancient times because of its delicious meat and high nutritional value. It is an important economic species in coastal fishery and aquaculture. In recent years, with the decline of marine fishing resources, the abalone breeding industry has developed rapidly. China has become the world's largest abalone breeding country, with an annual output of more than 200,000 tons, accounting for more than 90% of the world (Zhou et al., 2023). However, problems such as long growth cycle of abalone (usually more than 2 years of commodity specifications), prone to outbreaks in high temperature and hypoxia seasons, and germplasm degradation are becoming increasingly prominent. The deterioration of traits caused by improper inbreeding and breeding also brings hidden dangers to the industry. In order to meet the industry's demand for excellent abalone varieties, it is urgent to carry out abalone genetic breeding research to improve key traits such as growth rate, stress resistance and fertility (Ke et al., 2016).

Artificial domestication refers to the process in which wild groups are raised and bred through artificial environments, gradually adapting to and producing genetic changes. During the abalone domestication process, phenotypic traits undergo significant variations: on the one hand, excellent traits accumulate under artificial selection, such as faster growth, larger individuals and stronger heat and disease resistance, which appear one after another (Wang et al., 2024); on the other hand, adverse traits may also appear due to inbredness, such as the decrease in stress resistance caused by germplasm degeneration. Studies have shown that there are genetic differences between abalones in different domesticated generations from wild populations in terms of growth and survival traits.

This study aims to systematically summarize the characteristics of phenotypic trait variation during abalone domestication, as well as to reveal the genetic basis and molecular regulatory mechanisms behind these trait variations. First, we summarize the changes in the growth, stress resistance, behavioral reproduction and other traits under artificial domestication and selection; introduce the research progress of quantitative trait genetics in abalone, including QTL localization, candidate gene screening and molecular marker association analysis to analyze the genetic basis of key traits; focus on the molecular pathways that determine traits, such as the impact of IGF and mTOR pathways on growth metabolism, NF-κB and HSP-mediated immune and stress resistance networks, and genes and endocrine mechanisms related to reproductive regulation; finally, through three typical case analysis, we explore the problems and countermeasures encountered in actual breeding work, providing comprehensive data and analysis for abalone genetic breeding, which can provide scientific guidance for the cultivation of new high-yield and stress-resistant abalone varieties.

2 Variation Characteristics of Phenotypic Traits During Domestication

2.1 Variation of growth traits

Growth speed and size are the core traits that abalone breeding focuses on. During domestication, abalone populations from different sources and generations showed significant differences in growth traits. On the one hand, artificially selected breeding populations tend to grow faster and have larger individuals. For example, through family breeding, the shell length and weight heritability of South African abalone (*Haliotis midae*) is between 0.2 and 0.3, indicating that growth traits have moderate heritability and can be significantly improved through breeding. In actual breeding, South Africa has established a multi-generation family breeding program, and the growth rate of the third generation breeding group has increased by about 10 to 15% compared with the basic group. Similar results have been achieved in China's abalone breeding: through interspecies hybridization, the growth advantages of the "Green abalone (*Haliotis fulgens*)" produced are obvious, and the shell length growth rate is increased by more than 20% compared with the original varieties; the new "Fubao No. 1" breeding in Fujian is reported to grow at a rate of 30 to 50% faster than that of ordinary Ezo abalone. On the other hand, differences in growth performance among different domesticated populations are also related to genetic diversity. The study found that the individual growth of the trihybrid abalone population was highly differentiated, with the shell length of the largest individual and the smallest individual in the same population being nearly 3 times and the weight difference was 24 times, which suggests that there is significant separation of growth traits in the hybrid population. This phenomenon may originate from different combinations of growth-related genes produced by gene recombination after hybridization (Kijas et al., 2023).

2.2 Changes in stress resistance traits

Stress resistance mainly refers to the ability to resist disease and stress, including resistance to environmental stress such as pathogens, temperature, salinity, and hypoxia. The stress resistance traits of domesticated abalone population often undergo significant changes. On the one hand, artificial selection can significantly enhance the disease resistance of abalone (Luo et al., 2014). On the other hand, environmental stress tolerance can also be enhanced or weakened through domestication. Because the northern Ezo abalone adapts to cold water environment for a long time and has weak high temperature resistance, it is prone to large-scale deaths in summer water temperatures exceeding 24 ℃. By hybridizing Ezo abalone with other heat-resistant abalones, the cultured hybrids significantly increase the heat-tolerance threshold and can survive and grow normally in the southern summer. In addition, the Fujian scientific research team used the method of measuring heart rate and screening heat-resistant individuals, and selected individuals with stable response to high-temperature heart rate from the Ezo abalone population for breeding, and initially established high-temperature resistant strains. After two generations of selection, the survival rate of the obtained strains at high temperatures of 30°C was increased by about 25% compared with the unsuccessful population (Gan et al., 2021). However, it should be noted that excessive selection pressure may lead to a decrease in population genetic diversity, which affects the stable inheritance of stress resistance. In actual breeding, molecular marker assisted selection is often combined to maintain sufficient genetic diversity while improving stress resistance.

2.3 Adjustment of behavior and reproductive traits

The behavior and reproductive characteristics of abalone also change in artificial environments. Wild abalone often sticks to daytime and comes out to feed at night, and has the habit of getting dark and avoiding light; however, under long-term artificial domestication conditions, abalone's sensitivity to light and disturbance is reduced. It is reported that abalone gradually adapts to daytime feeding and increases daytime feeding in the breeding environment. The clustering and spawning behavior of abalone has changed in the domesticated population. Under wild conditions, abalone often lays eggs simultaneously under specific seasons and environmental stimulation, while artificial breeding groups may be selected and bred, and the breeding season may be extended or advanced. For example, the Nine-hole abalone (*Haliotis diversicolor*) line raised in indoor constant conditions has an increased number of annual reproduction in the gonad mature period earlier than the wild population. This may be related to artificial high nutritional feed supply and breeding. There are also studies that have observed changes in mate selection and mating behavior of domesticated abalone. In the wild, large male abalone often plays an advantage in reproduction, while in artificial groups, due to high density and controlled environment, small individuals also have the opportunity to reproduce, and the number of effective parents in the group increases. This is beneficial to maintaining genetic diversity to some extent. On the other hand, artificial domestication may have a dual impact on the fertility of abalone: First, good nutrition and environment improve the individual's absolute fertility (the amount of egg laying increases each time); but if the breeding of growth traits is overemphasized, there may be a trade-off effect of reducing reproductive investment, which needs to be avoided through balanced selection (Wang et al., 2019).

3 Genetic Basis Analysis of Trait Variation

3.1 Progress in genetic research on quantitative traits (QTL localization)

Important traits such as growth, survival, and stress resistance of abalone are often controlled by multiple genes and are quantitative traits. Through quantitative genetic analysis and QTL (quantitative trait localization) studies, the genetic basis of these traits can be analyzed and used for breeding. In recent years, many scholars have carried out family genetic assessment and linkage mapping research on the quantitative traits of abalone. In South African abalone (*H. midae*), Tshilate et al. (2024) constructed a high-density genetic linkage map, positioning 5 QTL sites affecting shell length, shell width and body weight, and the contribution rate of each QTL to phenotypic variation is 7.2%~11.4%. These QTLs are co-located on 18 linkage groups, and some sites have a pleiotropy effect on multiple traits. QTL was further localized to the genome, and several candidate genes were identified, such as *egf1*, *notch1*, *scube2*, etc., which were speculated to be related to the growth regulation of abalone. In Japanese Ezo abalone, early family selection experiments estimated that the heritability of growth traits was about 0.2~0.4, suggesting that breeding selection is feasible. Recently, mapping research based on high-throughput classification has been gradually carried out.

3.2 Screening and functional annotation of candidate genes

In addition to QTL studies based on linkage mapping, another idea is to screen candidate genes through comparative analysis, that is, to use omics data to find genes significantly related to trait variation and annotate them with functional annotations. Abalone genome and transcriptome data provide the basis for candidate gene screening. In recent years, genome sketches of multiple abalone species have been released one after another, such as Japanese abalone (*H. discuss hannai*), high-body abalone (*H. gigantea*), and South African abalone (*H. midae*), achieving gene prediction and annotation. These genomic data make it possible to screen candidate genes across the genome. For example, in view of the significant growth differentiation of hybrid abalone, Wang et al. (2023) localized GWAS-associated SNPs to genes based on the comparison of the transcriptomes of large and small individuals, and screened out 10 candidate genes closely related to growth differences. These genes include key regulators in pathways such as cell proliferation, development and energy metabolism, and are presumably involved in the differentiation of hybrid abalone growth phenotypes. Studies have shown that screening candidate genes through omics and combining functional analysis is an effective strategy to analyze the quantitative trait mechanism of abalone. In addition to growth, candidate genes for stress resistance traits have also been reported. Some scholars used transcriptome meta analysis of heat stress-responsive transcriptomes to identify 74 core heat response genes of abalone, including the heat shock protein family HSPs, the ubiquitin-proteasome system, etc., which were consistently upregulated in heat stresses in multiple species (Zhang et al., 2022). These genes have become important candidate targets for improving thermal resistance of abalone. Similarly, by comparing the gene expression of disease-resistant and susceptible lines, several immune-related candidate genes can also be screened out, such as pattern recognition receptors, antimicrobial peptides, etc.

3.3 Analysis of the association between phenotypic traits and genetic markers

Association analysis is a method to use natural variation at the population level to detect the association between traits and genotypes. In abalone, due to the lack of a large number of pure varieties and systematic breeding pedigrees, association analysis is usually based on high-density molecular marker data from population or family. High-throughput sequencing produces SNP markers are widely used in abalone association analysis and genome selection studies. Taking the thermal resistance trait as an example, Korean scholars genotyped a group of variegated abalone populations, and used GWAS analysis to detect multiple SNP sites related to high temperature resistance survival rates, which explained about 8% of the phenotypic variations in total (Lin et al., 2022). In addition to quantitative traits, qualitative traits such as shell color and markings were also correlated analysis in abalone. The membrane pattern separation of the tripartite hybrid abalone coat mentioned above, that is, through correlation analysis, it was found that a region on chromosome 15 had a very significant impact on the marking phenotype. This discovery enables subsequent use of molecular markers to predict shell color marks of hybrid progeny and improve breeding selection efficiency. In addition, association analysis is also applied to growth and reproductive traits.

4 Application of Abalone Genomics and Omics Tools

4.1 Current status of abalone genome sequencing and annotation

The genome of molluscs of abalone is relatively large and contains a high proportion of repeat sequences. However, the development of sequencing technology in recent years has promoted a breakthrough in sequencing of abalone genome. In 2017, researchers released the first genome sketch of *Haliotis discussion hannai*, with a total length of about 1.3 Gb and more than 23 000 genes encoding. Subsequently, the genomes of multiple species have been cracked one after another, especially in recent years, three-generation sequencing and Hi-C technology have been used to construct high-quality chromosomal-level abalone genomes (Figure 1) (Barkan et al., 2024). In terms of genomic annotation, a large number of genes and pathways related to traits are predicted in these sequences. Taking the Ezo abalone genome as an example, the annotation reveals that there are significantly amplified families of immune genes in its genome, such as detoxification enzymes, heat shock proteins, etc., which are related to the abalone's ability to adapt to environmental stress. The identification of candidate genes that affect shell color and growth (such as the *matrilin* gene associated with pearl formation) in the South African abalone genome provides clues to explain differences in morphological traits. It is worth mentioning that the Chinese scientific research team also conducted genome research on hybrid abalones. Zhao et al. used ternary hybrid abalone to construct the hybrid genome and compared the parent genome. They found that some fragments in the hybrid abalone genome have genetic contributions that tend to be biased towards a certain parent, which may explain the molecular basis of hybridization advantages (Zhao et al., 2023).

4.2 Application of transcriptomics in trait variation research

By analyzing gene expression profiles, transcriptomics can directly reflect the relationship between trait phenotype and gene expression regulation. In the study of abalone trait variation, transcriptome sequencing (RNA-Seq) has become a common tool. Many transcriptome studies targeting specific traits reveal key genes and pathways. For example, in order to understand the anti-thermal mechanism of abalone, there have been several transcriptome studies at home and abroad comparing the expression changes of abalone tissue before and after heat stress treatment. Tripp-Valdez et al. conducted integrated analysis of the heat-stress transcriptomes of 7 abalone species and 3 hybrid species, and identified 74 differentially expressed genes that recurred in at least 7 studies, among which the majority of genes in HSP, ubiquitin-proteasome and protein folding processing pathways. These core genes are considered conservative elements of abalone's heat stress response and have potential implications for improving heat resistance

(Valdez and Ángel, 2018). There are also a large number of transcriptome studies on immune and disease-resistant traits. Yang et al. (2019) analyzed the hematocrit of Nine-hole abalone under hypoxia stress and pathogen stimulation, and found that hypoxia will inhibit the expression of immune-related genes, causing abalone to develop an immunosuppressive state, specifically manifested as the downregulation of signaling pathways such as PI3K-AKT and NF-κB. Further RNAi interference of the *AKT* gene confirmed that downregulation of this gene will lead to reduced immune function.

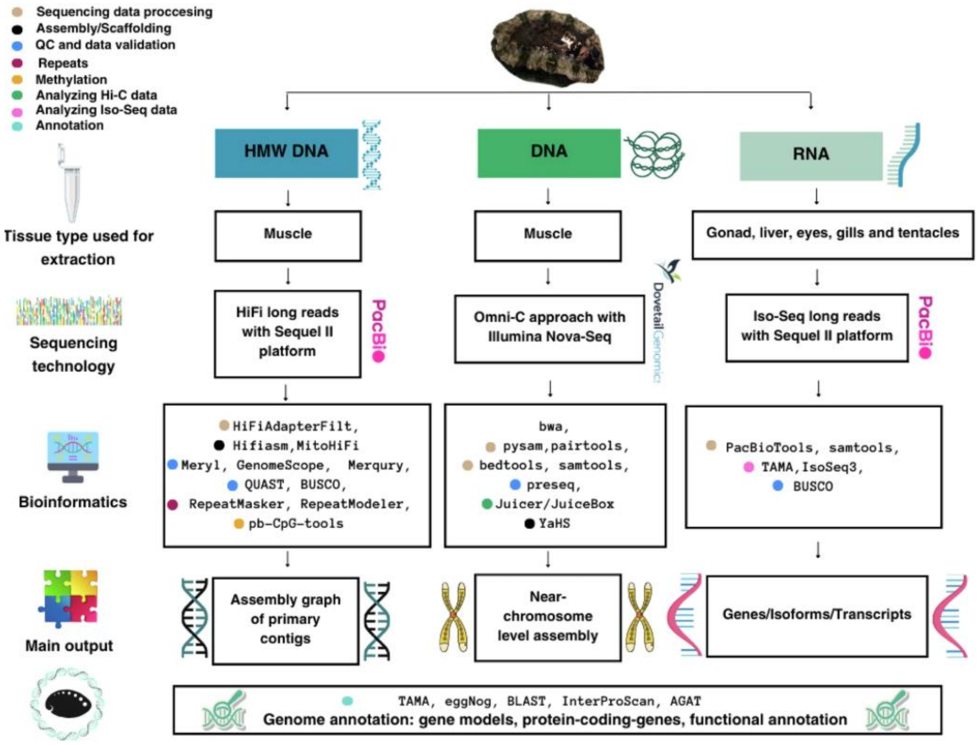

Figure 1 Chromosome-scale genome assembly of the tropical abalone (*Haliotis asinina*) (Adopted from Barkan et al., 2024)

4.3 Proteome and metabolomic data assisted genetic analysis

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5 Key Molecular Mechanisms and Signaling Pathways

5.1 Signal pathways that affect growth and metabolism (such as IGF, mTOR)

The growth and metabolism of abalone are regulated by multiple signaling pathways, among which the nutritional induction pathway represented by the IGF and mTOR pathways are the most critical. IGF signaling functions similarly to vertebrates in invertebrates, regulating individual growth and tissue development. Multiple IGF signaling component genes have been identified in abalone: such as IGF-I, IGF receptors and binding proteins. Among them, IGF binding protein-5 (IGFBP-5) is highly expressed in Ezo abalone nerves and gonads, and is believed to regulate growth and gonad development. When the *IGFBP-5* gene is knocked down, the growth of abalone embryos and larvae is inhibited, showing the importance of IGF signaling for the onogeny of abalone. The IGF signal activates the mTOR pathway through the downstream AKT. mTOR is a central regulator of cellular trophic and energy states that play a central role in abalone anabolic. Liu et al. (2021) found that arginine upregulates the activity of the abalone TOR pathway by activating the lysosomal membrane protein SLC38A9, promoting protein synthesis and growth. In addition, starvation and refeeding experiments show that the activity of the abalone mTOR pathway changes with the nutritional state. Short-term starvation will reduce the S6K phosphorylation level downstream of the TOR, inhibit anabolicity, and thus temporarily slow growth. After the resumption of feeding, mTOR is activated rapidly, cell proliferation and protein synthesis are accelerated, and compensatory growth is achieved. This shows that the mTOR pathway can affect the growth rate of abalone through nutritional regulation. In terms of energy metabolism, AMPK is a sensor of the energy state of cells, activated when energy is insufficient to inhibit synthesis and promote decomposition. Abalone is upregulated under stress such as hypoxia or hunger, thereby reducing growth-related energy consumption processes, which are manifested as growth stagnation. When the environment improves, the AMPK signal weakens and growth returns to normal.

5.2 Molecular networks that regulate immunity and stress resistance (such as NF-κB, HSP)

When abalone faces pathogenic and environmental stress, it activates a complex immune and anti-resistance molecular network, with the NF-κB signaling pathway and the heat shock protein (HSP) system being two key modules. NF-κB is the core transcription factor of the innate immune response, which can sense stimulation of bacterial lipopolysaccharides and induce downstream antibacterial gene expression. In abalone, multiple NF-κB pathway elements have been cloned, such as receptor TLR, adapter MyD88, IκB and NF-κB subunits. Studies have shown that the TLR/NF-κB pathway of abalone is rapidly activated in the face of pathogen infection, promoting the expression of immune effector molecules such as tumor necrosis factor and lysozyme, thereby improving the anti-infection ability of abalone. However, under environmental stress such as continuous high temperature or low oxygen, the NF-κB pathway response is blocked, and the expression of immune genes decreases, resulting in immunosuppression. The heat shock protein (HSP) family is another important molecular system for abalone to resist environmental stress. HSP includes molecular chaperones such as HSP70 and HSP90, which are highly expressed under stress such as heat stress and heavy metals, helping cells fold damaged proteins and prevent aggregation. When abalone is stimulated by high temperature, mRNA and protein levels of *HSP* genes (such as *HSP70*, *HSP90*) increase sharply (Yasa et al., 2020). Mediated by HSP, abalone cells tolerate higher temperatures and recover quickly after stress is relieved. It is worth noting that HSP expression itself is regulated by the heat shock transcription factor HSF1 (Zhang et al., 2020). In breeding practice, screening and retaining individuals with strong HSP response under heat stress is expected to cultivate high-temperature resistant strains.

5.3 Gene regulation mechanisms involved in reproductive regulation

The reproduction process of abalone is also finely regulated by genes and molecular pathways, including gonad development and maturation, gamete production and egg-laying behavior. The study found that multiple hormones and signaling factors are involved in this process. At the endocrine level, steroid hormones such as progesterone and estradiol may play a role in abalone gonad maturation, and their synthesis and effects involve a range of enzyme and receptor genes. At the same time, some neuropeptides and their receptors are upregulated in neural tissue during the abalone reproductive season, which is supposed to be a pro-reproductive signal. For example, GnRH-like peptides and dopamine may affect abalone gonad development and egg-laying behavior through the neuro-endocrine pathway. In addition to the hormone pathway, several specific genes have been shown to be closely related to the abalone reproductive process. IGF signal not only regulates growth, but also affects the reproductive axis. Studies have shown that the expression of IGFBP-5 in the abalone gonad maturation period is significantly increased, and it is speculated that it affects gametegenesis and maturation by regulating IGF accessibility (Bai et al., 2019).

6 Gene Editing and Functional Verification Technology

6.1 RNA interference and CRISPR technology attempts in abalone

To gain an in-depth understanding of the functional role of key genes on abalone traits, it is necessary to use functional genomic technology to manipulate and verify the target gene. Currently in abalone, RNA interference (RNAi) and CRISPR/Cas9 gene editing are the main attempts. RNA interference uses specific double-stranded RNA to induce gene silencing and has been successfully used in the study of abalone gene function. Zhang et al. (2020) efficiently silenced the HdHSF1 gene of Ezo abalone by injection of dsRNA, and observed significant changes in heat shock-related gene expression and heat-resistant phenotype, thereby verifying the function of HSF1 in abalone's anti-therm. The same method has been applied to the study of abalone *AKT* genes. After interference, the downstream immune genes of AKT are suppressed, confirming the key role of AKT in the immune pathway (Yao et al., 2025). The advantage of RNAi is that it is easy to operate and does not require genome change, but has limited maintenance time and is generally used for developmental stages or short-term trait analysis. Recently, the scientific research team of the Institute of Oceanography of the Chinese Academy of Sciences has made a breakthrough. The team established an improved abalone embryo semi-dry exposure microinjection technology, injecting Cas9 protein and gRNA into the Ezo abalone abalone fertilized egg, successfully knocking out the specific gene of the abalone and obtaining edited individuals that develop to the larval stage. They reportedly published this result in The CRISPR Journal, achieving the first accurate editing of the genome level of abalone species (Li et al., 2024). Although editing efficiency still needs to be improved, this attempt demonstrates that CRISPR technology can be used in abalone functional genome research.

6.2 Functional gene knockout/overexpression experimental cases

6.2.1 Knockout verification of genes related to growth regulation

Through genetic function research, several key genes that regulate abalone's growth have been identified and functionally verified. The mTOR pathway gene mentioned above is one of the hot topics of growth regulation. The study used RNAi to knock down the SLC38A9 transporter gene on the abalone mTOR pathway, which resulted in a significant reduction in the phosphorylation level of RPS6KP protein downstream of the TOR signal, and the abalone anabolic slowed down and the growth was inhibited. This functionally demonstrates that SLC38A9-mediated amino acid signaling is crucial for abalone growth. Similarly, genes on the IGF axis such as IGF1R receptor have also been verified by RNAi: interfering with IGF1R will reduce the growth rate of young abalone by about 15%, indicating that IGF signaling affects abalone growth by promoting cell proliferation (Li et al., 2021). In addition, some growth-related transcription factor genes such as Myostatin (myostatin) have also been studied in preliminary functional areas. Myostatin usually inhibits muscle growth. Researchers use antisense oligonucleotides to inhibit the expression of abalone Myostatin and found that the abalone foot muscle fiber diameter is significantly enlarged, consistent with other species, demonstrating that inhibition of Myostatin can help increase the meat production of abalone (Shiel et al., 2014). These functional validations provide gene targets for the next step of breeding.

6.2.2 Analysis of overexpression function of immune-related genes

In the field of immunity and disease resistance, some progress has been made in functional gene verification, but overexpression techniques are more challenging in abalone compared to knockout. Gene injection or transient expression of vectors is usually used to observe gene function. A successful case is the functional study of the abalone antimicrobial peptide *NDK* gene. The researchers constructed a eukaryotic expression vector of the abalone NDK gene and injected it into the abalone body cavity, and detected an increase in the content of NDK peptides in the abalone hemolymph. After artificial infection with the pathogen, the survival rate of abalone overexpressing NDK was 20% higher than that of the control group, proving that excessive NDK can enhance the antibacterial ability of abalone. Another example is the functional analysis of the heat shock protein HSP24. Some studies have increased the expression of endogenous HSP24 in abalone through heat induction, which is equivalent to a "overexpression" effect. It was found that the survival rate of pre-heat shock-treated abalone significantly increased the subsequent fatal high temperature stress ((Wan et al., 2012)). This functionally reflects the protective effect of small molecule heat shock proteins in anti-heat. Although this is strictly not a genetically modified overexpression, it provides functional evidence. To achieve true stable overexpression, strategies such as shellfish virus vectors can be used.

6.2.3 Exploration of the function of reproductive development regulatory genes

Compared with growth and immunity, the functional research on genes related to abalone reproduction is relatively weak, but there have been some explorations in recent years. To verify the role of the abalone IGFBP-5 gene mentioned above in reproduction, researchers conducted gene interference experiments. After knocking down IGFBP-5 in the neural tissue of female abalone, it was observed that the secretion of its gonadotropin mature hormone (presumably GnRH-like substance) was reduced, resulting in a delay in ovarian development by about 10 days, delaying egg laying than the normal group. This result supports a model of the role of IGF signaling in regulating gonad development (Wang et al., 2016). Similarly, functional studies have also been conducted for possible key hormone receptor genes in abalone reproduction, such as estrogen-associated receptor (*ERR*) genes. Some studies have used antagonists to inhibit ERR activity, which is equivalent to loss of function. It was found that the expression of yolk proteogen gene in abalone gonads has decreased and egg development is blocked, indicating that ERR signal may be involved in yolk synthesis and oocyte maturation. Furthermore, some studies have focused on meiotic regulatory genes during abalone gamete generation, such as *Cdc25*. Through drug inhibition of *Cdc25*, it was found that abalone oocytes were stagnant in the first premeiosis phase and could not mature, thus demonstrating the conservative role of cell cycle regulation in abalone egg maturation (Figure 2) (Ferencova et al., 2022). Although these functional studies are fragmented, they suggest several gene targets that can affect fertility.

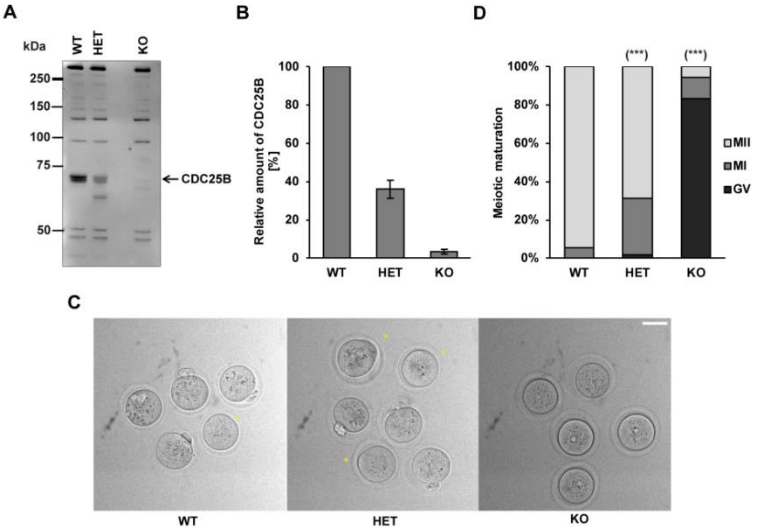

Figure 2 Cdc25b+/− oocytes resume meiosis but partially arrest in MI (Adopted from Ferencova et al., 2022)

6.3 Challenges and prospects in building a functional verification model system

Although technologies such as RNAi and CRISPR have been tried in abalone, there are still multiple challenges to establishing a mature functional gene verification model system. In terms of experimental materials: Abalone embryos and larvae are demanding and fragile to the environment, and it is not easy to obtain a large number of synchronously developed embryos for microscopy operations. Currently, most functional tests remain in the juvenile stage and have not developed a pattern that can cultivate edited individuals into adults and observe traits. Secondly, the abalone generation cycle is relatively long and generally has sexual maturity of 2 to 3 years, making it time-consuming and labor-intensive to establish a stable genetic modification genealogy. This is in sharp contrast to the rapid iteration of model organisms such as zebrafish and mice. Therefore, developing alternative models or cellular models of abalone may be one of the ways to improve validation efficiency. In addition, using close-range biological substitution is also one of the strategies. For example, bivalve shellfish such as scallops are more mature to edit genes, and some conservative gene functions can be verified and then deduced to abalone. The third challenge is the issue of gene manipulation efficiency and specificity. There is a situation where RNAi is away from the target and is not completely silent, and multiple sets of siRNAs need to be designed and combined with expression analysis to confirm the knockdown effect. CRISPR/Cas9 may be at risk of off-targeting, and highly efficient and specific gRNAs need to be screened and sequenced and verified (Li et al., 2024). At present, the large genome of abalone increases the possibility of off-target, and improving gene editing specificity is an important topic. In addition, polygenic traits often require multiple genes to change in synergistically in order to see significant changes in the phenotype, which poses a challenge to traditional single gene operation.

7 Actual Case Analysis

7.1 Research on disease-resistant breeding of *Haliotis discussion hannai*

Japanese abalone (i.e., Ezo abalone abalone, *Haliotis discuss hannai*) is an important breed of abalone in cold waters in East Asia. At the end of the 20th century, large-scale artificial breeding of wrinkled abalone was carried out in northern China and South Korea. However, bacterial and viral diseases that break out under intensive breeding conditions pose a serious threat to the industry. For example, Ezo abalone abalone furuncle disease and WS recovery syndrome have caused up to 30%~50% of farming losses. The representative of these is the cultivation of the disease-resistant abalone varieties of "Dongyou No. 1" in Fujian, China. This breed showed high resistance to major bacterial diseases in pathogen-challenged tests in laboratories and production ponds, with survival rates of 20~30% higher than that of ordinary strains under the same infection conditions. "Dongyou No. 1" passed the national variety approval in 2009, becoming the first national-level new aquatic product variety in Fujian Province. After its promotion, it significantly reduced the breeding mortality rate. In South Korea, researchers used family breeding to bind molecular markers to cultivate Ezo abalone abalone strains that are Abalone herpesvirus. By establishing multiple family lines of all siblings, they selected surviving individuals as their parents after artificially infected with the virus, and obtained disease-resistant strains with significantly improved survival rates through 2 generations. Their seedlings increased by about 15% compared with unselected seedlings in field trials (Figure 3) (Yasa et al., 2020). The case shows that through hybrid recombination and family selection, combined with artificial challenge screening, the disease resistance of abalone can be effectively improved.

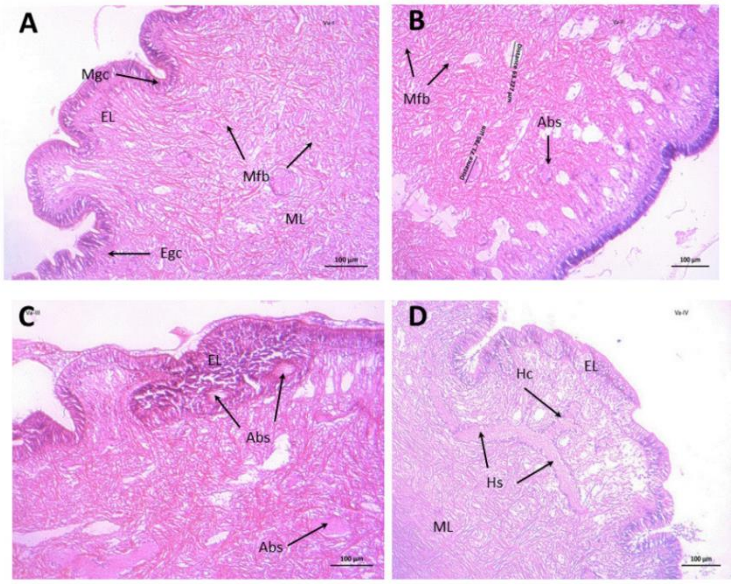

Figure 3 Histological section of abalone *H. squamata* foot (100× magnification). Control treatmen (A), infected with *V. alginolyticus* at concentrations of 1 × 105 cfu (B) 1 × 106 cfu (C) and 1 × 107cfu (D). Vacuolations, enlargement of hemolymph sinuses and abscesses werefound in infection treatments (Adopted from Yasa et al., 2020)

7.2 Multi-generation breeding program for *Haliotis midae*

South African pearl abalone (*Haliotis midae*) is the most important aquaculture shellfish in South Africa, and has achieved large-scale aquaculture since the 1990s. In order to improve its production efficiency, South Africa has implemented a systematic genetic improvement plan since the beginning of the 21st century. The plan is promoted by scientific research institutions and breeding enterprises, including establishing breeding family lines, estimating genetic parameters, and selecting parents with excellent performance to breed the next generation. The specific method is to select several pairs of pro-agrants from the breeding population every year for single-pair mating, and establish dozens of family lines of all compatriots. After the family seedlings were independently cultivated to a certain size, the shell length and weight were measured, and survival and growth performance were evaluated under the same conditions. BLUP and other methods were used to estimate the breeding values of each family, and based on this, several best families and individuals were selected as parent groups for the next generation (Difford et al., 2017). Since 2002, the South African abalone breeding program has been continuously selected and bred for more than 5 generations. Breeding results show that the growth traits of abalone have made significant progress: the average shell length and weight of the 4th generation breeding population increased by about 20% compared with the basal population, and the growth cycle was shortened by 4-6 months. At the same time, the feed conversion efficiency has also been improved, and the feed consumption per unit of weight gain has been reduced by about 10%. In terms of survival rate, although there is a large natural variation, the overall survival rate has also increased after several generations by eliminating early high death families. Genetic parameters study shows that the heritability of the shell length, width and weight of South Africa's abalone shell is between 0.2 and 0.25, and the survival rate is only about 0.1. However, in high-density breeding environment, the survival rate is positively correlated with growth traits, that is, the survival rate of faster-growing families is often higher. This provides convenience for both survival in choice. Breeding programs in South Africa also pay special attention to the application of molecular markers. Since the 2010s, researchers have developed microsatellite and SNP markers of South African abalone and used for family paternity testing and genetic diversity monitoring. The results show that through reasonable selection and large group breeding, sufficient genetic variation in the population can be maintained while improving the traits.

7.3 Multiomics study on tropical hybrid abalone in Hainan, China

One of China's innovations in abalone breeding is to cultivate tropical abalone varieties that are adapted to the high-temperature seas in the south. Traditional wrinkle plate abalone likes cold water and is not resistant to high temperatures, while tropical nine-hole abalone is small in individual and grows slowly. In order to take into account both large individuals and heat resistance, researchers carried out interspecies hybridization of abalone and created tropical hybrid abalone varieties. The most representative one is the "rebeaten No. 1" hybrid abalone (also known as "hybrid abalone I") cultivated by the Nanhai Institute of Oceanography, Chinese Academy of Sciences and other units. Its parent is a hybrid combination of female Ezo abalone × male variegated abalone. This hybrid abalone has the advantages of patriarchal heat resistance and maternal high yield. It can grow and reproduce normally in Hainan sea with high annual average water temperature, and is significantly improved compared with purebred wrinkled abalone. It is reported that the survival rate of "Reap Abalone No. 1" in the second half of the year under the water temperature of Hainan in summer was more than 90%, while the wrinkled abalone is less than 50%. In order to gain an in-depth understanding of the excellent trait mechanism of this hybrid abalone, researchers conducted a multiomic joint analysis of it. First of all at the genomic level, the genomic mutations of hybrid abalone and parents were compared using high-deep sequencing. It was found that most of the alleles of parents were preserved in the hybrid abalone genome and showed heterozygous advantages in the heat tolerance-related gene regions. For example, a hybrid abalone associated with HSP70 regulation simultaneously possesses different parental variants, which may confer stronger environmental resilience (Zhao et al., 2023). Next, in terms of the transcriptome, the differences in gene expression between hybrid abalone and purebred Ezo abalone under high temperature stress were compared. The results showed that the number of genes induced by hybrid abalone heat stress was greater, especially the gene expression level of the heat shock protein family was about 1.5 times that of purebred. This shows that hybrid abalone is more sensitive in molecular stress response and helps the formation of its heat-resistant phenotype (Zhang et al., 2022). Furthermore, metabolomic analysis also provides evidence. Hybrid abalone accumulates a large amount of metabolites such as lactic acid and succinic acid under high temperature stress, indicating that it maintains energy supply by enhancing anaerobic metabolism, while purebred wrinkle disc abalone has a collapse in energy metabolism and a large amount of pyruvate appears. These multiomic results are consistently pointing to: hybrid abalones can combine parental advantages to mobilize more effective molecular responses under stress conditions, thereby showing stress resistance and growth performance better than parents.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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