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From genes to color: Evolutionary insights and mechanistic pathways of the *yellow* gene in insect pigmentation

Blessie Pradeeka Gudelly [1], Shanthi Sharon Kolluri [2] and Komal Kumar Bollepogu Raja [3,*]

[1] *Depaartment of Surgery, Osmania Medical College, Hyderabad, Telangana, India.*
[2] *Department of Genetics, Loyola Academy, Hyderabad, Telangana, India.*
[3] *Department of Biotechnology, Malla Reddy Engineering College, Medchal, Telangana, India.*

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Abstract

Insect pigmentation plays a vital role in ecology and evolution, influencing survival, mate attraction, and communication. This review examines the complex mechanisms governing insect coloration, focusing on the roles of pigments (melanins and carotenoids) and structural colors. Specifically, the *yellow* gene in *Drosophila* is highlighted for its critical function in melanin biosynthesis and its evolutionary implications. Variations in the *yellow* gene's expression and regulation, influenced by both cis- and trans-regulatory elements, contribute to the phenotypic diversity of pigmentation patterns across insect species. The interplay between developmental genes and the *yellow* gene further illustrates how subtle genetic changes drive morphological diversity. While advances in CRISPR and other genetic tools have enhanced our understanding of pigmentation, challenges remain in deciphering the gene's complex interactions and the influence of environmental factors. Future research should focus on refining genomic techniques, exploring environmental impacts on pigmentation, and utilizing single-cell omics to gain deeper insights into the regulatory networks governing the *yellow* gene. This comprehensive understanding of insect pigmentation will not only enrich our knowledge of biodiversity but also inform conservation and biotechnological innovations.

Keywords: *Drosophila*; Y*ellow* gene; Pigmentation; Gene regulation

1. Introduction

Insect pigmentation, encompassing the vibrant spots and shade patterns that adorn the bodies of these tiny creatures, serve a multitude of crucial ecological and biological functions. The coloration of insects is primarily governed by pigments like melanin, carotenoids, and structural colors produced by microstructures within their exoskeletons. Melanin, a common pigment found in insects, provides shades ranging from black to brown, imparting protection against ultraviolet radiation and aiding in thermoregulation. Carotenoids, often responsible for red, orange, and *yellow* colors, serve as antioxidants and may play a role in attracting mates or deterring predators. Structural colors arise from the interaction of light with intricate surface structures on the insect's cuticle, creating iridescence or metallic hues that can confuse predators or signal mating readiness [1-3].

The evolutionary significance of insect pigmentation is profound. Camouflage patterns help insects blend into their environments, evading predators and enhancing survival rates. Conversely, bright warning colors like the bold stripes of wasps or the striking patterns of poisonous butterflies signal toxicity or distastefulness to potential predators—a defense mechanism known as aposematism [4]. Beyond survival advantages, insect pigmentation also serves vital roles in communication within species. Mating rituals often involve visual displays where color intensity or pattern intricacy

* Corresponding author: Komal Kumar Bollepogu Raja

signals genetic fitness or reproductive readiness. In social insects like ants or bees, subtle differences in pigmentation can denote caste or hierarchy, facilitating efficient colony functioning.

Understanding the mechanisms and functions of insect pigmentation not only enhances our appreciation of biodiversity but also informs efforts in conservation and biotechnological innovation, such as in the development of new materials inspired by nature's color palette. Thus, while small in scale, insect pigmentation looms large in the tapestry of natural wonder and scientific discovery.

Pigmentation in insects is primarily driven by the presence of pigments and the structure of the cuticle. Pigments can be classified into two main categories: carotenoids and melanins. Carotenoids contribute yellow, orange, and red colors, while melanins are responsible for black and brown hues [2, 3, 5]. Additionally, structural coloration—resulting from the microscopic arrangement of cuticular structures—can produce iridescent or metallic colors. The interplay between these pigments and structural features creates the diverse color patterns seen in insect species.

The genetic basis of insect pigmentation involves a complex network of genes that control pigment synthesis, distribution, and degradation. Key genes implicated in pigmentation include those encoding enzymes involved in pigment biosynthesis and regulatory genes that influence the expression of these enzymes. In *Drosophila melanogaster*, for example, genes such as *yellow*, *tan*, *ebony* and *dopa decarboxylase* are known to affect pigmentation [5-10]. These genes encode enzymes responsible for the production of melanin and other pigments, and their expression is tightly regulated to produce specific patterns.

2. The *yellow* Gene in *Drosophila* Pigmentation

The *yellow* gene in *Drosophila melanogaster* plays a crucial role in the pigmentation of the fruit fly's cuticle and is a prime example of how genetic factors influence coloration in insects [11]. This gene provides significant insight into the molecular mechanisms underlying pigmentation and its evolutionary implications. The *yellow* gene encodes a protein that is involved in the biosynthesis of pigments, particularly the production of melanin, which contributes to the brown and black colorations in the cuticle of *Drosophila*. The protein product of the *yellow* gene is a key enzyme in the pigment biosynthetic pathway. It facilitates the conversion of precursor molecules into melanin, influencing the overall color pattern of the fly [12, 13].

yellow gene expression is regulated at multiple levels, including transcriptional and post-transcriptional mechanisms. In *Drosophila*, the expression of the *yellow* gene is tissue-specific and varies between different developmental stages. The gene's expression is controlled by various transcription factors that bind to the gene's promoter region. Additionally, interactions with other genes, such as *black* and *tan*, modulate the activity of the *yellow* gene and its contribution to pigmentation[1, 6, 9, 11]. Mutations in the *yellow* gene lead to significant alterations in pigmentation. For instance, loss-of-function mutations result in a pale or yellowish appearance of the cuticle due to the reduced production of melanin. Conversely, gain-of-function mutations can cause an increase in pigment deposition, leading to darker pigmentation [12]. These mutations provide valuable tools for understanding the role of the *yellow* gene in pigment biosynthesis and its interactions with other pigmentation-related genes.

3. Evolutionary Perspectives

The *yellow* gene is not unique to *Drosophila* but is found across various insect species, indicating its evolutionary significance. Comparative studies of *yellow* across different species reveal how variations in this gene contribute to the diversity of pigmentation patterns observed in insects [2, 6, 9, 10]. Additionally, the *yellow* gene serves as a model for studying gene regulation, evolutionary adaptation, and the interplay between genetic and environmental factors in shaping pigmentation.

The evolutionary aspects of the *yellow* gene are also a focal point of recent studies. Changes in *yellow* gene expression have contributed to phenotypic diversity and pigmentation divergence among *Drosophila* species. These changes can result from alterations in the non-coding regulatory (cis-regulatory) regions of the gene or changes in the deployment of the trans-factors (trans-regulatory) that bind to these regions and regulate *yellow* gene expression. Both *cis*-regulatory elements (CREs) and transcription factor expression changes that regulate the *yellow* gene have been implicated in the evolution and diversification of color patterns in various *Drosophila* species [2, 6, 9, 10]. For instance, CREs have been identified that specifically modulate *yellow* gene expression in diverse anatomical regions including the wings, body, head, bristles, and mouthparts [9, 10, 14, 15]. This regulation is not isolated to pigmentation; the *yellow*

gene's involvement extends to other biological functions such as courtship behavior[16] and immune responses [17], underscoring its pleiotropic nature.

The evolutionary dynamics of the *yellow* gene highlight its role in shaping pigmentation diversity. Variations in CREs associated with the *yellow* gene have led to substantial differences in pigmentation patterns among *Drosophila* species. For example, the observed differences in abdominal pigmentation between *Drosophila melanogaster* and *Drosophila subobscura* are attributed to evolutionary modifications in a CRE located upstream of the *yellow* gene's transcription start site [11]. These variations are not merely cosmetic, but reflect deeper evolutionary shifts. Additionally, two independent studies have revealed that cis-regulatory changes occurring upstream of the *yellow* promoter and within its intron have facilitated the emergence of male-specific wing pigmentation spots in *Drosophila biarmipes* [18] and *Drosophila elegans* [19]. Conversely, similar mutations in orthologous CRE sequences have resulted in the loss of these pigmentation features in *Drosophila gunungcola* and *Drosophila mimetica* [20]. Such examples underscore the crucial role of CREs in driving pigmentation evolution.

The sexually dimorphic pigmentation pattern observed in *Drosophila melanogaster* provides a further illustration of the *yellow* gene's evolutionary flexibility [21]. In males, dark pigment is confined to the two posterior-most abdominal segments, a pattern that is consistent across many related species. However, in *Drosophila prostipennis*, this male-specific dark pigment has expanded to encompass two anterior abdominal segments. This expansion has been attributed to mutations in a specific *yellow* CRE [22]. This study exemplifies how subtle genetic changes can have pronounced effects on pigmentation patterns, contributing to the diverse array of visual signals observed in these species. Just recently, it was shown that the cis-regulatory architechture of the *yellow* gene enhancer is responsible for the induction of spot pattern on the abdomen of *Drosophila guttifera* and related species [6, 9, 10]. This spot pattern on the abdomen is driven by repression of an ancestral stripe element. Furthermore, using RNA in situ hybridization [8] and imaging of the abdomens [23], it was shown that the modular spot expression observed in closely related species is due to differences in *yellow* gene expression in these species. This study suggests that phenotypic differences observed in these closely related fruit flies is driven by differences in *yellow* gene expression pattern. In addition, a recent study explored how evolutionary changes in regulatory elements can quantitatively adjust pigmentation intensity in fruit flies [24]. The study showed that specific CREs associated with the *yellow* gene have evolved to modulate pigment levels in a precise manner. By comparing different *Drosophila* species, the study demonstrates that alterations in these regulatory regions can lead to significant variations in pigmentation intensity, reflecting adaptive responses to environmental or evolutionary pressures [24]. This research underscores the importance of regulatory evolution in shaping the diversity of pigmentation patterns observed in fruit flies.

Changes in the expression of developmental genes that regulate *yellow* have also been implicated in pigment pattern evolution in fruit flies, demonstrating the intricate interplay between genetic regulation and morphological development. These development genes comprise transcription factors and signaling molecules which are fundamental regulators of developmental processes. Later in development, they often undergo changes that significantly impact pigmentation patterns. Further, novel expression domains emerge in genes; a phenomenon known as 'co-option', through which developmental genes acquire novel functions. Co-option has been observed in the formation of wing spot pattern in *Drosophila guttifera* [25]. In this context, the *yellow* gene has acquired a Wingless (Wg)-responsive cis-regulatory element (CRE), integrating the Wg signaling pathway into its regulatory network. This co-option allows the *yellow* gene to be directly regulated by Wg, which menas that any changes in Wg expression will correspondingly affect black wing pigmentation.

Further studies reinforce the notion that alterations in the expression of toolkit genes are pivotal for the evolution of specific pigmentation traits. For instance, spatio-temporal changes in the expression of other toolkit factors such as En (Engrailed) and Dll (Distal-less) have been shown to play a significant role in the development of male-specific black wing spots in *Drosophila biarmipes* [18, 26]. These findings suggest that the toolkit genes En and Dll are not only crucial for general developmental processes but also for the fine-tuning of *yellow* gene in pigmentation patterns, contributing to the distinctiveness of male-specific traits. Just recently, it was shown that several developmental genes such as *decapentaplegic* (*dpp*), *abdominal-A* (*abdA*), *hedgehog* (*hh*), *zerknult* (*zen*) and *wg* collectively induce *yellow* to assemble the abdominal spot pattern of *Drosophila guttifera* [9, 10].

Taken together, changes in the expression of developmental genes that control *yellow* gene expression have been implicated in the formation of novel morphological patterns and therefore suggest that *yellow* gene is a hotspot of evolution and generation of novel patterns. Therefore, investigating *yellow* gene regulation and the principles gained by this research will allow a deeper mechanistic understanding of how diverse forms develop in organisms.

4. Limitations of *yellow* Gene Research

Research on the *yellow* gene faces several challenges due to its complex interactions with other genes and pathways involved in pigmentation. The *yellow* gene operates within a broader network of regulatory mechanisms, interacting with genes such as *black* and *tan*, which complicates the understanding of how these interactions collectively shape pigmentation patterns [27]. While significant insights have been gained, the full extent of these interactions remains partially understood, posing difficulties in delineating the precise role of the *yellow* gene in pigmentation. Additionally, the expression and function of the *yellow* gene can be influenced by genetic background and environmental factors, further complicating research [28]. Variability in pigmentation patterns across different *Drosophila* strains, influenced by both genetic and environmental differences, challenges the establishment of consistent models and the generalization of findings.

Moreover, the limitations of current functional analysis tools exacerbate these challenges. Although advanced gene-editing technologies like CRISPR/Cas9 have improved our ability to manipulate the *yellow* gene, issues such as off-target effects and incomplete knockouts can obscure results and hinder the accurate assessment of the gene's functions [29]. Finally, the evolutionary and comparative aspects of *yellow* gene research are constrained by limited genomic data and the complexity of evolutionary changes. Comprehensive cross-species comparisons necessary to understand adaptive pigmentation variations are still developing, highlighting the need for more extensive genomic resources and refined analytical approaches to fully elucidate the evolutionary role of the *yellow* gene.

5. Future Directions

To advance our understanding of the *yellow* gene and its role in pigmentation, several key areas of research should be prioritized. First, enhancing functional genomics through the development of more refined genetic tools is crucial. By employing improved CRISPR/Cas9 technologies with greater targeting specificity and integrating these with high-throughput sequencing methods, researchers can gain deeper insights into the gene's function and its interactions within the pigmentation network [29]. Additionally, incorporating systems biology approaches can facilitate the modeling of complex interactions between the *yellow* gene and other components of the pigmentation pathway. Computational models and integrative omics techniques, such as transcriptomics and proteomics, can provide a comprehensive view of how alterations in the gene network impact overall pigmentation.

Further research should also explore how environmental factors influence the expression and function of the *yellow* gene. Experiments manipulating environmental variables, like temperature and diet, could reveal important insights into the adaptive significance of pigmentation patterns. Expanding comparative evolutionary studies to include a broader range of species and populations will help elucidate how evolutionary pressures shape the *yellow* gene and its functions. Moreover, the characterization of regulatory elements, including enhancers, repressors, and epigenetic modifications, is essential for understanding the precise control of *yellow* gene expression. Techniques such as chromatin immunoprecipitation (ChIP-seq) and reporter assays can aid in mapping these regulatory elements and elucidating their roles in pigmentation regulation.

6. Advancing *yellow* Gene Research through Single-Cell Omics

Single-cell omics technologies offer unprecedented insights into the complex regulation of the *yellow* gene, essential for understanding its role in pigmentation. Single-cell transcriptomics, such as single-cell RNA sequencing, allows for detailed examination of *yellow* gene expression across individual cells, revealing cell-specific patterns that bulk analyses might miss. This precision helps identify how the *yellow* gene interacts with other genes and regulatory elements within different cell types and developmental stages. Additionally, single-cell ATAC-seq provides crucial information on chromatin accessibility, pinpointing active enhancers and silencers that modulate *yellow* gene expression. Single cell data sets for different *Drosophila* tissues have been generated till date [30-35]. By employing single-cell multi-omics approaches, researchers can track dynamic changes in gene expression and chromatin states over time, further elucidating the *yellow* gene's contribution to pigmentation.

Furthermore, integrating single-cell genomics techniques, such as single-cell DNA sequencing, with functional genomics and epigenomics, enhances our understanding of genetic variability and regulatory mechanisms affecting the *yellow* gene. Single-cell CRISPR/Cas9 screens enable precise manipulation of the gene's regulatory regions, revealing their functional roles. Meanwhile, single-cell epigenomic profiling of DNA methylation and histone modifications sheds light on the epigenetic landscape influencing *yellow* gene activity. Combining these insights with multi-omics data offers a comprehensive view of how *yellow* gene expression is regulated and its impact on pigmentation at multiple molecular

levels. This integrated approach not only advances our knowledge of the *yellow* gene but also sets the stage for exploring its roles in various cellular and developmental contexts.

7. Conclusion

In summary, single-cell omics and genomics provide powerful tools for dissecting the complex regulation of the *yellow* gene and its role in pigmentation. These approaches offer unprecedented resolution and insights into cellular and molecular mechanisms underlying pigmentation, paving the way for more detailed and accurate models of gene function.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflicting interests.

Authors' Contributions

The manuscript was prepared by B.P.G, S.S.K. and K.K.B.R. and reviewed by K.K.B.R.

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