Vol-3 Issue-1 2025
Scientific Research Journal of Biology and Life Science
ISSN: 2584-0606, Peer Reviewed Journal

Genetic Mechanisms of Pigment Synthesis Pathways in Fish and Crustaceans

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Abstract:
The pigmentation of fish and crustaceans constitutes a vital component of aquatic ecosystems. A thorough understanding of the genetic mechanisms that underpin the synthesis pathways of these pigments is essential. This review presents a comprehensive analysis of the gene and genome duplications that have been identified as significant factors influencing the evolutionary trajectories of pigment production pathways in both fish and crustaceans. Through a systematic examination of the genes, enzymes, and regulatory networks involved in pigment production, researchers have elucidated the intricate processes that govern the development, arrangement, and expression of pigmentation traits in various fish and crustacean species. The evolution of these pigment production pathways has revealed the intricate composition of coloration in fish and crustaceans, which encompasses a diverse array of pigments, including structural colors, carotenoids, and melanins. Furthermore, the application of machine learning algorithms and network analysis techniques offers a valuable approach to enhancing the understanding of the cellular dynamics and interactions that influence pigmentation in these organisms. The insights gained from this research may have significant implications for improving the sustainability of aquaculture, informing conservation strategies, and fostering advancements in biotechnology and biomimicry.

Keywords: *Genetic mechanisms, pigment synthesis, chromatophores, gene expression, pigmentation pathways*

Introduction

The diverse and vibrant colors exhibited by fish and crustacean species have long captivated both scientists and enthusiasts. The coloration of fish and crustaceans serves multiple functions crucial to their survival, reproduction, and communication within their surroundings (Ertl et al., 2013). Examples of these functions include the cryptic patterns observed in bottom-dwelling species, as well as the iridescent hues characteristic of tropical reef fish and crustaceans. A comprehensive understanding of the origins of this remarkable diversity necessitates an examination of the genetic factors that regulate the evolution of pigment production pathways in both fish and crustaceans (Irion & Nüsslein-Volhard, 2019). Gene and genome duplications have been recognized as pivotal factors influencing the evolutionary trajectories of pigment production pathways in fish and crustaceans (I. Braasch, M. Schartl, & J.-N. Volff, 2007). These systems facilitate adaptive responses to changing ecological constraints and selection pressures by providing the genetic building blocks necessary for the development of novel traits. Fish and crustaceans have broadened their repertoire of color-producing molecules by acquiring duplicate copies of genes that encode pigment-synthesizing enzymes through the process of gene duplication. Moreover, polyploidy, or whole-genome duplication events, has occurred throughout the evolutionary history of fish and crustaceans, creating additional opportunities for genetic variety and innovation. The functional diversity of duplicated genes is influenced by a series of evolutionary processes known as sub functionalization and neofunctionalization, which are often initiated by gene duplication events (Kuzmin, Taylor, & Boone, 2022). Subfunctionalization involves the partitioning of ancestral gene functions between duplicate copies (Kuzmin et al., 2022). while

neofunctionalization results in the acquisition of novel functions by one of the gene duplicates (Kuzmin et al., 2022). These processes enable fish and crustaceans to adapt to a diverse array of biological niches and visual environments, thereby enhancing specialization and refinement of their pigment production pathways (Behringer & Duermit-Moreau, 2021). Furthermore, the regulation of gene expression involved in pigment synthesis is significantly influenced by evolutionary dynamics of regulatory elements. Modification to the cis-regulatory regions that govern gene expression can alter pigment patterns, even when the underlying enzymatic machinery remains unaltered (Dsilva & Galande, 2024). The remarkable diversity in coloration observed in fish and crustaceans can be attributed to the evolutionary changes that duplicated regulatory components, such as enhancers and transcription factor binding sites, may undergo. These alterations result in variations in gene expression patterns.

This review aims to provide a comprehensive overview of the genetic mechanisms underlying the evolution of pigment synthesis pathways in fish and crustaceans, specifically focusing on gene and genome duplication. By synthesizing evidence from molecular genetics, comparative genomics, and evolutionary biology, we seek to elucidate the intricate processes driving the emergence and diversification of coloration in these organisms. A deeper understanding of these genetic mechanisms will offer insights into the origins of biodiversity and the adaptive strategies that enable fish and crustaceans to thrive in various aquatic ecosystems.

Genetic Basis of Pigment Synthesis in Fish and Crustaceans

Fish and crustaceans possess four types of chromatophores: melanophores, xanthophores, iridophores, and leucophores (Schartl et al., 2016). The principal pigments found within those chromatophores include eumelanin in melanophores, pteridines and carotenoids in xanthophores, guanine in iridophores, and uric acid in leucophores. With the exception of carotenoids, which are obtained from dietary sources, these pigments are primarily synthesized within fish and crustaceans. Specifically, pteridine, guanine, and uric acid are derived from purines, while eumelanin is synthesized from tyrosine. Purines are produced either through de novo synthesis or recycled via the salvage pathway.

De Novo Purine Synthesis

One of the primary metabolic pathways involved in the synthesis of purine nucleotides, which are essential building blocks of nucleic acids such as DNA and RNA, is known as de novo purine synthesis. Purines play a critical role in various cellular functions, including signaling, energy metabolism, and the transfer and storage of genetic information. Consequently, the de novo synthesis of purines is subject to stringent regulatory mechanisms and is highly conserved across all domains of life. This pathway is particularly important in embryonic development, where it contributes significantly to pigmentation. Notably, mutations in enzymes associated with de novo purine synthesis lead to defects in pigmentation (Ng, Uribe, Yieh, Nuckels, & Gross, 2009). In contrast, mutants affecting de novo pyrimidine synthesis exhibit normal pigmentation (Willer, Lee, Gregg, & Link, 2005).

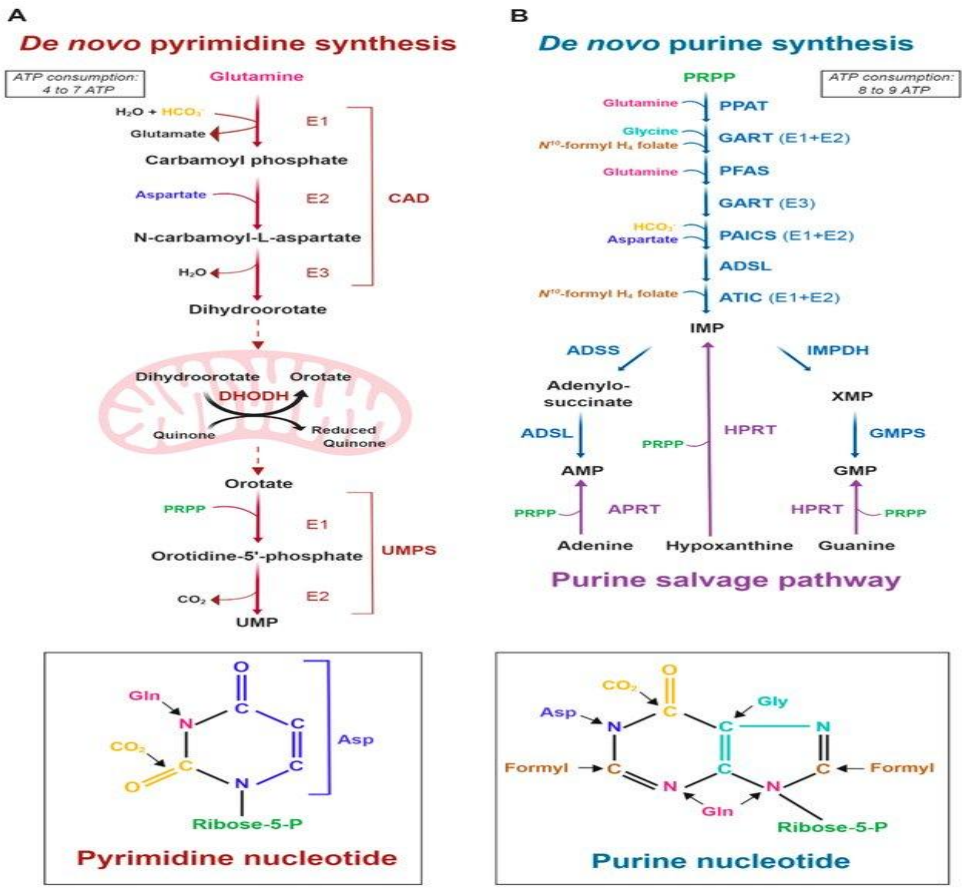

Fig. 1. The de novo purine synthesis pathway. IMP is synthesized from PRPP (5-phospho-D-ribosyl-1-pyrophosphate) via multiple enzymatic pathways, in which gart and paics proteins (green) catalyze multiple steps (Ng et al., 2009).

Phosphoribosyl diphosphate serves as the initial substrate for the de novo synthesis of purine, leading to the production of purine nucleotides, specifically adenosine monophosphate (AMP) and guanosine monophosphate (GMP). Within the de novo purine synthesis process, inosine monophosphate (IMP) acts as a critical intermediate at a branch point (Fig. 1). The pathway diverges from IMP to form AMP and GMP. In the de novo synthesis of IMP, which serves as the precursor for both AMP and GMP, the enzymes gart and paics are essential. Phosphoribosylglycinamide synthetase, phosphoribosylglycinamide formyl transferase, and phosphoribosylaminoimidazole synthetase are encoded by the gart genes, whereas the paics gene encodes phosphoribosylaminoimidazole carboxylase and phosphoribosylaminoimidazole succinocarboxamide synthetase. Mutations in gart and paics are associated with defects in pigmentation and microphthalmia. Nevertheless, some homozygous mutants of paics and gart can survive to adulthood, and their pigmentation presents a phenotype that closely resembles that of the wild type. It is probable that both the nucleotide salvage pathway and the de novo purine synthesis pathway play crucial roles in adult pigmentation. Research utilizing two morpholino antisense oligos (MO) targeting GMP synthase (gmps) and adenylosuccinate synthase (adss) indicates that the GTP pathway is not necessary for pigmentation, whereas the ATP pathway is essential (Tetsuaki Kimura, 2021). When gmps is downregulated, pigmentation deficiencies in xanthophores and iridophores were observed. Conversely, knocking down adss does not result in pigmentation issues; rather, only eye defects become evident. Furthermore, nearly all xanthophore and iridophore pigmentation is lost in the presence of morpholino targeting IMP dehydrogenase 1a (impdh1a).

Melanophore Pigment

Melanin, the pigment responsible for the diverse array of colours and patterns observed in various species, is produced by melanophores, which are specialized pigment cells located in the skin of fish and crustaceans. Within these melanophores, melanosomes, specialized organelles, are abundant and play a critical role in the synthesis of melanin. The intricate process of melanin production involves several biochemical reactions, including the conversion of tyrosine into dopaquinone, a precursor to melanin, followed by the polymerization of dopaquinone to produce either eumelanin or pheomelanin.

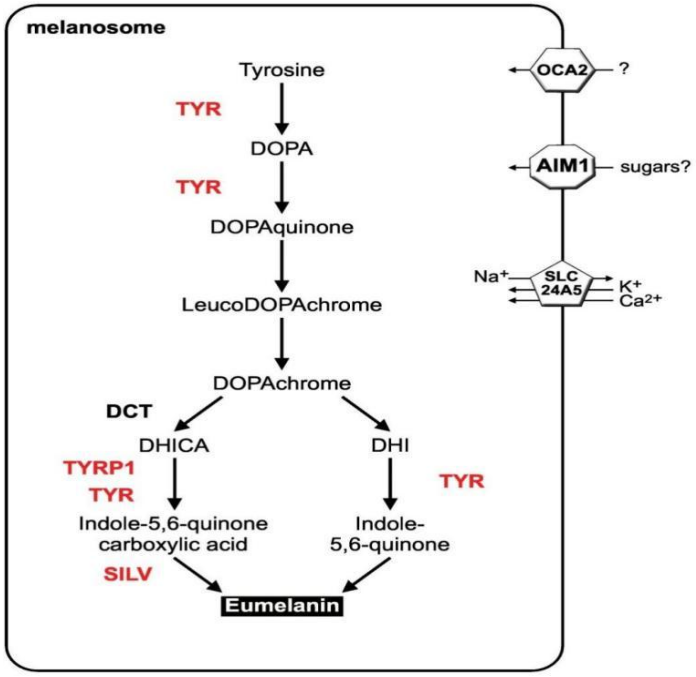

Fig. 2. Eumelanin synthesis pathway in fish. Eumelanin is made from tyrosine by an enzymatic process in the melanosome. OCA2 and SLC45A2 transport the substrates of eumelanin. SLC24A5 is an anion transporter and is dispensable for tyrosinase activity. Enzymes are shown in red (I. Braasch, M. Schartl, & J. N. Volff, 2007).

Iridophore Pigments

Fish and crustaceans possess specialized pigment cells known as iridophores, which are characterized by a range of reflective properties that impart an iridescent appearance (Frohnhöfer, Krauss, Maischein, & Nüsslein-Volhard, 2013). These structures exhibit remarkable optical characteristics that refract and scatter light, resulting in a dazzling spectrum of colors that shift and shimmer in response to variations in viewing angle and ambient lighting conditions. Iridophores are typically composed of crystalline arrangements of guanine or other reflective compounds. They play a significant role in the vibrant coloration of fish and crustaceans, contributing to some of the most striking and iridescent hues observed in aquatic environments. In contrast to traditional pigments that are derived from biological processes, iridophores produce colour through complex structural mechanisms that manipulate light to create shimmering iridescent displays. There are two distinct pathways for guanine synthesis: one that initiates from IMP and another that begins with GTP. The first pathway involves the conversion of IMP to xanthosine monophosphate (XMP) by IMP dehydrogenase, followed by the conversion of XMP to guanosine monophosphate (GMP) by GMP synthase, the dephosphorylation of GMP to guanosine by 50'-nucleotidase, and the subsequent conversion of guanosine to guanine by polynucleotide phosphorylase (PNPase). In the second pathway, ribonucleoside-triphosphate reductase reduces GTP to deoxyguanosine triphosphate (dGTP), which is then hydrolyzed to deoxyguanosine by dGTPase, with PNPase facilitating the conversion of deoxyguanosine to guanosine. Thus, PNPase is essential to the pathway leading to guanine production (Tetsuaki Kimura, 2021).

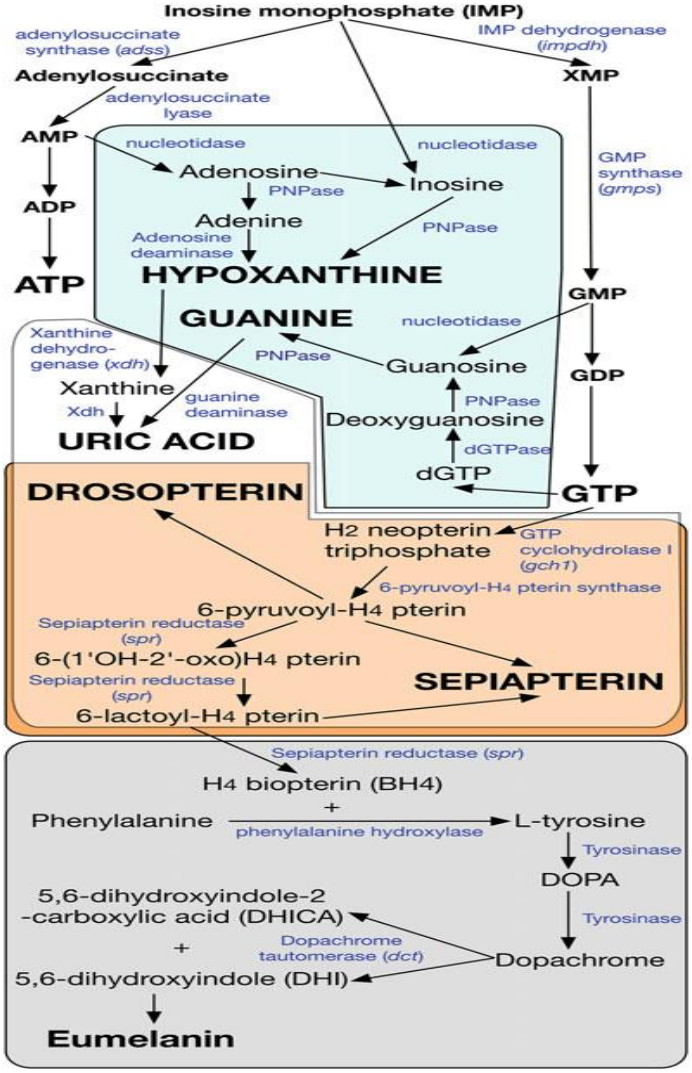

Fig. 3. Fish and crustacean embryonic pigment production mechanism. IMP is used to make GTP and ATP. Pigment cells share the process of converting purines into pigment. Red represents the enzymes. Iridophore is indicated by the gray backdrop, leucophore by white, xanthophore by yellow, and melanophore by black. Fish and crustacean leucophores possess white and yellow pigments throughout the embryonic stage due to their xanthophore characteristics.

Xanthophore Pigment

Specialized pigment cells known as xanthophores are found in a variety of organisms, most prominently in fish, amphibians, reptiles, and certain invertebrates such as crustaceans. These cells are responsible for the synthesis and storage of xanthophore pigments, which exhibit a color range from yellow to orange (Parichy, 2021). The term "xanthophore" is derived from the Greek words "xanthos," which means yellow, and "phoros," which means bearer or carrier, aptly reflecting their primary function. Xanthophores contain pteridines and carotenoids, which serve as yellow and orange pigments.

Pteridine Synthesis

Pteridines are synthesized from GTP and are stored within the pteridine-containing organelle known as pterinosome (Tetsuaki Kimura, 2021). The synthesis of pteridines encompasses three interconnected pathways: the regeneration of tetrahydrobiopterin (BH4), the production of yellow pteridine pigment, and the de novo synthesis of BH4 from GTP. The initial and rate-limiting step of pteridine production during BH4 de novo synthesis is catalyzed by GTP cyclohydrolase 1 (Gch1). The activity of Gch1 is regulated by a feedback regulatory protein (Gchfr), which is dependent on H4biopterin. Subsequent

steps in pteridine synthesis are catalyzed by sepiapterin reductase (Spr) and 6-pyrovoyltetrahydroptein synthase (Spr). The final three stages in the synthesis of BH4 are also catalyzed by Spr. During the regeneration of BH4, dihydropteridine reductase (Dhpr) and pterin-4 alpha-carbinolamine dehydratase (Pcbd), which functions as a dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1), facilitate the reaction. Specifically, Pcbd dehydrates H4biopterin-4acarbolamine to form qH2biptrerin, which is subsequently reduced to BH4 by Dhper. Additionally, the formation of pteridine pigments is catalyzed by Spr and xanthine oxidase/xanthine dehydrogenase (Xod/Xdh).

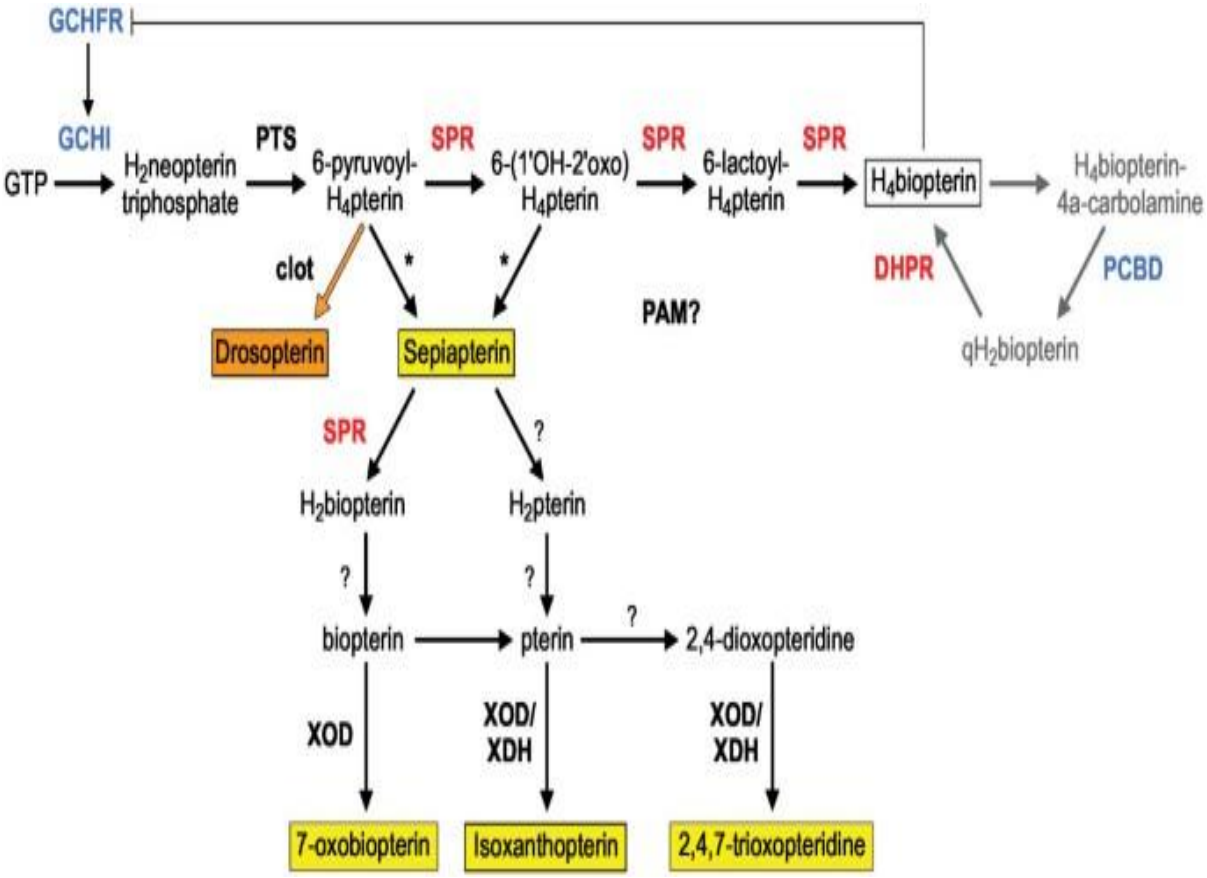

Fig. 4. Pteridine synthesis pathway

Carotenoids

In animals, carotenoids function as essential dietary components or are synthesized endogenously, playing a crucial role in producing the vibrant colors observed in feathers, skin, scales, and other tissues. The remarkable diversity of coloration seen in birds, fish, reptiles, crustaceans, and insects frequently arises from the presence and distribution of various carotenoid compounds (Tetsuaki Kimura, 2021).

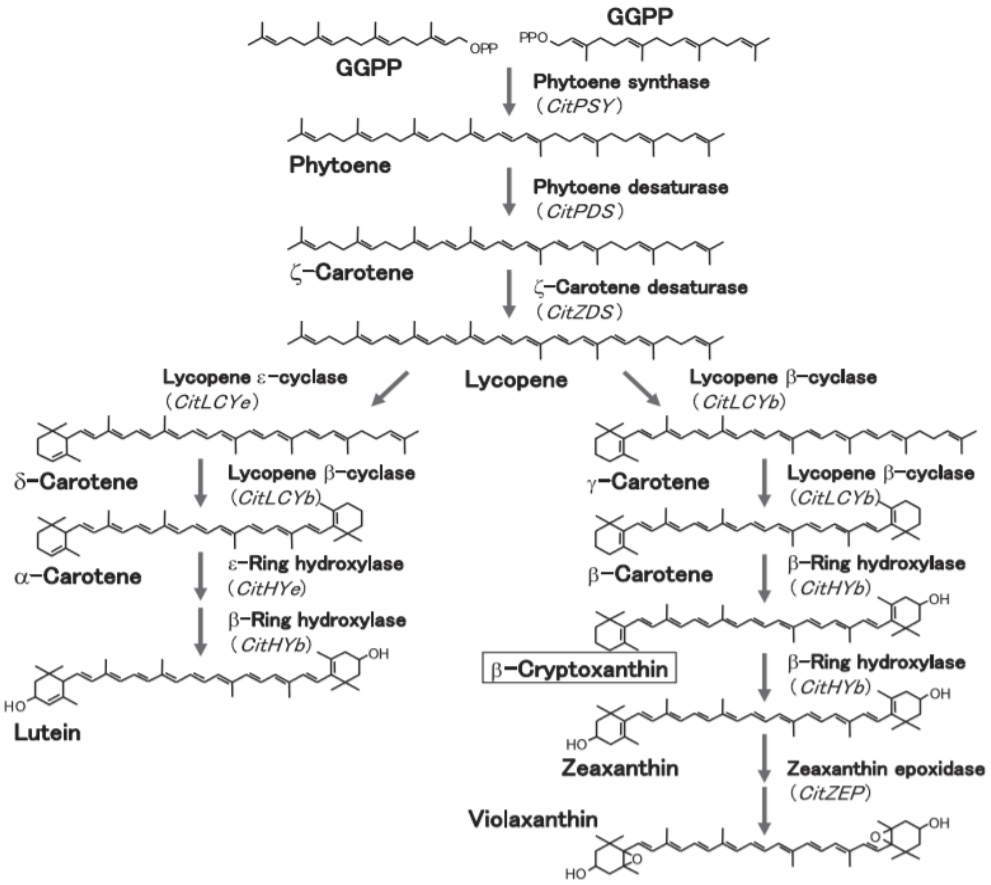

Fig. 5. Carotenoid biosynthetic pathway in plants. GGPP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; Ɛ-LCY, lycopene Ɛ-cyclase; β-LCY, lycopene β-cyclase; β-CHX, β-carotene hydroxylase; Ɛ- CHX, β-carotene hydroxylase; ZEP, zeaxanthin epoxidase.

Vertebrates are incapable of synthesizing carotenoids; instead, fish and crustaceans obtain these compounds through their diet. In fish and crustaceans, xanthophores contain yellow pigments known as carotenoids. Typically, carotenoid pigments are stored in lipid-rich xanthophore droplets. Thus, carotenoids are internalized, deposited, and metabolized following diet intake. Due to their hydrophobic nature, carotenoids are transported to xanthophores via lipoprotein in the blood stream. According to (Toews, Hofmeister, & Taylor, 2017), scavenger receptors identify these lipoproteins and facilitate the transport of carotenoids into xanthophores. In mutants of the scarb1 (scavenger receptor class B, member 1) in fish and crustaceans, the characteristic yellow hue of xanthophores is diminished (Liang et al., 2025). Additionally, the gene perilipin 6 (plin6) has been implicated in carotenoid transport and deposition (Granneman et al., 2017). Plin6 is responsible for transporting and concentrating carotenoids within lipids. Fish and crustaceans lacking plin6 exhibit a significantly lower concentration of carotenoids. β-carotene oxygenases are responsible for the cleavage of carotenoids in vertebrates. β-carotene oxygenase 1 (BCO1) plays a critical role in the visual system. Mutations in BCO2 in sheep have been shown to affect the accumulation of antioxidants in adipose tissue (Våge & Boman, 2010). In fish and crustaceans, the orthologs bco1 and bco2b are expressed in xanthophores (Saunders et al., 2019). This suggests that these genes are associated with the ability of xanthophores to synthesize yellow pigments from dietary β-carotene.

Leucophore pigments

Uric acid-filled white granules are present in leucophores (Goda, Miyagi, Kitamoto, Kondo, & Hashimoto, 2023). During the larval stage, leucophores contain orange pigments. Similar to xanthophores, these orange pigments are found in drosopterinosomes, which also contain red pigments such as drosopterin, isodrosopterin, and neodrosopterin (Tetsuaki Kimura, 2021). As they mature from larvae to adulthood, leucophores shed their orange pigments, resulting in a white coloration. (T. Kimura et al., 2014) reported a leucophore pigmentation mutant designated "wl" (white leucophore), in which slc2a11b is identified as the causative agent. This mutant exhibits white leucophores and an absence of yellow xanthophores from the embryonic stage through the early larval stage. In contrast, adult wl mutants possess xanthophores that are orange in color. Thus, it is likely that leucophores experience only a loss of pteridines rather than a transition of pigment from pteridines to carotenoids, considering that xanthophores and leucophores share a common precursor. Research conducted by Lewis and colleagues demonstrated that in the adult fish and crustacean species, two distinct forms of leucophores exist: melanoleucophores and xantholeucophores (Lewis et al., 2019). Melanoleucophores directly originate from melanophores, while xantholeucophores arise from cells that resemble xanthophores. Both types of leucophores can be found in the adult unpaired fins. Xantholeucophores contain carotenoids and pteridines, exhibiting orange pigments similar to those of xanthophores. Furthermore, xantholeucophores are characterized by the presence of pterinosome-like organelles and carotenoid vesicles.

Regulatory Elements and Transcription Factors in Pigment Gene Expression

The expression of pigment genes in organisms such as those responsible for synthesizing carotenoids, melanin, and other pigments is meticulously regulated by a complex network of regulatory elements and transcription factors. These regulatory components are essential for maintaining appropriate pigmentation through development, responding to environmental cues, and initiating physiological reactions to both internal and external stimuli. Additionally, they play a critical role in modifying the spatial and temporal expression patterns of pigment gene expression.

Promoter Regions and Enhancers

Specific genomic sequences known as enhancers and promoter regions adjacent to pigment genes function as docking sites for transcription factors and other regulatory proteins (Panigrahi & O'Malley, 2021). Consensus sequences located within promoter regions are typically recognized by general transcription factors, which initiate the recruitment of RNA polymerase and the assembly of the transcriptional machinery. In contrast, enhancers may reside at a considerable distance from the genes they regulate; they can engage in DNA looping interactions with promoter regions to facilitate the recruitment of transcriptional activators or co-activators.

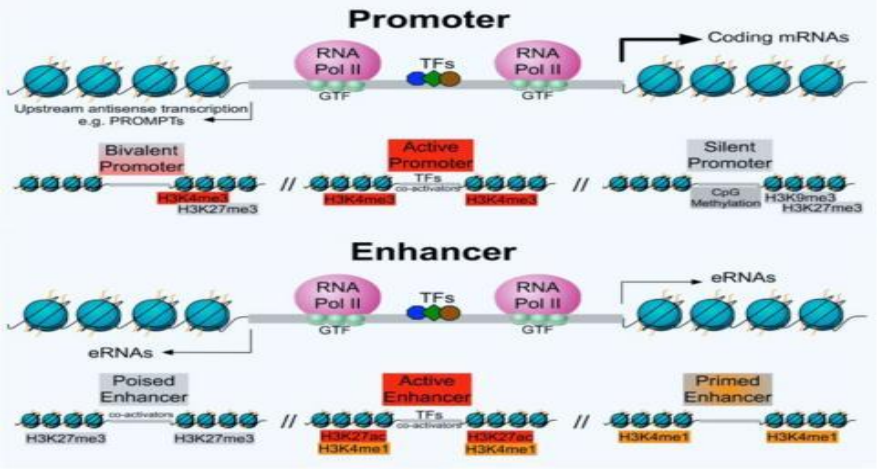

Fig. 6. Promoters and Enhancers Relationship

Transcription Factors

Transcription factors are proteins that bind to specific DNA sequences within regulatory regions to either activate or repress gene expression (Weidemüller, Kholmatov, Petsalaki, & Zaugg, 2021). In the context of pigment gene regulation, transcription factors such as the melanocyte-specific transcription factor MITF (microphthalmia-associated transcription factor) play pivotal roles in coordinating the expression of melanogenic enzymes involved in melanin synthesis. MITF regulates the transcription of genes encoding tyrosinase, tyrosinase-related protein 1 (TRP-1), and dopachrome tautomerase (DCT), among others, by binding to conserved DNA motifs within their promoter regions.

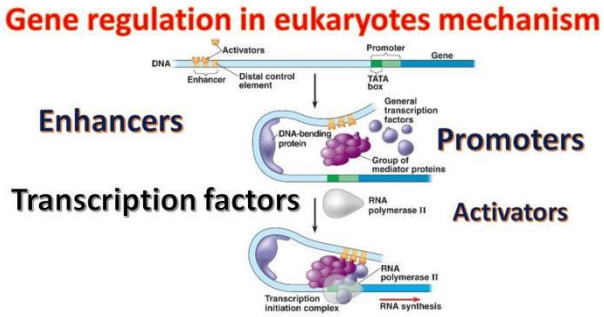

Fig. 7. Transcription factor binding

Signaling Pathways Environmental Stimuli

Signaling pathways initiated in response to developmental cues, hormone signals, cellular stress, or environmental stimuli can significantly influence the expression of pigment genes. For example, melanocyte-stimulating hormone (MSH) and other hormonal signals activate the cyclic AMP (cAMP) signaling pathway, which subsequently enhances melanogenesis in melanocytes by promoting the expression of MITF (Rodríguez & Setaluri, 2014). Similarly, environmental stimuli such as ultraviolet (UV) radiation can induce the expression of melanogenic enzymes by generating reactive oxygen species (ROS) and activating DNA damage response pathways.

Fig. 8. Environmental signaling pathways with external cues and internal repercussions.

Genes for Enzymes Involved in Pigment Synthesis

Table 1. Genes for Enzymes Used in Pigment Synthesis

Gene name	Abbreviation	Pathway
Tyrosinase	TYR	Melanin Synthesis
Tyrosinase-related protein	TYRP	Melanin Synthesis
GTP Cyclohydrolase	GCH	Pteridine Synthesis
Sepiapterin reductase	SPR	Pteridine Synthesis
Xanthine Dehydrogenase	XDH	Pteridine Synthesis
Beta-Carotene Oxygenase	BCO2	Carotenoid Synthesis

Dopachrome Tautomerase	DCT	Melanin Synthesis
IMP Dehydrolase	Impgh	Guanine synthesis (iridophore)
GMP Synthase	Gmps	Guanine synthesis (iridophore)
Carotenoid Oxygenases	CO	Carotenoid Synthesis
Dihydropteridine reductase	DHPR	Pteridine Synthesis
Pterine-4a-Carbinolamine Dehydratase	PCD	Pteridine Synthesis
Polynucleotide phosphorylase	PNPase	Guanine synthesis (iridophore)
Melanocortin 1 receptor	**MC1R**	Melanin Synthesis
Phytoene synthase	**PSY**	Carotenoid Synthesis
Phytoene desaturase	**PDS**	Carotenoid Synthesis
Carotenoids isomerase	**CRTISO**	Carotenoid Synthesis

Gene Expression Regulation in Response to Environmental Cues

The ability of living organisms to regulate gene expression in response to a variety of external stimuli is vital for their adaptation and survival within their environments. Environmental cues encompass a wide range of factors, including light, temperature, nutrition, infections, toxins, and social interactions (Li, Yang, Li, Zhu, & Li, 2021). The dynamic interaction between these stimuli and an organism's genetic composition shapes its phenotype and behavior. The processes by which information encoded in a DNA sequence is selectively transcribed into RNA and subsequently translated into proteins are collectively referred to as the regulation of gene expression. Environmental cues significantly influence this regulatory framework at multiple levels, allowing organisms to swiftly and selectively modify patterns of gene expression. This adaptive flexibility is essential for maintaining homeostasis, optimizing physiological responses, and adapting to changing environmental conditions. Environmental cues can influence both the activity and abundance of transcription factors, which are proteins that bind to specific DNA sequences to regulate the initiation of transcription. By interacting with enhancers or promoters, transcription factors can either activate or repress the expression of target genes in response to these environmental signals. Moreover, environmental factors can induce alterations in chromatin structure through epigenetic modifications, including DNA methylation, histone acetylation, and chromatin remodeling. These modifications alter the accessibility of DNA to the transcriptional machinery, thereby influencing gene expression patterns without altering the underlying DNA sequence. Environmental cues can affect the expression of small non-coding RNAs, such as microRNAs (miRNAs), which regulate gene expression post-transcriptionally by binding to target mRNAs and inhibiting their translation or promoting their degradation. Variations in environmental conditions can also influence the activity and abundance of RNA-binding proteins, which modulate mRNA stability, localization, and translation efficiency through interactions with specific RNA sequences. Furthermore, environmental signals can initiate various post-translational modifications of proteins, including phosphorylation, acetylation, ubiquitination, and proteolytic cleavage. These modifications can influence protein activity, stability, subcellular localization, and interactions with other molecules, thereby affecting cellular responses to environmental cues. Cells detect environmental signals through specialized receptors located on their surfaces, including G protein-coupled receptors and receptor tyrosine kinases. The activation of these receptors initiates intracellular signaling cascades that ultimately lead to changes in gene expression via transcription factors or other regulatory molecules. Environmental stimuli can also trigger the production of second messengers, such as cyclic AMP (cAMP) or calcium ions, which transmit signals to downstream effectors and regulate gene expression through various signaling pathways.

Pigmentation of fish and crustaceans, defined as the patterns of color exhibited by various species within these groups, represents a compelling area of research that encompasses both genetic diversity and adaptability (Fujii, 2000). The coloration of fish and crustaceans is critically important to their survival

in aquatic environments for several reasons. These include the ability to camouflage and evade predators or to ambush prey, facilitate communication with conspecifics for mating or establishing social hierarchies, regulate body temperature through the reflection or absorption of sunlight, provide protection against ultraviolet (UV) radiation, and attract mates through visually striking displays. The diverse pigmentation patterns observed in fish or crustaceans reflect their interactions with both their environment and their fellow species. Fish and crustaceans exhibit complex coloration resulting from intricate interaction among genetic factors. The coloration patterns of these organisms are predominantly determined by genes that regulate pigment synthesis, transport, and distribution. For instance, dark pigments such as eumelanin and pheomelanin are produced in response to regulation of genes that encode melanin-producing enzymes, including tyrosinase. Genes that regulate structural coloration influence iridescence and metallic reflections, while genes involved in carotenoid metabolism govern the presence of red, orange, and yellow hues.

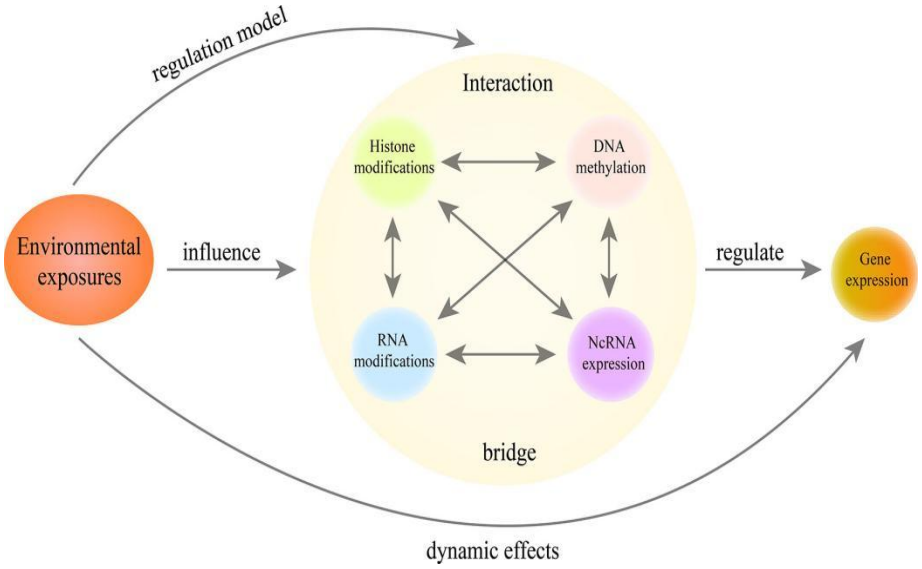

Fig. 9. Environmental influence on gene expression

Pigmentation of fish and crustaceans consists of a variety of pigments, each contributing to a distinct color. Melanin, produced in specialized cells known as melanophores, imparts dark hues such as brown and black. Red, orange, and yellow colors are attributed to carotenoid pigments, which can be synthesized de novo or obtained from the diet. Additionally, specific structures within the irises, scales, or skin of fish and crustaceans interact with light to produce metallic reflections or iridescence, which results in structural colors. The phenotypic diversity observed in fish and crustaceans populations is primarily attributed to genetic variation within pigmentation genes. Mutations in these genes that influence pigment production, distribution, or functionality can lead to a wide range of color abnormalities. This genetic variation is shaped by the forces of natural selection, genetic drift, and gene flow, which collectively determine the distribution of pigmentation traits among populations and species. Furthermore, the pigmentation of fish and crustaceans is closely linked to their environmental adaptation. Species inhabiting diverse environments may exhibit distinct pigmentation patterns that enhance their ability to camouflage and evade predators. For instance, animals residing in open water may employ countershading to blend in with the ambient light, whereas those inhabiting rocky or forested environments might utilize disruptive coloration to evade detection. Additionally, seasonal variations in pigmentation can occur to facilitate thermoregulation or facilitate mate recognition. Understanding the genetic basis of pigmentation in fish and crustaceans, as well as its adaptive significance, is crucial for the conservation of biodiversity and for enhancing our comprehension of ecological interactions and evolutionary processes. Research into the genetics of pigmentation offers valuable insight into how organisms adapt to their environments and how differential selection on pigmentation traits may lead to the emergence of new species.

Emerging Technologies and Approaches in Studying Fish Pigmentation Genetics

Comprehending the complexities of coloration patterns and their ecological significance necessitates a thorough understanding of fish and crustacean pigmentation. Recent technological advancements have fundamentally transformed the study of pigmentation genetics in fish and crustaceans, enabling researchers to investigate the underlying mechanisms through innovative and sophisticated tools.

Gene Editing

Fish and crustacean pigmentation can be elucidated through functional genomics techniques such as transcriptomics and proteomics, which facilitate the identification of the genes and regulatory mechanisms involved in pigmentation. Transcriptomic analysis reveals gene expression patterns associated with the formation and regulation of pigmentation under various developmental stages and environmental conditions (Wittkopp, Vaccaro, & Carroll, 2002). Furthermore, researchers can employ gene-editing technologies such as CRISPR-Cas9 to precisely modify pigmentation-related genes, thereby allowing for the validation of gene function and the assessment of the relevance of these genes to coloration phenotypes.

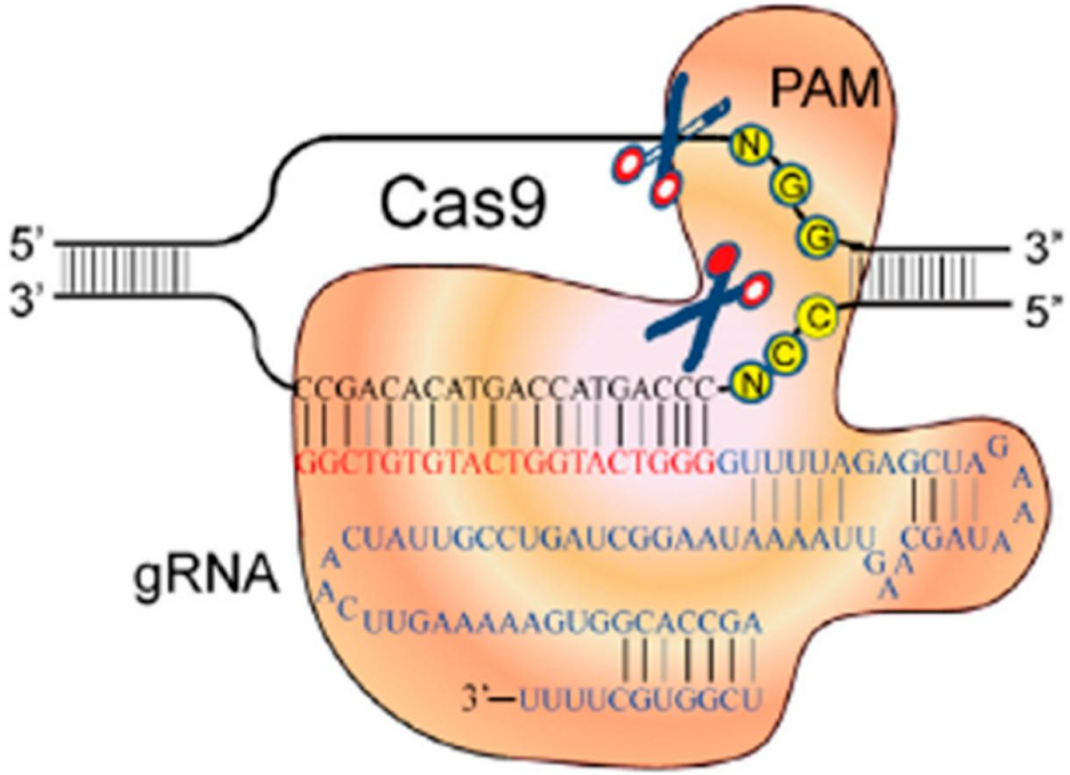

Fig. 10. Genome editing with CRISPR/Cas9

The CRISPR/Cas9 system comprises a modified single guide RNA (sgRNA/gRNA), which includes a trans-activating crRNA (tracrRNA) and a targeting CRISPR RNA (crRNA), along with a Cas9 endonuclease (Filippova, Matveeva, Zhuravlev, & Stepanov, 2019). Thus, the Cas9 protein and sgRNA represent the two essential components of this system. The Cas9 nuclease is directed to its specific target sequence by a precisely engineered guide RNA consisting of approximately 20 base pairs. One of the major advantages of this system, compared to zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN), is that it requires only a modification of the 20-nucleotide sgRNA "spacer" sequences to target new genomic sites. This significantly simplifies the editing process.

Single-cell Analysis

Single-cell sequencing methods facilitate the study of gene expression profiles at the individual cell level, providing unprecedented insights into the cellular heterogeneity that underlies pigmentation patterns (Chen, Jin, Huang, & Chen, 2016). By employing single-cell RNA sequencing (scRNA-seq), researchers can analyze the molecular profiles of pigment-producing cells, such as chromatophores and melanocytes, thereby elucidating their contributions to overall coloration. This approach enhances our understanding of the cellular dynamics and interactions that influence pigmentation in fish and crustaceans.

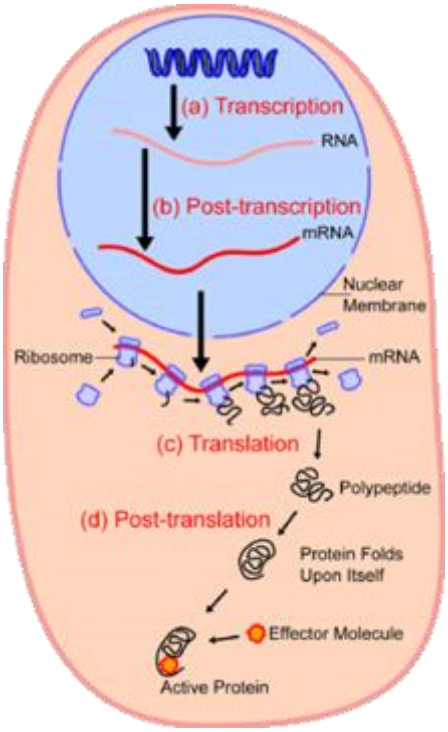

Fig. 11. Single cell showing central dogma required to quantify RNA, DNA, and protein

Computational Biology and Modeling

Computational modeling and bioinformatics tools facilitate the integration and analysis of large-scale genomic and transcriptomic datasets related to fish and crustacean coloration (Rendleman, Choi, & Vogel, 2018). Machine learning algorithms and network analysis techniques are employed to generate gene regulatory networks and predictive models for pigmentation phenotypes. These computational methods enhance our understanding of the evolution and adaptation of pigmentation by providing valuable insights into the complex relationships that exist among genes, environmental factors, and phenotypic expression.

Epigenetics and Regulatory Mechanisms

Histone modifications, non-coding RNAs, and DNA methylation are examples of epigenetic processes that play a crucial role in regulating gene expression during the development of pigmentation (Bure, Nemtsova, & Kuznetsova, 2022). Recent research is focused on investigating the epigenetic landscape of pigmentation-related genes and its interactions with environmental signals. Epigenetic alterations serve as dynamic regulators of pigmentation phenotypes, mediating plasticity and adaptation to fluctuating environmental conditions.

Implications for Conservation and Aquaculture

The genetics of coloration in fish and crustaceans can provide valuable insights for the identification and protection of genetically diverse populations and subspecies. By investigating the genetic variation associated with pigmentation traits, conservationists can prioritize the preservation of unique color

morphs and populations that may possess significant adaptive features. Furthermore, pigmentation patterns in fish and crustaceans can change over time, with such alterations serving as indicators of environmental stressors, including pollution, habitat degradation, and climate change. Monitoring pigmentation phenotypes in wild fish and crustacean populations can guide conservation efforts aimed at mitigating environmental threats and can serve as early warning signals for ecosystem disruption.

By understanding the genetic foundations of pigmentation adaptability, conservationists can employ assisted evolution strategies to enhance fish and crustacean populations faced with environmental challenges. By intentionally breeding individuals with adaptive pigmentation traits, conservation initiatives can accelerate the development of populations that are better suited to changing environmental conditions. Moreover, the application of genetics to fish and crustacean pigmentation enables aquaculturists to selectively breed individuals with desirable color characteristics for commercial production. Fish and crustaceans produced through aquaculture operations can achieve higher market value and enhanced aesthetic appeal by being selectively bred for their vibrant colors, consistent pigmentation patterns, and resistance to colour anomalies. The pigmentation characteristics of fish and crustaceans are closely linked to their overall health and fitness. For example, certain pigments may provide antioxidant benefits or protection against ultraviolet (UV) radiation, thereby improving health and reducing the risk of disease. By employing selective breeding strategies, aquaculture practices can leverage the genetics of pigmentation to optimize the welfare, health, and growth of fish and crustaceans. Moreover, by understanding consumer preferences regarding coloration, aquaculturists can tailor production to meet market demands. Consequently, aquaculture operations can enhance the marketability and consumer acceptance of their products by raising fish and crustaceans with specific coloration patterns or hues.

Conclusion

In summary, research on the genetic mechanisms underlying pigment synthesis pathways in fish and crustaceans has elucidated the molecular basis of color variation and its ecological significance. Through a systematic examination of the genes, enzymes, and regulatory networks involved in pigment production, researchers have uncovered the complex processes that govern the development, organization, and expression of pigmentation traits in fish and crustacean species. The elucidation of pigment production pathways has revealed the intricate composition of coloration in fish and crustaceans, which encompasses a diverse array of pigments, including structural colors, carotenoids, and melanins. Understanding the genetic regulation of these pigments has enhanced our comprehension of the evolutionary processes, such as natural selection, genetic drift, and gene flow, that contribute to color diversity. Furthermore, genetic research has provided conservation organizations with valuable tools for maintaining biodiversity and ecosystem health. By identifying the genetic markers associated with pigmentation traits, researchers can assess population genetic structures, detect adaptive variation, and formulate management strategies for threatened or endangered species.

Moreover, the incorporation of genetic information in aquaculture has enhanced selective breeding programs aimed at producing fish with desirable coloration characteristics for commercial purposes. By leveraging the genetic mechanisms underlying pigment synthesis, aquaculturists can optimize the growth, health, and marketability of fish and crustaceans while simultaneously addressing consumer preferences for aesthetically appealing products. Overall, investigations into the genetic mechanisms governing pigment synthesis pathways in fish and crustaceans represent a crucial aspect of applied genetics, ecology, and evolutionary biology. Continued exploration of the molecular basis of fish and crustacean colorations will undoubtedly deepen our understanding of the complex interactions among genes, environment, and phenotype that shape biological diversity. Furthermore, this knowledge has the potential to enhance aquaculture sustainability, inform conservation strategies, and stimulate innovations in biotechnology and biomimicry.

ACKNOWLEDGMENTS

This work was supported by Kibogora Polytechnic University, Department of Sciences, PO. BOX 50 Nyamasheke, Rwanda.

In partnership with the National Engineering Research Center of Marine Facilities Aquaculture, Zhejiang Ocean University, Zhoushan, 316022, China.

CONFLICT OF INTEREST

All the authors declared no potential interest.

AUTHOR CONTRIBUTION

Murwanashyaka Michel: Writing original draft, writing review & editing.

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DATA AVAILABILITY

Enquiries about data availability should be directed to the authors.

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