ANNUAL REVIEWS

Annual Review of Genetics

Mechanisms Underlying the Formation and Evolution of Vertebrate Color Patterns

Claudius F. Kratochwil[1,*] and Ricardo Mallarino[2,*]

[1]Institute of Biotechnology, HiLIFE, University of Helsinki, Helsinki, Finland; email: claudius.kratochwil@helsinki.fi

[2]Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA; email: rmallarino@princeton.edu

Annu. Rev. Genet. 2023. 57:135–56

First published as a Review in Advance on July 24, 2023

The *Annual Review of Genetics* is online at genet.annualreviews.org

https://doi.org/10.1146/annurev-genet-031423-120918

*These authors contributed equally to this article

Keywords

coloration, pigmentation, pattern formation, color patterns

Abstract

Vertebrates exhibit a wide range of color patterns, which play critical roles in mediating intra- and interspecific communication. Because of their diversity and visual accessibility, color patterns offer a unique and fascinating window into the processes underlying biological organization. In this review, we focus on describing many of the general principles governing the formation and evolution of color patterns in different vertebrate groups. We characterize the types of patterns, review the molecular and developmental mechanisms by which they originate, and discuss their role in constraining or facilitating evolutionary change. Lastly, we outline outstanding questions in the field and discuss different approaches that can be used to address them. Overall, we provide a unifying conceptual framework among vertebrate systems that may guide research into naturally evolved mechanisms underlying color pattern formation and evolution.

1. INTRODUCTION

Animal coloration (see the sidebar titled Common Terms Used in Pigmentation Research) has been a captivating area of research that has attracted attention from various disciplines, including biophysics, genetics, developmental biology, evolutionary biology, and ecology. Because it plays a fundamental role in mediating intra- and interspecific communication, coloration is a key trait that allows species to adapt and survive in different environments. While the ultimate causes driving coloration differences within and between species have been extensively studied for decades (4, 5, 9), the proximate causes—in particular the underlying cellular and developmental mechanisms by which these processes occur—often remain poorly understood.

COMMON TERMS USED IN PIGMENTATION RESEARCH

Color refers to the visual perception of different wavelengths of light, which can be seen as different hues, such as red, blue, or green. In the context of animal coloration, color refers to the hue of an animal's integument (e.g., fur, feathers, scales, and skin layers) and can include pigment-based and/or structural coloration.

Pigment is a molecule that absorbs specific wavelengths of light, giving a cell, tissue, or organism its color. Pigmentation refers to the presence, amount, and distribution of pigments in the body of an organism. It may include brown to black eumelanin (often referred to as melanin); yellow to orange pheomelanin (in mammals and birds), carotenoids, luteins, and zeaxanthins; and red to purple anthocyanins, porphyrins, and turacins. The guanine crystals that underlie the metallic blue structural color of many vertebrates do not involve pigments but are often referred to as such.

Coloration is the general appearance of an animal's integument. It can encompass aspects of hue, saturation, and brightness, as well as patterns and textures.

In animals, color can be generated through deposition of pigments or by structural features that interfere with light (see the sidebar titled Common Terms Used in Pigmentation Research). In this review, we use color as a generic term to refer to both structural and pigment-based coloration. On a spatial scale, animals can vary in their overall body color or the distribution of color across their body (i.e., color patterns; see the sidebar titled Terms Used in Pigment Pattern Formation). Genetic studies in natural populations of different vertebrate species have identified

TERMS USED IN PIGMENT PATTERN FORMATION

Color pattern and pigmentation pattern, terms that are often used interchangeably, refer to the arrangement, distribution, or spatial variation of pigments in an animal's integument. Spatial variation in coloration can also be created by spatial variation in light-interfering structures or in the texture and morphology of the integument and its appendages (e.g., feather or scale characteristics).

A prepattern refers to an initial arrangement of cells with different properties, such as gene expression or cell distributions, that acts as a blueprint for the formation of the later-emerging color pattern. Prepatterns can be created through various mechanisms but necessarily need some kind of non-cell-autonomous channeling of positional information.

Positional information refers to the distribution of the different molecular or biochemical signals providing cells with information about their position within a developing tissue. It can be established in several ways, including the release of morphogens (which are signaling molecules that diffuse through a tissue and create a gradient) or by physical means.

key genes responsible for promoting differences in overall body coloration (57, 59, 62, 64, 74). Largely owing to decades of research in traditional laboratory model species (2, 61), we now have a comprehensive mechanistic understanding of how such genes generate differences in overall body color. For example, the melanistic color displayed by some populations of rock pocket mice (*Chaetodipus intermedius*) inhabiting dark lava substrates is caused by coding changes in the Melanocortin 1 receptor (*Mc1r*) (57), a G protein–coupled receptor that resides on the surface of pigment-producing cells, for example, melanocytes, and binds paracrine factors to trigger a well-characterized signaling cascade leading to pigment production (10).

Less understood, however, are the molecular and developmental processes that mediate differences in color patterns among individuals of the same species and between members of different species. Because of their unparalleled diversity, visual accessibility, and widespread occurrence among species, color patterns have the potential of revealing critical insights into the mechanisms underlying biological organization. Recent advances in genomics and experimental approaches (12, 40), as well as the relative ease with which they can now be employed in wild-derived species (but also in domestic animals that have been artificially selected for color phenotypes), now offer the possibility of gaining mechanistic insights into the processes underlying the astonishing diversity of color patterns found in nature (**Figure 1**).

This review discusses the state of the research on the natural mechanisms underlying color pattern formation in the different vertebrate groups by establishing the general principles that govern their development and evolution. Moreover, we integrate newer research directions that move toward more complex color patterns and how they can provide mechanistic insights into

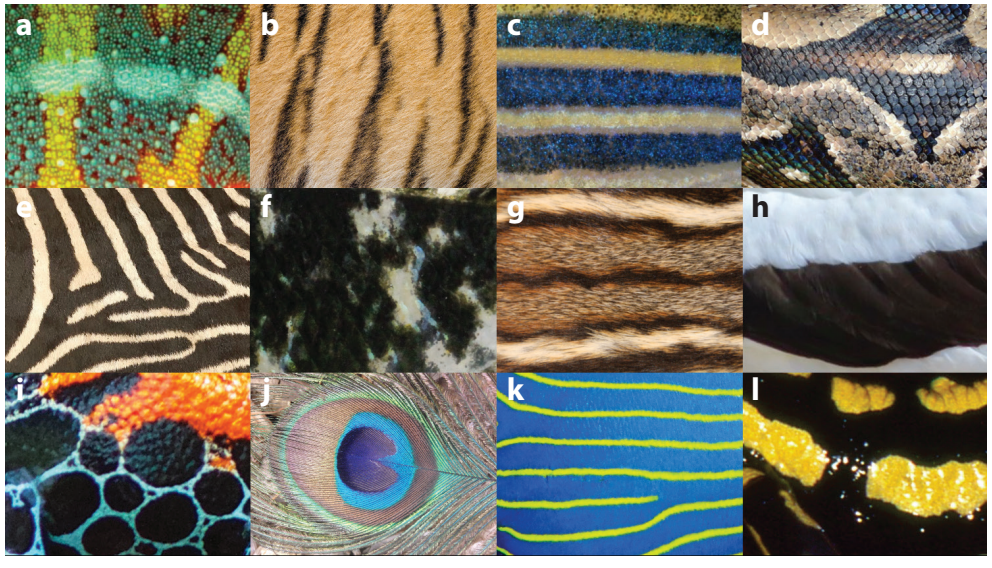

Figure 1

Color pattern diversity in vertebrates. Vertebrates display a vast array of color patterns, as exemplified by patterns found in the (*a*) panther chameleon, *Furcifer pardalis*; (*b*) tiger, *Panthera tigris*; (*c*) zebrafish, *Danio rerio*; (*d*) red-tailed boa, *Boa constrictor*; (*e*) Grévy's zebra, *Equus grevyi*; (*f*) orange blotch morph of the Lake Victoria cichlid fish, *Haplochromis chilotes*; (*g*) Eastern chipmunk, *Tamias striatus*; (*h*) Nazca booby, *Sula granti*; (*i*) red-and-blue poison dart frog, *Dendrobates reticulatus*; (*j*) Indian peafowl, *Pavo cristatus*; (*k*) emperor angelfish, *Pomacanthus imperator*; and (*l*) fire salamander, *Salamandra salamandra*. Panel *a* adapted from Reference 73 (CC BY 4.0), and panel *c* adapted from Reference 21 (CC BY 4.0). Photos provided by Mathias Appel (panel *b*), Alex Popovkin (panel *d*), Dan Rubenstein (panel *e*), Nidal Karagic (panel *f*), Ricardo Mallarino (panel *g*), Cassie Stoddard (panel *h*), Claudius Kratochwil (panel *i*), Satdeep Gill (panel *j*), Kevin Lino (panel *k*), and Stanislav Doronenko (panel *l*).

how these patterns are generated. We discuss open key questions in the field, such as the molecular basis of reaction–diffusion patterns and how they might interact with prepatterns (see the sidebar titled Terms Used in Pigment Pattern Formation), and cover recent advances in experimental approaches that can be used to address these questions. On a broader evolutionary scale, we discuss the interrelationship between types of patterns, modes of pattern formation, and their evolvability. Overall, we provide a unifying framework among vertebrate systems that can be used as a starting point to characterize and obtain a comprehensive understanding of the complex processes underlying color pattern formation and evolution.

2. FEATURES AND VARIATIONS OF VERTEBRATE COLOR PATTERNS

Color patterns can be defined as spatial variation in the pigment-based and structural properties of a tissue. The terms color pattern and pigmentation pattern are often used interchangeably, with pigmentation patterns (especially in mammals) mostly referring to melanic (i.e., eumelanin- and pheomelanin-based) patterns. Color patterns usually involve pigments other than melanin (e.g., carotenoids) or the structural coloration caused by light-interfering micro- and nanostructures. In this review, we use color pattern as a general term for spatial variation in both pigmentary and light-interfering tissue properties.

Describing and characterizing animal color patterns are difficult tasks, owing to the sheer diversity of colors and patterns (**Figure 1**) and the fact that their appearance can be influenced by various factors, such as environmental light conditions. Nevertheless, certain characteristics and features have been used to categorize patterns (**Figure 2**). For example, color patterns can be periodic, such as the banded patterns of snakes, or aperiodic, such as the white head of a bald eagle (**Figure 2*a***). Other patterns can be gradual, such as the countershading found in many species, or found in specific parts of the body, such as ornaments in birds. The orientation of patterns is another factor contributing to pattern diversity, with patterns often appearing in horizontal or vertical configurations (**Figure 2*b***). The complexity of color patterns can also vary widely, from simple patterns, such as evenly spaced stripes, to the intricate and complex patterns found in many tropical reptiles and fish (**Figure 2*c***). Patterns can vary in their degree of heterogeneity, both across an individual's body and between individuals, even in the absence of genetic variation (such as those seen in many domestic animals) (**Figure 2*d***). Patterns can also be modified or reorganized or can disappear throughout an organism's life, but there can also be mechanisms in place to maintain their consistency (**Figure 2*e***). Additionally, color patterns can be observed at different scales. Small-scale variations in pigment distribution or structural elements within individual hairs, feathers, or scales create micropatterns. Micropatterns ultimately contribute to the appearance of macropatterns, which are visible at a larger, macroscopic scale (i.e., along the body of an organism) (**Figure 2*f***). Patterns can also often co-occur, with one pattern (e.g., spots or stripes) being superimposed on another pattern (e.g., a dorsoventral gradient) (*6*), or fade from one pattern (e.g., stripes) into another (e.g., spots).

The remarkable variety of color patterns seen across animals raises intriguing questions about their developmental and evolutionary origins: How do genetic, molecular, and developmental factors generate this diversity, and how might these factors influence the evolvability and evolutionary dynamics of such patterns?

The color of the integument, which includes skin, feathers, hairs, and scales, ultimately results from the multilayered organization of cells and extracellular components with different pigmentary properties and structural components. In vertebrates, these are mostly generated by specialized pigment-producing cells. Color patterns arise from spatial differences in the properties and arrangements of pigment-producing cells. In mammals and birds, different forms of melanin

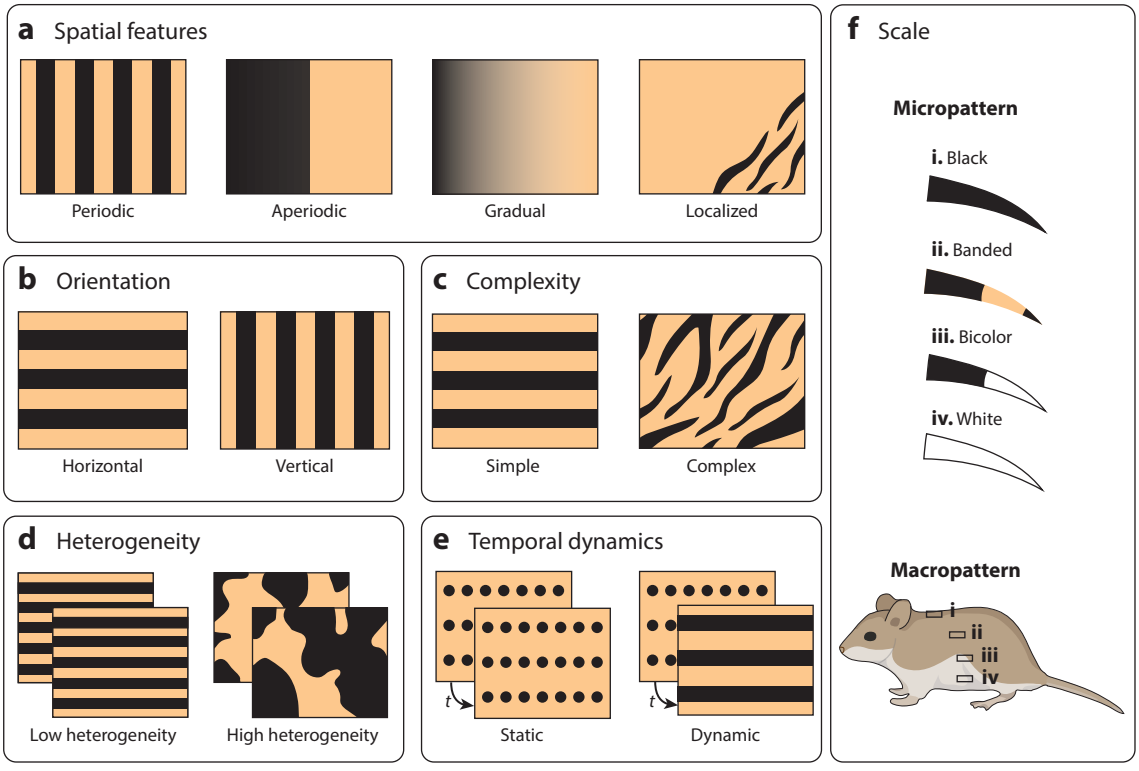

Figure 2

Classification of color patterns. (*a*) Color patterns can vary in their spatial arrangement, which includes periodic, aperiodic, and gradual patterns as well as those that are localized to specific body parts. (*b*) Periodic patterns can also differ in their orientation. (*c*) Patterns can be simple or complex, and this complexity is often measured using entropy as a proxy. (*d*) Another aspect of pattern complexity is pattern heterogeneity, which can be characterized by quantifying differences in body parts, left-right asymmetry, and interindividual variation (in genetically similar or identical individuals). (*e*) Some patterns also exhibit dynamic changes on different timescales, including changes during ontogeny, seasonal changes, or plastic changes that can occur at different speeds, ranging from milliseconds to seconds (physiological color change) and minutes to weeks (morphological color change). (*f*) Color patterns can be found at different scales. Micropatterns of hairs and feathers can contribute to macropatterns, but not all macropatterns are based on micropatterns. Achieving a comprehensive classification of color patterns is a major challenge that must be overcome to better understand the processes that shape the development and evolution of these patterns.

(brown to black eumelanin and yellow to red pheomelanin) are produced by specialized pigment cells termed melanocytes (**Figure 3*a***). Melanin is synthesized by melanocytes and is packaged into melanosomes, specialized organelles that are deposited into the skin, hair, or feathers. As we discuss in Section 3, patterns can be generated at various stages of this process. Mammalian fur patterns, for instance, are commonly produced by spatiotemporal modulation of melanin deposition into growing hair (2, 51). This process generates spatial variation in the overall pigmentation, resulting in the diverse coloration across different regions of the animal's body. Both hairs and feathers often have micropatterns that shape the color pattern of the animal. For example, hair often has alternating bands of pheomelanin or eumelanin (**Figure 2*f***), while feathers can also differ in the presence of nanostructures that can shape micropatterns through light interference (27).

In contrast to birds and mammals, coloration in reptiles, amphibians, and fish is determined by chromatophores. Color patterns result from the spatial variation in chromatophore types, properties, and arrangements within their multilayered integument (25, 58, 73) (**Figure 3*b***).

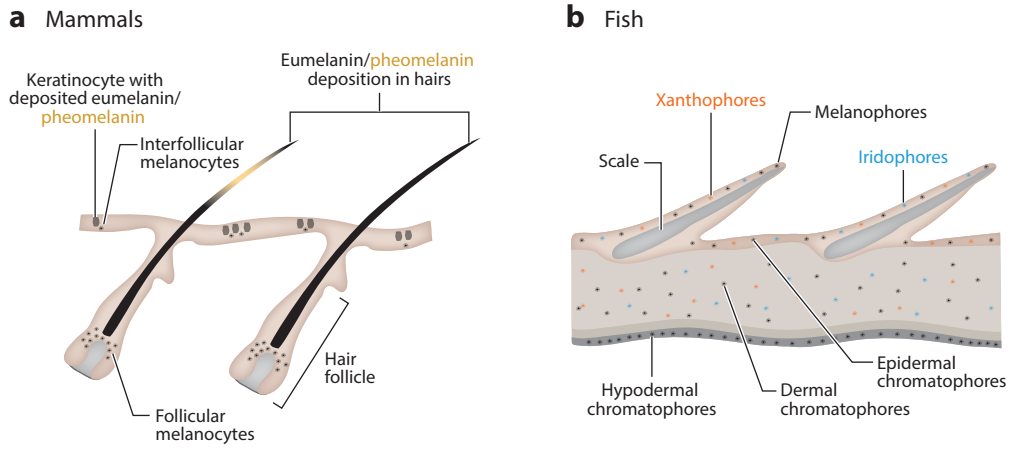

Figure 3

The physiological basis of color patterns differs across vertebrates. (*a*) Color patterns in mammals and birds are primarily determined by variations in the pigmentation and micro- and nanostructures of their epidermal appendages, such as hairs and feathers. The deposition of pigments by melanocytes, including eumelanin and pheomelanin, occurs during the growth of hairs or feathers. The resulting macroscopic patterns are often generated by the micropatterns of the appendages, such as the size and distribution of pheomelanin-enriched portions of the hairs. In feathers, micropatterns and emerging macropatterns can result from complex morphological variations at the nano- and microscales of the feather barbs and barbules that extend from the central shaft of the feather. Some species also have melanocytes residing between hair follicles (so-called interfollicular melanocytes); these, however, rarely contribute to color patterns. (*b*) In other vertebrates, including amphibians, reptiles, and fish, color patterns are created by variations in the three-dimensional arrangement of pigments and light-interfering substances within the integument. Several types of cells, including brown to black melanophores, yellow to red xanthophores/erythrophores, and reflective iridophores, influence the color of the tissue. Additionally, there can be significant variation in the cellular characteristics of these cells, such as the intracellular localization of pigments. Although not pigmented themselves, scales can also generate micropatterns that affect the coloration of animals through their structural properties as well as the distribution and presence of chromatophores in the surrounding epidermis.

Chromatophores can store and sometimes also dynamically reposition the pigment-bearing organelles (46, 71). In reptiles, amphibians, and fish, dark chromatophores are referred to as melanophores (instead of melanocytes), and they lack the ability to produce pheomelanin (68). Yellow to red pigmentation is instead created by specialized cells referred to as xanthophores (with red-pigment-producing cells often called erythrophores). Additionally, iridophores generate iridescent coloration through the synthesis of light-scattering crystals. Other chromatophores, including white leucophores (34, 43) and blue cyanophores (17), have also been described, with likely more cell types awaiting discovery. Color patterns in these clades are therefore rarely shaped by switches between the type of pigment produced and deposited, but rather by the three-dimensional distribution of the cells within the integument, as well as by the highly plastic cellular characteristics of the chromatophores.

3. THE ONTOGENETIC SEQUENCE OF COLOR PATTERN FORMATION

During the formation of a color pattern, spatial variation of some characteristics must be acquired. In general, as suggested by Kaelin & Barsh (31), the development of color patterns can be divided into two processes: pattern establishment and pattern implementation. Some patterns undergo these processes sequentially, with establishment occurring first, followed by implementation, while in others these can occur in a single, combined step (one-step establishment and implementation) (**Figure 4**).

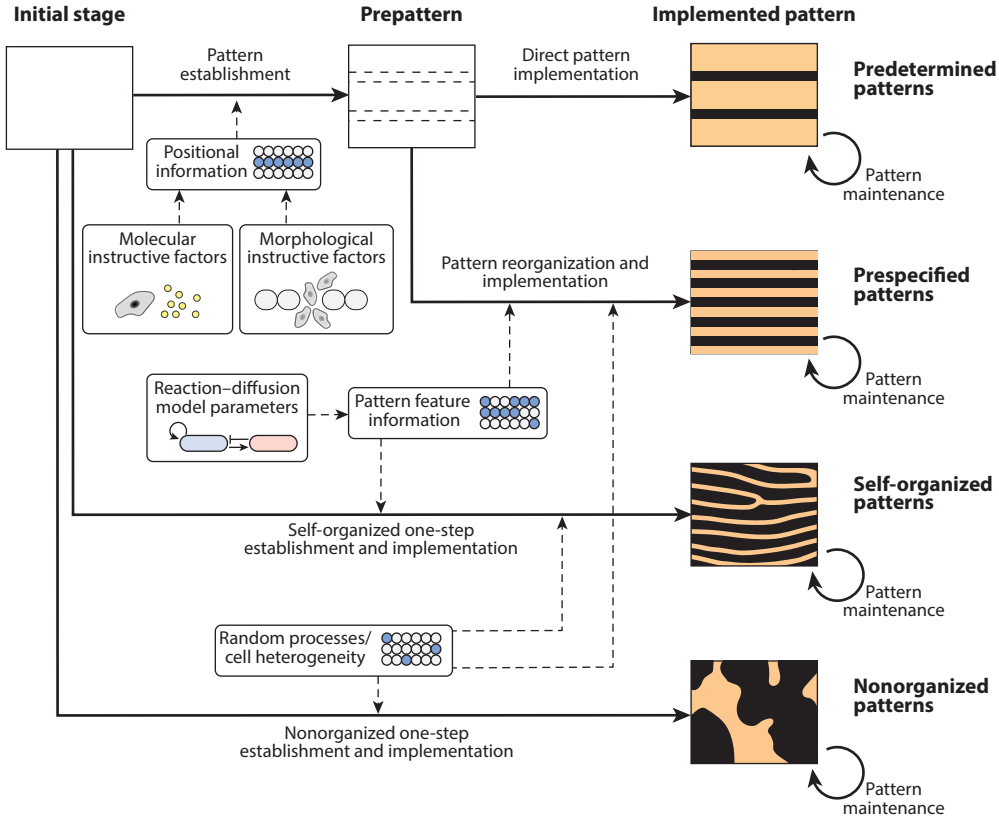

Figure 4

The formation of color patterns. The mechanisms by which color patterns form can be divided into at least four different categories. Predetermined and prespecified patterns develop in two steps. The first step, referred to as pattern establishment, leads to a prepattern. The prepattern can form through molecular instructive factors (e.g., paracrine factors) or morphological instructive events (e.g., constrained migratory pathways). The implemented pattern then forms through a process called pattern implementation, during which the pattern becomes visible (e.g., pigments are produced). If the prepattern is the direct blueprint of the adult implemented pattern, we refer to it as a predetermined pattern. When additional processes (e.g., reaction–diffusion processes, random processes, or other mechanisms that are not specifically depicted in this figure) result in a reorganization of the pattern (e.g., the formation of additional stripes or only partial pigmentation of the stripe area), we refer to it as a prespecified pattern. Self-organized and nonorganized patterns develop in a one-step process (establishment and implementation) without any non-cell-autonomous instructive factors. Self-organized patterns form autonomously, for example, through the interaction of cells. The parameters of the genetically encoded reaction–diffusion model define some characteristics of the pattern. Nonorganized patterns are rare in natural populations (but common in domestic animals) and are usually caused by genetic mosaicism or migration defects. The patterns are nonorganized because they are largely affected by stochastic events (e.g., where chromatophores end up and how much they proliferate). Stochastic events might also affect prespecified and self-organized patterns.

During pattern establishment, a prepattern is formed. These prepatterns lack any spatial variation in pigmentation or color, but they serve as a blueprint for the eventual color pattern that will develop. The emergence of the color pattern from the prepattern is referred to as pattern implementation. Through the implementation phase, further reorganization of the pattern can occur. We refer to patterns with limited or no reorganization as predetermined patterns and to patterns involving reorganization as prespecified patterns (**Figure 4**). We use the terms specified and determined similarly to the way that they have been used in cell differentiation to refer to the degree of flexibility or fixity in the formation of color patterns. Specified refers to a stage where

the color pattern is not yet fully fixed or immutable, while determined refers to a stage where the color pattern has become fully fixed, with a one-to-one or almost one-to-one correspondence between the prepattern and the implemented pattern. The process of pattern implementation can involve spatial variation in chromatophore/melanocyte number, type, concentration, and localization, which ultimately leads to the appearance of a pattern with visible spatial variation in light interference or pigmentation.

Patterns that are formed in a one-step establishment and implementation process are either fully self-organized (without any prepattern) or fully nonorganized (**Figure 4**). Self-organized patterns, such as those seen in leopards, tigers, and some tropical reef fish species, emerge, for example, through paracrine factors or physical cell–cell interactions that follow Turing reaction–diffusion models (35). Importantly, there might be other mechanisms that may or may not be consistent with reaction–diffusion predictions. Nonorganized patterns are unpredictable owing to stochastic events and can have different underlying causes, including genetic mosaicism [mostly described in domestic animals such as tortoiseshell or calico cats (19) and Lemon Frost leopard geckos (20)] or migration defects [also mainly described in domestic and lab animals, including piebald patterns in horses, mice, and dogs, but also found in the wild, e.g., in the orange blotch phenotype in several cichlid fish species (63, 72) (**Figure 1*f***)].

In the next section, we describe the two different phases (pattern establishment and pattern implementation) and four different categories (predetermined, prespecified, self-organized, and nonorganized patterns) of color pattern formation in more detail. We provide examples and speculate about the molecular processes that might constrain or facilitate their evolution.

3.1. The Establishment Phase of Color Patterns

Pattern establishment is the phase in which the foundation (i.e., the prepattern) for the later-developing spatial variation in color is generated. The formation of such prepatterns is instructed by positional information (see the sidebar titled Terms Used in Pigment Pattern Formation) provided by non-cell-autonomous mechanisms. This information can be provided by instructive paracrine factors released from other tissues or developing organs (**Figure 5*a***). For example, developing somites provide instructive information that leads to the formation of a prepattern in the integument of galliform birds (landfowl including chickens, pheasants, and quails) (22). The position of the somites and the signals they produce therefore establish a prepattern that determines the position of the longitudinal stripes.

Morphological instructive factors that constrain or channel processes, such as the migration of cellular precursors, can also constitute a prepattern (**Figure 5*b***). A good example of morphological constraints shaping color patterns can be seen during stripe pattern formation in zebrafish, in which the horizontal myoseptum that divides the dorsal and ventral muscle groups (myotomes) provides one of the main migrating routes for chromatophore precursors. The positioning of the myoseptum therefore determines both the position and orientation of the forming stripes (14).

Once a prepattern is established, the final pattern can be implemented in a way that faithfully recapitulates the blueprint provided by the prepattern (i.e., a predetermined pattern). However, it is also possible for the initial blueprint to be further modified and reorganized (i.e., prespecified patterns). In the case of a prespecified pattern, the final pattern may have elements lacking or additional elements, such as extra stripes or random patterns that are added to or superimposed on the initial prepattern. As a result, the final pattern will resemble the prepattern but will not look exactly the same. There are several factors that can modify a pattern once a prepattern blueprint is established. These include, for example, cell-autonomous mechanisms that may act to promote differences in cellular behaviors (**Figure 5*c***). In a recent study, Inaba and colleagues

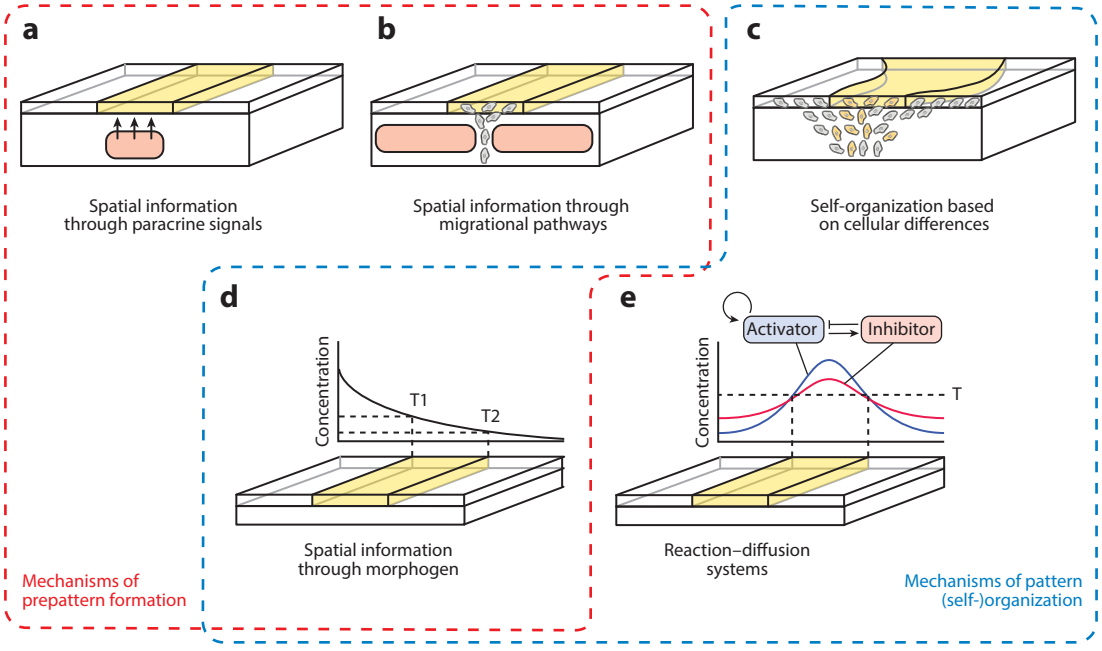

Figure 5

Color pattern establishment. Several processes can influence the establishment and self-organization of patterns during pattern implementation (or one-step establishment and implementation). (*a*) Localized paracrine signals can provide spatial information that leads to the formation of a prepattern. (*b*) Morphological structures can similarly constrain developmental processes, such as cell migration, to lead to a prepattern. (*c*) Cell–cell variation can control cell behavior in a cell-autonomous manner, resulting in the formation of nonorganized patterns such as blotches or piebald patterns. (*d*) Morphogen gradients can provide positional information that leads to prepatterns or can reorganize prepatterns. (*e*) Reaction–diffusion systems can directly generate color patterns without a preexisting prepattern or reorganize a prepattern, resulting in an implemented pattern that is a modified version of the prepattern. T1 and T2 refer to two thresholds below or above which different tissue responses are triggered.

(28) demonstrated that melanocytes play an instructive role in producing the stripe patterns seen in Japanese quails. Specifically, the interplay between melanocyte cell-autonomous mechanisms and paracrine signals emanating from the dermis seems to be a determining factor in patterning the stripes of these birds (28).

Differential signaling along a tissue, including cases in which morphogen gradients are present, can also act to organize and control the formation of color patterns (**Figure 5*d***). Recent work in African striped mice showed that the Wnt modulator *Sfrp2* can act as an orienting signaling mechanism that guides the establishment of the parallel stripes seen in the coats of these rodents. Specifically, during mid-embryogenesis, a dorsoventral gradient of *Sfrp2* regulates the distribution of developing hair follicles and establishes a prepattern that foreshadows the pigmentation stripes that will later appear. Upon disruption of *Sfrp2*, pigmentation stripes still form, but their boundaries are altered (29).

In some cases, late-acting reaction–diffusion systems may be present once an initial pattern has been established, causing it to be further modified (**Figure 5*e***). This is illustrated in the example discussed above, in which the positioning of the zebrafish myoseptum establishes an initial prepattern by guiding chromatophore migration, but the spacing and width of the resulting stripe pattern are determined by interactions among chromatophores (58, 61). Evidence for this model comes from the *choker* mutation. In *choker* mutants, which lack the horizontal myoseptum, both the

direction and positioning of stripes are disturbed, while the spacing and width of melanic stripes and yellow interstripes are comparable to wild-type zebrafish (14).

Numerous studies have explored how the spacing and width of stripe patterns are formed in zebrafish. Research on mutant lines has indicated that the characteristics of the pattern are largely determined by interactions between three types of chromatophores: melanophores, xanthophores, and iridophores (14, 60, 77). In the fin, interactions between melanophores and xanthophores are sufficient to generate stripe patterns (36).

A recent study on a monogenic color pattern trait in anole lizards also demonstrates how modulation of the migratory behavior of chromatophore progenitors might trigger transitions between patterns, in this case from a diamond-like pattern to a chevron-like pattern (13). Specifically, this might be realized by molecular changes that alter the parameters within this reaction–diffusion-like pattern formation process. This study illustrates how different phenotypes arise from a prespecified pattern (**Figure 4**) through the molecular tinkering of a single gene affecting self-organization parameters (13).

Interestingly, there are also examples that suggest the existence of nonimplemented prepatterns. In African cichlid fishes, horizontal stripes are likely formed in a similar fashion as in zebrafish, with migration of chromatophore progenitors along myosepta (23). Variation in the presence or absence of stripes seems to be mainly driven by adult (and not embryonic) variation in the melanocortin signaling antagonist *agrp2/asip2b* (38). The antagonist has no effect on the positioning of the stripes but acts to block the implementation of the pattern. This explains the frequent repeated evolution of the trait (39) and suggests that preexisting prepatterns may facilitate the rapid and repeated (re)evolution of color patterns, even if they appear quite complex. A more recent study in frogs, where the paralog *asip1* is also associated with stripes in frogs, might support the notion that this mechanism is indeed more prevalent (18).

3.2. The Implementation of Color Patterns

The implementation of color patterns involves multiple mechanisms that ultimately lead to the appearance of spatial variation in coloration. These mechanisms include, for example, changes in the relative abundance of pigments, in cellular and subcellular features, in deposition, and in the morphology of the integument. Generally, due to the differences in pigment cell and appendage characteristics, the mechanisms of color pattern implementation differ substantially among vertebrates. In mammals and birds, pigmentation is mainly generated through deposition of pigments into epidermal cells (i.e., keratinocytes) or into feathers and hairs (15, 75). Spatial variation in the pigmentation of a tissue can therefore be modified by changing pigment synthesis, deposition, or decay. For example, the rate at which pigment is produced can vary between cells and regions of the skin. Changes in pigment production result in alterations in pigmentation intensity, which can change the different properties of the color (**Figure 6*a***). In the case of (eu)melanin, the resulting color can range from black (high eumelanin density) to brown (low eumelanin density) or white (no eumelanin). In addition to these changes, the rate at which pigments are deposited (or how quickly they are degraded) can cause variation in pigmentation (**Figure 6*b***). An additional mechanism, which is commonly found in mammals and birds, involves molecular switches in the type of pigment that is synthesized (**Figure 6*c***). Specifically, melanocytes can switch between the production of eumelanin or pheomelanin, with the type of pigment produced being primarily determined by the interactions between *Mc1r* and its antagonist *Agouti* (2, 7). When *Agouti* is not present, α-melanocyte-stimulating hormone (α-MSH) can readily bind to *Mc1r*, leading to the activation of cyclic adenosine monophosphate (cAMP) signaling and synthesis of eumelanin. However, when *Agouti* is present, which typically occurs for a short period of time during hair/feather growth, it

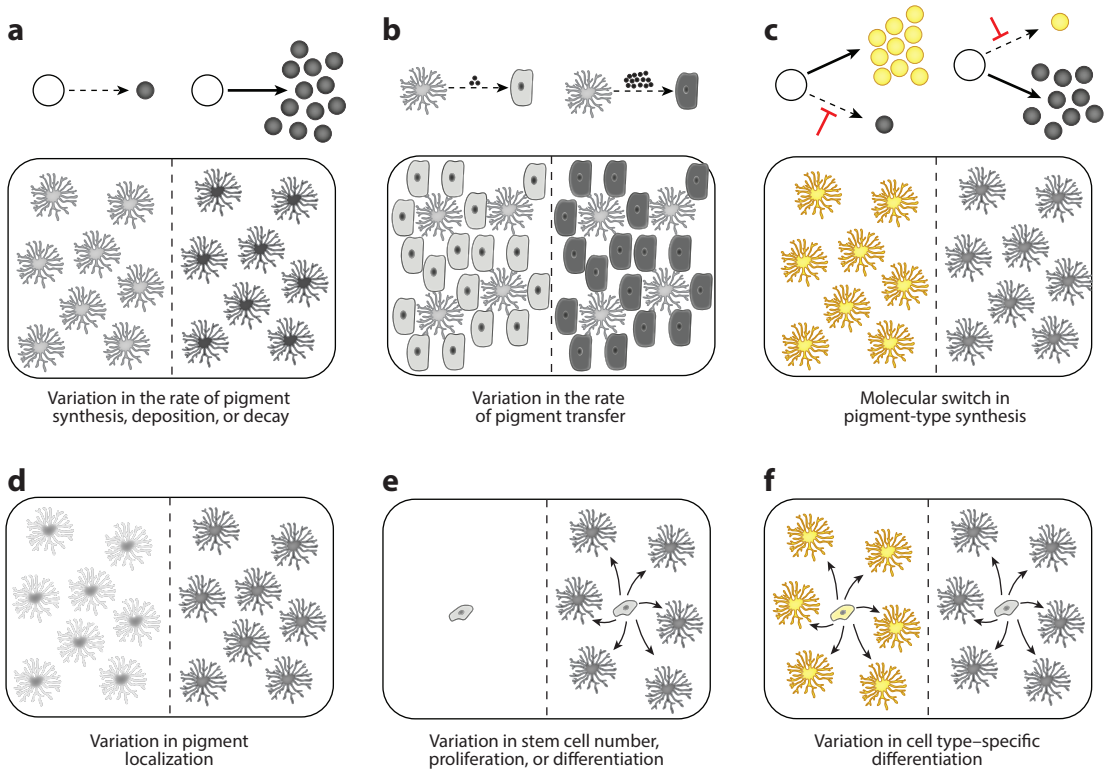

Figure 6

Color pattern implementation. Different cellular and intracellular processes can underlie color patterns. (*a*,*b*) Variation in the pigment (*a*) produced or (*b*) deposited/transferred to other cells can drive differences in pigmentation and the formation of patterns. (*c*) A molecular switch in pigment production from one pigment to another can also underlie the formation of a color pattern. (*d*) In some species, chromatophores can change the intracellular distribution of melanin through (*left*) aggregation and (*right*) dispersal of melanosomes. (*e*) Differences in the proliferation or differentiation of chromatophores can cause spatial variation in their numbers. (*f*) Differences in the type of chromatophores produced can also cause a color pattern.

preferentially binds to *Mc1r*, and this causes a switch to light (yellow to red) pheomelanin production (2). Other vertebrates, including amphibians, reptiles, and fish, have additional mechanisms that can change the intracellular positioning of pigment-bearing organelles or the size of the cells (**Figure 6*d***). For example, aggregation of melanin at the center of cells makes the skin appear lighter or reveals colors beneath the cells, while dispersal of melanin can result in darker skin (70). If melanophores are unevenly distributed across the skin or differ in their ability to aggregate or disperse melanin, a color pattern will emerge (or this process will contribute to an existing pattern) (44). In chameleons, it has also been shown that an active compression of cells can result in the color changes often observed in these animals (73). Also, in fish, including zebrafish (21) and neon tetra (26), differences in color patterns can be generated by changes in the orientation of light-interfering guanine platelets or their spacing (26).

At the cellular level, changes in color can also be the consequence of variation in chromatophore number or type. Chromatophore numbers can vary due to differences in the quantity of chromatophore progenitors or in their proliferation and rate of differentiation (**Figure 6*e***). Reptiles, amphibians, and fish have a diverse set of chromatophore cell types (68), with independent cell types contributing to specific colors (e.g., yellow to red xanthophores and erythrophores

as well as silver-blue iridophores). Spatial variation in the distribution of each of these different cell types results in a wide range of color pattern diversity (**Figure 6*f***).

Patterns are often generated by a combination of these mechanisms. For example, vertical bar patterns in cichlid fish are shaped by variations in chromatophore types, intracellular pigment localization, and pigment production (44). In *Peromyscus* mice, variation in color pattern between populations living in different substrates is produced by both changes in the position of the dorsoventral pigmentation boundary and the amount of pigment that is deposited into individual hairs (1, 47, 48). Both processes are controlled by isoform-specific differences in *Agouti* expression: During development, a ventral-specific *Agouti* isoform establishes an embryonic prepattern that sets the dorsoventral pigmentation boundary. During postnatal stages, a hair cycle–specific isoform regulates the amount of pheomelanin deposited into each hair (78). Remarkably, natural selection can promote independent changes in either of these traits by targeting different regions in the *Agouti* locus, both in coding and in noncoding regions, underscoring the modular nature of color-producing mechanisms (1, 48, 50, 52).

4. THE MAINTENANCE OR MODIFICATION OF COLOR PATTERNS

As discussed above, most processes that operate to establish and implement color patterns take place during embryonic development and initial postnatal days. As such, most studies have focused on examining the processes occurring at these critical stages. However, a key topic that remains largely unexplored concerns the set of mechanisms by which color patterns are maintained (or modified) throughout the life of an organism. In some species, the color pattern seen during early postnatal stages remains consistent across life, regardless of the number of times hairs, feathers, or scales cycle or are lost. This contrasts with what is seen in other species, in which embryonic and juvenile patterns are drastically different from adult ones. Key insights into what promotes the maintenance of color patterns and what causes temporal dynamics of coloration (**Figure 2*e***) in the different groups of vertebrates have come from studies in snowshoe hares, fish, and lizards.

Snowshoe hares undergo seasonal changes in hair color. During spring, the hare's entire white coat is replaced by a shorter coat that ranges from tan to brown. In late autumn, the reverse process takes place, and the tan/brown coat is replaced by the characteristic white winter coat. This transition is regulated by seasonal expression of the pigmentation gene *Agouti*. Specifically, differences in *Agouti* regulation underlie the loss of pigmentation in hairs that grow after the autumn molt (30). Although the seasonal changes seen in snowshoe hares occur throughout the entire body and, as such, do not constitute what we define as a color pattern, these findings are interesting because they suggest that temporal variation in the transcription of pigmentation genes may be a common mechanism by which melanocyte behavior is modified throughout multiple hair or feather cycles. For example, it is likely that the keratinocytes and/or melanocytes in mammalian and avian species displaying marked differences between embryonic/juvenile and adult patterns (e.g., tapirs or quails) undergo drastic changes in their epigenetic and signaling environments throughout the life of the organism. These dramatic changes contrast with other species in which the color pattern seen during early postnatal stages remains consistent across the life of an animal, regardless of the number of times hairs or feathers cycle or are lost [e.g., African striped mice or estrildid finches (a subfamily of small passerine, or perching, birds)]. This consistency in color implies that skin cells in these species acquire epigenetic changes during pattern establishment that are permanently incorporated into the signaling centers controlling pigment production, leading to predictable transcriptional patterns that are sustained throughout life.

Since fish and reptiles have multiple cell types involved in color pattern establishment, factors other than or in addition to transcriptional changes may be responsible for color pattern maintenance or changes. For example, in a recent study, Liang et al. (45) found that the ontogenetic,

sex-linked color change in the African cichlid fish *Melanochromis auratus* can be attributed to changes across multiple levels of biological organization, including chromatophore number, intracellular dispersal of pigments, and tilting of reflective guanine platelets. Neural innervation has been suggested to have a role in triggering the color pattern dynamics. In another teleost fish, the anemone fish, the thyroid hormone has been suggested to play a role in the formation and plasticity of their iconic white-orange bar pattern (65). In reptiles, Zhang and colleagues (81) examined ontogenetic changes in the coloration of lizard tails and found that light scattering in juveniles is caused by premature guanine crystals in underdeveloped iridophore cells. As individuals become adults, different tail coloration patterns emerge as a result of the reorganization of these guanine crystals during chromatophore maturation.

While the examples discussed above exemplify the marked plasticity that color patterns in fish and reptiles can experience, several studies also point to the existence of mechanisms that actively control pattern stasis. For example, ablating different regions of zebrafish skin with laser irradiation led to the regeneration of color patterns, demonstrating that pattern regeneration occurs through autonomous mechanisms (80). In the case of cichlids, *agrp2* may act throughout the life of the fish to actively and permanently repress the implementation of one pattern (e.g., horizontal stripes) but not other patterns (e.g., vertical bars and dorsoventral patterning) (38). It is possible that the same factors that contribute to the maintenance of patterns may, when deregulated, also be responsible for triggering morphological color change (i.e., temporal dynamics on longer timescales ranging from days to weeks). Moreover, on an evolutionary scale and as speculated for the cichlid fish stripe example, they might be evolutionary hotspots that drive the diversification and repeated evolution of these patterns (37).

5. OUTSTANDING QUESTIONS AND HOW THEY CAN BE ADDRESSED

Despite key insights into the mechanistic basis of color pattern formation obtained from several recent groundbreaking studies, many of them covered here, there are questions and topics that remain elusive. We argue that breaking down the formation of color patterns into pattern establishment and pattern implementation is helpful in not only dissecting the individual steps involved in their development but also comprehending the processes that contribute to their robustness, variability, and evolvability. Below, we highlight four key outstanding questions and discuss how different models and novel methodological approaches can be used to address them.

5.1. What Provides Positional Information During Prepattern Establishment?

The investigation of the earlier steps of color pattern formation—when prepatterns are formed—requires a precise molecular dissection of the processes operating during pattern establishment. To do so, it is necessary to identify the tissues and/or organs responsible for providing positional information. Critical first steps in this direction have been taken in studies of a few species. For example, as discussed above, zebrafish myosepta guiding the migration of chromatophore progenitors provide positional information for stripe formation (14). However, how the migratory patterns of chromatophores vary within vertebrates and how this influences adult patterns remain unclear. In addition, it is possible that there are differences in the timing of migration events, as well as in the guiding cues that affect pigment progenitor migration. To obtain insights into these poorly understood processes, one can take a comparative approach. Specifically, lineage-tracing experiments using Cre/loxP-mediated recombination in neural crest–derived progenitors, such as those used in zebrafish (69), could be used in phenotypically different but closely related species.

The *choker* zebrafish mutant is an interesting example in which only the establishment of the pattern but not its implementation is affected. Searching for more mutants affecting pattern

establishment, either in laboratory mutants or in species displaying naturally occurring variation in pattern establishment, could provide valuable information on the mechanisms by which prepattern formation leads to color pattern diversity. For example, the recent work by Haupaix et al. (22) represents an interesting example of a tissue (i.e., the developing somitic mesoderm), providing positional information. The molecular identity of the instructive factor from the somitic mesoderm remains elusive, making this a logical next step to pursue. Moreover, on an evolutionary scale, it would be fascinating to study how well this instructive factor is conserved across species or how changes may contribute to color pattern variation.

In many cases, such as in the examples of zebrafish and galliform birds discussed above, there is a clear existence of a prepattern conveying positional information. In other cases, however, similar prepatterns have not been identified. For example, it is unclear whether the vertical pattern (often also referred to as vertical bars) commonly found in different vertebrate groups, particularly in different snake and fish species, involves the existence of a prepattern. Studies in zebrafish demonstrate that clones from the same pigment cell progenitors are restricted to specific anterior-posterior regions, suggesting the existence of a constraint limiting the anterior-posterior spread of cells (69). The zebrafish, *Danio rerio*, does not have vertical patterns, but it is possible that this feature provides instructive information in other species, including some of the relatives of the zebrafish that do have them. Mechanistically, it remains unclear whether the limited anterior-posterior spread of clonal populations occurs through restricted migration (**Figure 5*b***) or if it is simply due to self-organization or random clustering of the clones (**Figure 5*c***).

On an evolutionary scale, the study of how variation and loss of prepatterns during evolution influence color pattern characteristics, such as their complexity and heterogeneity, would be interesting. In many species (e.g., tigers or zebras), color patterns show a high degree of interindividual variation, such that they are used to recognize individuals of the same species, analogous to the way that fingerprints are used to identify humans. That is, although all tigers and zebras have stripes organized in a similar dorsoventral fashion, such patterns vary substantially among individuals, and even among littermates. In stark contrast, other species (e.g., chipmunks or African striped mice) show remarkable interindividual consistency in their color patterns, making it very difficult to distinguish individuals based on this trait. Even though similar genes may operate in implementing the patterns seen in zebras, tigers, and rodents, the marked differences in the spatial organization suggest that the upstream mechanisms controlling the establishment of these patterns are fundamentally different. For example, in recent studies in African striped mice described above, the *Sfrp2* modulatory signal is always highest in the dorsalmost region of the embryo and decreases ventrally (29), which likely acts to constrain the prepattern, leading to reduced coat variation among individuals. By contrast, in domestic cats, while a Wnt modulator (*Dkk4*) is also responsible for establishing the prepattern, the expression of this gene does not occur in a predictable (i.e., constraining) spatial pattern (32), likely explaining why there is higher interindividual color pattern variation.

In summary, we believe that future research should focus on identifying the tissues, molecular agents, and developmental mechanisms that provide positional information during prepattern establishment. Doing so will provide a better understanding of how changes in these prepatterns underlie and facilitate within- and between-species variations in nature.

5.2. How Do Prepatterns Become Implemented Patterns?

Pigment pattern implementation is ultimately driven by processes that cause variations in cell identities, states, and their respective gene expression profiles. As such, a fundamental question that remains largely unanswered is how genes involved in implementing color patterns achieve spatially

restricted expression patterns. Key insights into this can be obtained by comparing gene expression profiles across anatomical regions differing in coloration. For example, transcriptomic comparisons between yellow and black regions in cheetahs identified the paracrine signaling molecule *Edn3* as a key factor controlling differences in hair color (33). In rodents, *Alx3* has repeatedly evolved in distantly related striped species to regulate differences between dark and light hair (49). Recent developments in single-cell RNA sequencing technologies now allow the investigation of these questions at higher resolution. In mammals, for example, dermal papilla cells are responsible for controlling the secretion of key paracrine signaling factors that regulate melanocyte behavior, such as *Edn3* and *Agouti*. Because dermal papilla cells represent a small fraction of the cells that constitute the dermis, bulk RNA analyses may not provide adequate resolution for detecting subtle expression differences (see, e.g., 38).

Once candidate genes mediating pattern implementation are identified, a possible next step is to uncover the regulatory mechanisms controlling the spatially restricted expression of such genes. Recently, several experimental strategies have been devised [e.g., chromatin immunoprecipitation sequencing (ChIP-seq), assay for transposase-accessible chromatin using sequencing (ATAC-seq), deoxyribonuclease (DNase), and cleavage under targets and tagmentation (CUT&Tag)] to identify genomic regions displaying hallmark signatures of active *cis*-regulatory elements, regions of the genome that mediate differences in spatiotemporal gene expression patterns. Due to its relative technical simplicity and low input sample requirements, ATAC-seq has emerged as one of the most widely used methods, particularly for research in nontraditional model organisms. In the context of studying pigment pattern formation, this technique has already proven to be tremendously useful in understanding pigment gene regulation in various butterfly species (41, 42, 54), as it allows researchers to profile chromatin states from tissues collected from different regions of the body, the same tissue at different developmental time points, or the same tissue in organisms differing in their phenotypes. We anticipate that this strategy will become widely used in future studies of vertebrate pigmentation. For example, as discussed in previous sections, a variety of pigmentation phenotypes across different taxa—from the dorsoventral pigmentation boundary in many vertebrates to the stripe patterns present in juvenile galliform birds—result from spatial-specific differences in *Agouti* expression (22, 52). By interrogating chromatin states from different skin regions, it is possible to uncover the upstream molecular factors binding to *Agouti cis*-regulatory elements and establish the mechanisms responsible for achieving spatial-specific expression of this gene. As is the case with transcriptomic approaches, methods for profiling chromatin states at the single-cell level are increasingly being used, providing the ability to study the questions outlined here at much higher cellular resolution.

By integrating multiple experimental strategies, such as those discussed above, it is possible to identify the genes and pathways associated with pigment pattern establishment and implementation. However, to distinguish correlation from causation and gain a mechanistic understanding of how such genes and molecular pathways act to promote differences in pigmentation, it is critical to incorporate functional experiments aimed at testing gene function. In recent years, studies in pigment pattern formation have employed a variety of functional approaches to directly test hypotheses about gene function, ranging from transgenic in vivo manipulations to in vitro cellular assays to grafting experiments. For example, because they allow for the delivery of viruses carrying genes of interest into developing mammalian embryos, in utero lentiviral injections have proven to be a powerful approach to test the function in nontraditional rodents. Using this technique, Manceau et al. (52) established that ectopic expression of *Agouti* leads to differences in melanocyte maturation, indicating this gene is responsible for promoting the dorsoventral differences in pigmentation seen in deer mice. More recently, Mallarino et al. (49) used this same approach, coupled with in vitro assays in melanocytes, to show that *Alx3* acts to repress melanocyte differentiation,

explaining why hair follicles of the light stripes in African striped mice fail to differentiate and produce melanin. Unlike mammals, avian embryos are readily accessible, allowing researchers to use different approaches that are difficult to implement in other groups. Recently, Haupaix et al. (22) used heterospecific grafting experiments between different galliform species to show that the somitic mesoderm can autonomously instruct *agouti* expression and control the position and width of yellow stripes. Lastly, as in almost every area of biology, CRISPR-Cas9 genome editing has revolutionized pigmentation research in nonmodel organisms by allowing scientists to manipulate genomes and understand gene function in an unprecedented way (53). CRISPR-Cas9 allows for experimental testing of hypotheses related to the genes and mutations identified through association mapping and transcriptomics. It therefore provides new avenues for investigating not only the relationship between genotype and phenotype but also the cellular and developmental mechanisms that connect them. For example, by knocking out *agrp2* in an African cichlid fish, a recent study demonstrated that losses and gains of horizontal stripe color patterns are driven by recurring regulatory changes in a single gene (39). The study demonstrates that stripe patterns reemerge when *agrp2* is knocked out in a stripeless species and provides convincing evidence that *agrp2* is required for striped pattern repression. CRISPR approaches have also been recently adopted in studies of wild-derived mammal species to address questions related to color pattern formation. In a recent preprint, Johnson et al. (29) developed and employed in vivo gene editing methods in African striped mice, representing the first time this has been achieved in a wild-derived mammal. Because this approach uses viruses to deliver genome editing reagents directly into the pregnant uterus, bypassing the common drawbacks associated with traditional ex vivo transgenic approaches used in laboratory mice, this work may open the door to performing functional studies in other nontraditional mammal species.

5.3. What Factors Shape the Characteristics of Self-Organized and Nonorganized Patterns?

Even though theoretical simulations involving Turing reaction–diffusion systems closely recapitulate many animal patterns seen in nature, the molecules mediating such interactions remain largely elusive. The most detailed cellular and molecular insights come from the stripes of the zebrafish model. However, how patterns in other species form is unclear. In many cases, such as in African cichlid fishes, the distribution of chromatophores does not exhibit the strict spatial organization seen in zebrafish because the different cell types tend to co-occur in the same regions. This suggests that the formation of these patterns is not driven by cell–cell interactions and that the mechanisms involved are fundamentally different from those observed in zebrafish. Also, the egg spots, colorful markings on the anal fin of many male cichlid fish species (66), might constitute interesting case studies to investigate the molecular mechanisms of self-organized patterns. Consequently, the precise processes underlying pattern formation and implementation in these organisms remain unclear and should be comparatively investigated (45). Since transgenic and knockout approaches are being developed in a growing number of nontraditional fish species, this avenue may constitute a powerful strategy for performing comparative analysis of self-organizing patterns (67).

Nonorganized patterns (i.e., patterns that are largely formed by stochastic events) are rare in nature but do occur. For example, the *pax7a* mutation in cichlid fishes leads to irregular melanophore blotches (63), a phenotype that resembles the piebald patterns seen in many domestic animals (3, 31). Similarly, rare mutations in the gene *Taqpep* are responsible for causing the coat patterns in cheetahs to go from spotty (in the spotted cheetah) to striped (in the king cheetah) (33). Studying these natural mutants may reveal insights into what factors influence the phenotype of the pattern. For example, what determines the proportion and size of melanic regions and

whether there are certain regions that are always pigmented or always devoid of pigmentation remain unknown.

5.4. How Does Color Pattern Development Constrain or Facilitate Pattern Variation and Evolutionary Diversification?

As we have discussed in this review, color patterns have evolved in different species within each of the major vertebrate clades. Among each group, color patterns vary considerably in complexity and design, ranging from simple markings to more elaborate reticulate designs. However, despite this variation, some of the patterns seen among members of the same taxonomic group show certain features that seem to be fixed, implying that variation is constrained by processes controlling their development. In an effort to gain insights into this, Hidalgo et al. (24) surveyed plumage color patterns in a large variety of passerine birds and found that, despite extensive phenotypic variation in adults, these differences could be traced back to a limited set of regions in the embryos. Moreover, a defined set of molecules varied in expression within such regions and correlated with differences in adult coloration. Thus, between-species color pattern differences result from the differential regulation of pigment production within a limited set of skin regions. This result suggests that, despite the vast diversity of color patterns seen in passerine birds, embryonic development constrains the set of possible phenotypes that can be generated. As shown by Hidalgo et al. (24), detailed pattern cataloging coupled to developmental characterization can reveal key insights into the developmental constraints underlying pattern diversity. Whether this same pattern holds for other vertebrate groups remains to be shown. More generally, studies and meta-analyses that compare color pattern formation and implicated genes across organisms might improve our understanding of color pattern evolution. A newly developed database, Gephebase (8), could be mined to establish whether color pattern evolution (in contrast to overall changes in color) is more frequently associated with *cis*-regulatory changes (11). In addition, it would be valuable to expand such meta-analyses to the specific developmental processes and types of color pattern formation that we discuss here.

A major challenge to studying the evolutionary dynamics and interindividual variation of color patterns is the phenotypic characterization of the patterns themselves. Recently, a wide range of tools have been developed to comprehensively compare color patterns across species (76, 79). The heterogeneity of patterns, both within an individual and between individuals, poses a challenge in distinguishing the characteristics that are predetermined by prepatterns from those that are influenced by stochastic or reaction–diffusion model dynamics occurring independently or superimposed on prepatterns. For example, if random factors drive the variation of the position or orientation of a stripe pattern with respect to homologous landmarks, but genetic factors determine the thickness and spacing of the stripes, extracting measures of those elements that are genetically determined might be challenging without any prior information. Using measures of pattern complexity (56) or a combination of various measures to create high-dimensional morphospaces (55) has already been demonstrated to be a suitable strategy for identifying the characteristics that are modified during evolutionary diversification. However, these comparisons become even more challenging when comparing species that differ markedly in their morphology or have complex patterns that are driven by micropattern variation (22) or by independent, superimposed color patterns [e.g., stripe and dorsoventral pattern variation in zebrafish (6) or horizontal and vertical patterns in cichlids (16, 38)]. As methods for color pattern cataloging improve and become automated, we anticipate these approaches will increase in precision and expand to a broader taxonomic sampling, providing key insights into the interplay between developmental constraints and pattern diversity. Ultimately, it might be possible to link features such as heterogeneity and complexity of color patterns to developmental or genetic characteristics.

6. CONCLUSION

Although numerous groundbreaking studies have shed light on the genetic and developmental mechanisms that underlie animal color patterns, our understanding of these processes is far from complete. To provide a more comprehensive framework, we have proposed a classification of the development of color patterns into two distinct stages—pattern establishment and pattern implementation—and four mechanistic trajectories—predetermined, prespecified, self-organized, and nonorganized patterns. Moreover, we have discussed the various mechanisms that operate throughout development and postnatal stages to establish, implement, maintain, and modify color patterns. Lastly, we have suggested a set of outstanding questions that can serve to guide future studies in the field. We anticipate that this conceptualization will aid in comprehending the underlying processes that contribute to the diversity and features of animal color patterns and inspire new research directions.

SUMMARY POINTS

1. The classification of animal color patterns is challenging, as they vary in spatial arrangement, orientation, complexity, heterogeneity, temporal dynamics, and scale.
2. Many color patterns form in two steps: The first, referred to as pattern establishment, leads to the formation of a prepattern, or blueprint; the second, known as pattern implementation, interprets the information laid out by the blueprint and executes the pigmented pattern. Both processes can also occur in a single step, without the formation of a prepattern.
3. Prepatterns are instructed by spatial information, which can be provided by morphological (e.g., migratory corridors) and/or molecular (e.g., paracrine factors) cues.
4. The implementation of a pattern can be further or solely influenced by stochastic and reaction–diffusion model parameters.
5. Pattern implementation takes place through multiple mechanisms leading to the appearance of spatial variation in coloration, such as changes in cellular and subcellular features, deposition, the morphology of the integument, and the relative abundance of pigments.
6. While color pattern establishment and implementation typically occur during embryogenesis/early postnatal stages, there are several processes that act throughout an animal's life to either modify or consistently maintain color patterns.

FUTURE ISSUES

1. What are the mechanisms by which prepatterns are established?
2. How do prepatterns become translated and reorganized into the implemented pattern?
3. What factors influence the characteristics of self-organized and nonorganized patterns?
4. How do different mechanisms of color pattern formation either limit or enable interspecies variation and evolutionary diversification?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by an Academy of Finland Fellowship (347309) and a Sigrid-Jusélius Foundation grant to C.F.K. and a National Institutes of Health grant (R35GM133758) to R.M. We express our gratitude to Marie Manceau and David Parichy for their valuable feedback and insightful suggestions.

LITERATURE CITED

1. Barrett RDH, Laurent S, Mallarino R, Pfeifer SP, Xu CCY, et al. 2019. Linking a mutation to survival in wild mice. *Science* 363(6426):499–504
2. Barsh GS. 1996. The genetics of pigmentation: from fancy genes to complex traits. *Trends Genet*. 12:299–305
3. Brancalion L, Haase B, Wade CM. 2022. Canine coat pigmentation genetics: a review. *Anim. Genet.* 53(1):3–34
4. Caro T, Mallarino R. 2020. Coloration in mammals. *Trends Ecol. Evol.* 35(4):357–66
5. Caro T, Stoddard MC, Stuart-Fox D. 2017. Animal coloration: production, perception, function and application. *Philos. Trans. R. Soc. B* 372(1724):20170047
6. Ceinos RM, Guillot R, Kelsh RN, Cerdá-Reverter JM, Rotllant J. 2015. Pigment patterns in adult fish result from superimposition of two largely independent pigmentation mechanisms. *Pigment Cell Melanoma Res*. 28(2):196–209
7. Cortés R, Navarro S, Agulleiro MJ, Guillot R, García-Herranz V, et al. 2014. Evolution of the melanocortin system. *Gen. Comp. Endocrinol.* 209:3–10
8. Courtier-Orgogozo V, Arnoult L, Prigent SR, Wiltgen S, Martin A. 2020. Gephebase, a database of genotype–phenotype relationships for natural and domesticated variation in Eukaryotes. *Nucleic Acids Res*. 48(D1):D696–703
9. Cuthill IC, Allen WL, Arbuckle K, Caspers B, Chaplin G, et al. 2017. The biology of color. *Science* 357(6350):eaan0221
10. D'Mello SAN, Finlay GJ, Baguley BC, Askarian-Amiri ME. 2016. Signaling pathways in melanogenesis. *Int. J. Mol. Sci.* 17(7):1144
11. Elkin J, Martin A, Courtier-Orgogozo V, Santos ME. 2022. Meta-analysis of the genetic loci of pigment pattern evolution in vertebrates. bioRxiv 2022.01.01.474697. **https://doi.org/10.1101/2022.01.01.474697**
12. Feigin C, Li S, Moreno J, Mallarino R. 2022. The GRN concept as a guide for evolutionary developmental biology. *J. Exp. Zool. B Mol. Dev. Evol.* 340(2):92–104
13. Feiner N, Brun-Usan M, Andrade P, Pranter R, Park S, et al. 2022. A single locus regulates a female-limited color pattern polymorphism in a reptile. *Sci Adv*. 8(10):eabm2387
14. Frohnhöfer HG, Krauss J, Maischein H-M, Nüsslein-Volhard C. 2013. Iridophores and their interactions with other chromatophores are required for stripe formation in zebrafish. *Development* 140(14):2997–3007
15. Galván I, Solano F. 2016. Bird integumentary melanins: biosynthesis, forms, function and evolution. *Int. J. Mol. Sci.* 17(4):520
16. Gerwin J, Urban S, Meyer A, Kratochwil CF. 2021. Of bars and stripes: A Malawi cichlid hybrid cross provides insights into genetic modularity and evolution of modifier loci underlying colour pattern diversification. *Mol. Ecol.* 30(19):4789–803
17. Goda M, Fujii R. 1995. Blue chromatophores in two species of callionymid fish. *Zool. Sci.* 12(6):811–13
18. Goutte S, Hariyani I, Utzinger KD, Bourgeois Y, Boissinot S. 2022. Genomic analyses reveal association of *ASIP* with a recurrently evolving adaptive color pattern in frogs. *Mol. Biol. Evol.* 39(11):msac235

19. Grahn RA, Lemesch BM, Millon LV, Matise T, Rogers QR, et al. 2005. Localizing the X-linked orange colour phenotype using feline resource families. *Anim. Genet.* 36(1):67–70
20. Guo L, Bloom J, Sykes S, Huang E, Kashif Z, et al. 2021. Genetics of white color and iridophoroma in "Lemon Frost" leopard geckos. *PLOS Genet* 17(6):e1009580
21. Gur D, Bain EJ, Johnson KR, Aman AJ, Pasolli HA, et al. 2020. In situ differentiation of iridophore crystallotypes underlies zebrafish stripe patterning. *Nat. Commun.* 11(1):6391
22. Haupaix N, Curantz C, Bailleul R, Beck S, Robic A, Manceau M. 2018. The periodic coloration in birds forms through a prepattern of somite origin. *Science* 361(6408):eaar4777
23. Hendrick LA, Carter GA, Hilbrands EH, Heubel BP, Schilling TF, Le Pabic P. 2019. Bar, stripe and spot development in sand-dwelling cichlids from Lake Malawi. *EvoDevo* 10:18
24. Hidalgo M, Curantz C, Quenech'Du N, Neguer J, Beck S, et al. 2022. A conserved molecular template underlies color pattern diversity in estrildid finches. *Sci. Adv.* 8(35):eabm5800
25. Hirata M, Nakamura K-I, Kondo S. 2005. Pigment cell distributions in different tissues of the zebrafish, with special reference to the striped pigment pattern. *Dev. Dyn.* 234(2):293–300
26. Hiroshi N, Noriko O, Ryozo F. 1990. Light-reflecting properties of the iridophores of the neon tetra, *Paracheirodon innesi*. *Comp. Biochem. Physiol. A Physiol.* 95(3):337–41
27. Inaba M, Chuong C-M. 2020. Avian pigment pattern formation: developmental control of macro- (across the body) and micro- (within a feather) level of pigment patterns. *Front. Cell Dev. Biol.* 8:620
28. Inaba M, Jiang T-X, Liang Y-C, Tsai S, Lai Y-C, et al. 2019. Instructive role of melanocytes during pigment pattern formation of the avian skin. *PNAS* 116(14):6884–90
29. Johnson MR, Li S, Guerrero-Juarez CF, Miller P, Brack BJ, et al. 2023. *Sfrp2* is a multifunctional regulator of rodent coat patterns. bioRxiv 2022.12.12.520043. **https://doi.org/10.1101/2022.12.12.520043**
30. Jones MR, Mills LS, Alves PC, Callahan CM, Alves JM, et al. 2018. Adaptive introgression underlies polymorphic seasonal camouflage in snowshoe hares. *Science* 360(6395):1355–58
31. Kaelin CB, Barsh GS. 2013. Genetics of pigmentation in dogs and cats. *Annu. Rev. Anim. Biosci.* 1:125–56
32. Kaelin CB, McGowan KA, Barsh GS. 2021. Developmental genetics of color pattern establishment in cats. *Nat. Commun.* 12(1):5127
33. Kaelin CB, Xu X, Hong LZ, David VA, McGowan KA, et al. 2012. Specifying and sustaining pigmentation patterns in domestic and wild cats. *Science* 337(6101):1536–41
34. Kimura T, Nagao Y, Hashimoto H, Yamamoto-Shiraishi Y-I, Yamamoto S, et al. 2014. Leucophores are similar to xanthophores in their specification and differentiation processes in medaka. *PNAS* 111(20):7343–48
35. Kondo S, Miura T. 2010. Reaction-diffusion model as a framework for understanding biological pattern formation. *Science* 329(5999):1616–20
36. Kondo S, Watanabe M, Miyazawa S. 2021. Studies of Turing pattern formation in zebrafish skin. *Philos. Trans. R. Soc. A* 379(2213):20200274
37. Kratochwil CF. 2019. Molecular mechanisms of convergent color pattern evolution. *Zoology* 134:66–68
38. Kratochwil CF, Liang Y, Gerwin J, Franchini P, Meyer A. 2022. Comparative ontogenetic and transcriptomic analyses shed light on color pattern divergence in cichlid fishes. *Evol. Dev.* 24(5):158–70
39. Kratochwil CF, Liang Y, Gerwin J, Woltering JM, Urban S, et al. 2018. Agouti-related peptide 2 facilitates convergent evolution of stripe patterns across cichlid fish radiations. *Science* 362(6413):457–60
40. Kratochwil CF, Meyer A. 2015. Closing the genotype-phenotype gap: emerging technologies for evolutionary genetics in ecological model vertebrate systems. *Bioessays* 37(2):213–26
41. Lewis JJ, Geltman RC, Pollak PC, Rondem KE, Van Belleghem SM, et al. 2019. Parallel evolution of ancient, pleiotropic enhancers underlies butterfly wing pattern mimicry. *PNAS* 116(48):24174–83
42. Lewis JJ, Van Belleghem SM, Papa R, Danko CG, Reed RD. 2020. Many functionally connected loci foster adaptive diversification along a neotropical hybrid zone. *Sci. Adv.* 6(39):eabb8617
43. Lewis VM, Saunders LM, Larson TA, Bain EJ, Sturiale SL, et al. 2019. Fate plasticity and reprogramming in genetically distinct populations of *Danio* leucophores. *PNAS* 116:11806–11
44. Liang Y, Gerwin J, Meyer A, Kratochwil CF. 2020. Developmental and cellular basis of vertical bar color patterns in the East African cichlid fish *Haplochromis latifasciatus*. *Front. Cell Dev. Biol.* 8:62
45. Liang Y, Meyer A, Kratochwil CF. 2020. Neural innervation as a potential trigger of morphological color change and sexual dimorphism in cichlid fish. *Sci. Rep.* 10(1):12329

46. Ligon RA, McCartney KL. 2016. Biochemical regulation of pigment motility in vertebrate chromatophores: a review of physiological color change mechanisms. *Curr. Zool.* 62(3):237–52
47. Linnen CR, Kingsley EP, Jensen JD, Hoekstra HE. 2009. On the origin and spread of an adaptive allele in deer mice. *Science* 325(5944):1095–98
48. Linnen CR, Poh Y-P, Peterson BK, Barrett RDH, Larson JG, et al. 2013. Adaptive evolution of multiple traits through multiple mutations at a single gene. *Science* 339(6125):1312–16
49. Mallarino R, Henegar C, Mirasierra M, Manceau M, Schradin C, et al. 2016. Developmental mechanisms of stripe patterns in rodents. *Nature* 539:518–23
50. Mallarino R, Linden TA, Linnen CR, Hoekstra HE. 2016. The role of isoforms in the evolution of cryptic coloration in *Peromyscus* mice. *Mol. Ecol.* 26(1):245–58
51. Manceau M, Domingues VS, Linnen CR, Rosenblum EB, Hoekstra HE. 2010. Convergence in pigmentation at multiple levels: mutations, genes and function. *Philos. Trans. R. Soc. B* 365(1552):2439–50
52. Manceau M, Domingues VS, Mallarino R, Hoekstra HE. 2011. The developmental role of Agouti in color pattern evolution. *Science* 331(6020):1062–65
53. Matthews BJ, Vosshall LB. 2020. How to turn an organism into a model organism in 10 'easy' steps. *J. Exp. Biol.* 223(Suppl. 1):jeb218198
54. Mazo-Vargas A, Langmüller AM, Wilder A, van der Burg KRL, Lewis JJ, et al. 2022. Deep cis-regulatory homology of the butterfly wing pattern ground plan. *Science* 378(6617):304–8
55. McCluskey BM, Liang Y, Lewis VM, Patterson LB, Parichy DM. 2021. Pigment pattern morphospace of *Danio* fishes: evolutionary diversification and mutational effects. *Biol. Open* 10(9):bio058814
56. Miyazawa S. 2020. Pattern blending enriches the diversity of animal colorations. *Sci Adv*. 6(49):eabb9107
57. Nachman MW, Hoekstra HE, D'Agostino SL. 2003. The genetic basis of adaptive melanism in pocket mice. *PNAS* 100(9):5268–73
58. Nüsslein-Volhard C, Singh AP. 2017. How fish color their skin: a paradigm for development and evolution of adult patterns: Multipotency, plasticity, and cell competition regulate proliferation and spreading of pigment cells in Zebrafish coloration. *Bioessays* 39(3):1600231
59. Orteu A, Jiggins CD. 2020. The genomics of coloration provides insights into adaptive evolution. *Nat. Rev. Genet.* 21(8):461–75
60. Patterson LB, Parichy DM. 2013. Interactions with iridophores and the tissue environment required for patterning melanophores and xanthophores during zebrafish adult pigment stripe formation. *PLOS Genet*. 9:e1003561
61. Patterson LB, Parichy DM. 2019. Zebrafish pigment pattern formation: insights into the development and evolution of adult form. *Annu. Rev. Genet.* 53:505–30
62. Protas ME, Hersey C, Kochanek D, Zhou Y, Wilkens H, et al. 2006. Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. *Nat. Genet.* 38(1):107–11
63. Roberts RB, Moore EC, Kocher TD. 2017. An allelic series at *pax7a* is associated with colour polymorphism diversity in Lake Malawi cichlid fish. *Mol. Ecol.* 26(10):2625–39
64. Rosenblum EB, Römpler H, Schöneberg T, Hoekstra HE. 2010. Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *PNAS* 107(5):2113–17
65. Salis P, Roux N, Huang D, Marcionetti A, Mouginot P, et al. 2021. Thyroid hormones regulate the formation and environmental plasticity of white bars in clownfishes. *PNAS* 118(23):e2101634118
66. Santos ME, Braasch I, Boileau N, Meyer BS, Sauteur L, et al. 2014. The evolution of cichlid fish egg-spots is linked with a *cis*-regulatory change. *Nat. Commun.* 5:5149
67. Santos ME, Lopes JF, Kratochwil CF. 2023. East African cichlid fishes. *EvoDevo* 14(1):1
68. Schartl M, Larue L, Goda M, Bosenberg MW, Hashimoto H, Kelsh RN. 2016. What is a vertebrate pigment cell? *Pigment Cell Melanoma Res*. 29(1):8–14
69. Singh AP, Dinwiddie A, Mahalwar P, Schach U, Linker C, et al. 2016. Pigment cell progenitors in zebrafish remain multipotent through metamorphosis. *Dev. Cell* 38(3):316–30
70. Sköld HN, Aspengren S, Cheney KL, Wallin M. 2016. Fish chromatophores—from molecular motors to animal behavior. *Int. Rev. Cell Mol. Biol.* 321:171–219
71. Sköld HN, Aspengren S, Wallin M. 2002. The cytoskeleton in fish melanophore melanosome positioning. *Microsc. Res. Tech.* 58(6):464–69

72. Streelman JT, Albertson RC, Kocher TD. 2003. Genome mapping of the orange blotch colour pattern in cichlid fishes. *Mol. Ecol.* 12(9):2465–71
73. Teyssier J, Saenko SV, van der Marel D, Milinkovitch MC. 2015. Photonic crystals cause active colour change in chameleons. *Nat. Commun.* 6:6368
74. Theron E, Hawkins K, Bermingham E, Ricklefs RE, Mundy NI. 2001. The molecular basis of an avian plumage polymorphism in the wild. *Curr. Biol.* 11(8):550–57
75. Tobin DJ. 2008. Human hair pigmentation–biological aspects. *Int. J. Cosmet. Sci.* 30(4):233–57
76. Van Belleghem SM, Papa R, Ortiz-Zuazaga H, Hendrickx F, Jiggins CD, et al. 2018. patternize: An R package for quantifying colour pattern variation. *Methods Ecol. Evol.* 9(2):390–98
77. Volkening A. 2020. Linking genotype, cell behavior, and phenotype: multidisciplinary perspectives with a basis in zebrafish patterns. *Curr. Opin. Genet. Dev.* 63:78–85
78. Vrieling H, Duhl DM, Millar SE, Miller KA, Barsh GS. 1994. Differences in dorsal and ventral pigmentation result from regional expression of the mouse agouti gene. *PNAS* 91:5667–71
79. Weller HI, Van Belleghem SM, Hiller AE, Lord NP. 2022. Flexible color segmentation of biological images with the R package recolorize. bioRxiv 2022.04.03.486906. **https://www.biorxiv.org/content/10.1101/2022.04.03.486906v3**
80. Yamaguchi M, Yoshimoto E, Kondo S. 2007. Pattern regulation in the stripe of zebrafish suggests an underlying dynamic and autonomous mechanism. *PNAS* 104(12):4790–93
81. Zhang G, Yallapragada VJ, Shemesh M, Wagner A, Upcher A, et al. 2022. Ontogenetic color switching in lizards as a by-product of guanine cell development. bioRxiv 2022.01.12.475993. **https://doi.org/10.1101/2022.01.12.475993**