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Not just black and white: pigment pattern development and evolution in vertebrates

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Abstract

Animals display diverse colors and patterns that vary within and between species. Similar phenotypes appear in both closely related and widely divergent taxa. Pigment patterns thus provide an opportunity to explore how development is altered to produce differences in form and whether similar phenotypes share a common genetic basis. Understanding the development and evolution of pigment patterns requires knowledge of the cellular interactions and signaling pathways that produce those patterns. These complex traits provide unparalleled opportunities for integrating studies from ecology and behavior to molecular biology and biophysics.

Keywords

chromatophore; neural crest; pigment pattern; animal colour

1. Introduction

In vertebrates, pigmentation and coloration vary widely from black and white stripes of zebra to muted browns in house sparrows to bright colors and bold patterns of tropical fish. Closely related species may show highly divergent patterns, while distantly related species can appear strikingly similar. Understanding the genetic and developmental basis of variation in form is of central importance to developmental biology. Pigment patterns present an ideal system in which to study how developmental changes generate differences in form both within and between species.

Our current understanding of pigment pattern development owes a great deal to amateur “geneticists” of the 17th and 18th century, who cultivated unique or striking coat colors in “fancy” mice [1,2]. Upon the rediscovery of Mendel's work, these mice afforded opportunities to study modes of inheritance and genetic interaction. A century later, over 100 coat color loci have been identified in mouse and nearly half of these have now been cloned [3]. This work identified genes important for the specification, differentiation and morphogenesis of pigment cells as well as those involved in pigment synthesis. Despite this

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progress, little is known about the molecular or cellular processes that generate complex patterns such as stripes and spots, or how alterations in those processes might produce different phenotypes.

Vertebrate pigment cells are derived from the neural crest, a transient population of cells formed during embryogenesis that gives rise to a wide range of additional cell types including glia, neurons, bone and cartilage [4]. In birds and mammals, unpigmented pigment cell precursors known as melanoblasts migrate from the neural crest to the epidermis and into developing feather or hair follicles [5,6]. Mature pigment cells known as melanocytes synthesize melanin pigment, package it into melanosomes and then transfer those melanosomes to keratinocytes for deposition into developing feathers and hairs. Though mammals and birds have only one pigment cell type, the melanocyte, these cells can produce either eumelanin (black/brown) or pheomelanin (yellow/red) and can switch rapidly between the synthesis of these two pigment types [7]. In contrast to mammals and birds, poikilotherm vertebrates such as fish and amphibians have multiple pigment cell types known as chromatophores: black melanophores, yellow xanthophores, red erythrophores, iridescent iridophores, white leucophores and blue cyanophores [8,9,10]. Rather than transferring their pigments to other cell types, chromatophores retain their pigments intracellularly. Despite these differences, many of the genetic pathways critical for melanocyte differentiation and morphogenesis are conserved in melanophores and other chromatophore types [8,11].

In addition to their utility for studying genetic and cellular mechanisms, pigment patterns also provide ample opportunities to explore relationships between phenotype and environment. While the diverse patterns of domestic animals have mainly aesthetic value, the pigment patterns of wild animals are of great importance to their fitness. These patterns serve a variety of functions including camouflage and warning coloration and influence aspects or behavior such as mate recognition, mate choice, and shoaling preference [12,13,14,15]. Their clear ecological significance makes pigment patterns potential targets of both natural selection and sexual selection. Thus far, the genetic bases of adaptive traits have been identified only in a few cases [16,17,18,19]. Pigment patterns present many exciting opportunities to study the genetics and development of these important traits.

In birds and mammals, pigmentation can vary across the entire body or across individual hairs or feathers. By varying the type of melanin produced in different regions of the body a wide range of patterns may be achieved. Poikilotherms, likewise, can arrange their chromatophores to achieve broad swaths of uniform color or complex patterns. In the following sections we review how various types of colors are achieved in different vertebrates, and then discuss how colors are arranged to produce various patterns.

2. Colors

2.1. Black, Brown and White: Melanism/Albinism

One of the most common pigment polymorphisms found in vertebrates is melanism [20]. In mammals, increased production of eumelanin and a corresponding reduction in pheomelanin synthesis generates a melanic phenotype, which may be dark brown or entirely black. This

switch is primarily controlled by the interaction of two genes: the *melanocortin receptor 1* (*Mc1r*) and *agouti*. Mc1r is a seven transmembrane G protein-coupled receptor expressed by melanocytes and agouti is a small paracrine signaling molecule expressed by dermal papilla cells [21,22]. In the absence of agouti, α-MSH (α melanocyte-stimulating hormone) stimulates Mc1r to synthesize eumelanin. When agouti is present, it inhibits Mc1r activity, causing the melanocyte to synthesize pheomelanin instead of eumelanin. In many mammals, including mice, *agouti* is normally expressed during the mid-portion of hair growth resulting in hairs with a black (eumelanin) base and tip and a yellow (pheomelanin) band in the middle.

Alterations to these two proteins are the most frequent causes of melanism in mammals (Table 1). In mice, dominant *Mc1r* or recessive *agouti* alleles increase eumelanin synthesis generating a black coat. Deletions in *agouti* are associated with recessive melanic phenotypes in domestic cats [23], horses [24] and Japanese quail [25]. Dominant black phenotypes are associated with *Mc1r* mutations in many mammalian species including pocket mice [18], pigs [26], sheep [27], jaguars and juguarundis [23]. Dominant *Mc1r* mutations in birds also cause melanism, but the effects appear more variable than in mammals (bananaquits [28]; artic skuas and lesser snow geese [29]; chickens [30,31]). While an association between black coloration and *Mc1r* mutations is observed in many species, in only a few cases has functional work determined that these mutations alter *Mc1r* activity [27,32]. This type of demonstration is critical since mutations in genes other than *Mc1r* or *agouti* can also generate melanism. One notable example is the cause of dominant black in domestic dogs. Linkage analysis and molecular cloning revealed that black coat color in domestic dogs results from a mutation in the β-defensin gene, *CBD103* [33,34]. *CBD103* is highly expressed in dog skin and competitively inhibits agouti binding to Mc1r. Mice over-expressing *CBD103* in an *agouti* background develop black coats, suggesting that *CBD103* expression in skin could produce black coats in other mammals as well [34].

In poikilotherms, α-MSH and MCH (melanin-concentrating hormone) have different effects depending on the duration of their signals. Short-term expression causes melanosomes to disperse (making the animal appear darker) or to aggregate (making the animal appear lighter) respectively, a set of processes known as physiological color change [35] (see "Green" section below). Longer-term expression of α-MSH and MCH causes alteration to the number of melanophores, known as morphological color change [36]. These alterations to melanization occur through some of the same pathways as mammalian melanism, but the mechanism in poikilotherms is reversible and does not involve genetic alteration. For example, darkening that occurs during adaptation to a black background in Mozambique tilapia is not associated with a change in the sequence or expression level of Mc1r[37].

Albinism is characterized by reduced melanin in the skin, hair and eyes. Hair follicles of albino mice contain melanocytes, but do not produce melanin. Like melanism, albinism has evolved repeatedly in widely divergent taxa, often through mutations in the same set of genes (Table 1). In humans, albinism is associated with mutations in several genes including *Tyrosinase* and *Tyrosinase related protein-1*, two enzymes required for melanin synthesis [38]. Mutations in ocular and cutaneous albinism 2 (*Oca2*) are another frequent cause of albinism in humans. Cave dwelling populations of Mexican tetra evolved albinism

independently multiple times. At least two of these independent populations contain genomic alterations in Oca2 that abrogate the protein's function in cell-culture assays [19].

2.2. Yellow-Orange-Red

Like black phenotypes, changes in a variety of genes can cause yellow, orange, or red coloration in vertebrates (Table 1), but unlike black, these colors can be produced by a wide range of pigments. In mammals and birds, loss of function mutations in *Mc1r* or dominant mutations in *agouti* cause increased pheomelanin synthesis, generating a yellow or red coat or feathers. Constitutive activation of *agouti* in mice and Japanese quail produce pheomelanic phenotypes [39]. Mutations in *Mc1r* are associated with yellow or red coats in several breeds of domestic dog [40,41], pigs [26], cows [42], horses [43], chickens [30] and Kermode bears [44]. A point mutation in Mc1r causing reduced α-MSH binding affinity is partially responsible for the light coloration of some populations of beach mice [45]. The differences in pigmentation between light beach mice and dark inland mice are quantitative differences and are produced by the differential activity of at least two major genes [46]. These results highlight the difficulties in determining the genes responsible for phenotypic variation between species, since in many cases changes in the activity of multiple genes contribute to the difference in phenotype.

While yellowish or rufous feathers in birds are often colored by some combination of pheomelanin and eumelanin [47], many striking yellow, orange and red feather colors are due instead to the presence of carotenoid pigments [48]. Animals cannot synthesize carotenoid pigments the way they synthesize melanin or other pigments, and therefore must obtain carotenoids from a dietary source. Carotenoids can be metabolized to alter the colors they produce (e.g., [49]) and in some cases, animals preferentially utilize certain dietary pigments over others (e.g., [50]).

In poikilotherms, colors in the yellow-to-red range are produced by xanthophores and erythrophores. Generally, xanthophores are yellow and erythrophores are red pigment-containing cells, although, both colors can be produced by a range of pteridine and carotenoid pigments and both xanthophores and erythrophores can contain both pigment types [48,51]. Xanthophore color can even vary between two closely-related species, for example they appear yellow in zebrafish *Danio rerio* and orange in its close relative, the pearl danio, *D. albolineatus* [52]. Differences between xanthophores and erythrophores beyond their color have been difficult to identify in part because models of xanthophore study, such as zebrafish and the medaka *Oryzias latipes*, both lack erythrophores. Within the *Danio* clade, the phylogenetic distribution of species with erythrophores suggests this cell type may have arisen or been lost independently multiple times [52,53].

Red coloration is less commonly generated in other ways, such as hemoglobin visible through the skin (e.g., [54]) and other rare pigments (see [55] for review). One additional red pigment type has been discovered: pterorhodin, a dark-red pteridine dimer, contained strangely enough in melanophores! This pigment was identified in only two groups of frogs, the phyllomedusids from the New World and the hylids from Australia [56,57]. Its presence in melanophores of these frogs, located within curiously large melanosomes, raises a number of questions about the evolutionary origin of pigment cells, the evolution of pigment

within the cells, and the evolution of the frog groups in question [57] – all of which remain to be answered.

2.3. Blue and other Structural Colors

With the exception of the extremely rare cyanophore [10], blues observed in vertebrates are not due to the presence of pigment. Instead, they are structural colors, caused by the reflection of short-wavelength light off nanostructures in the animal's skin or feathers [58]. Blue-greens, ultraviolet-blues, iridescent colors, and some whites also fall in this category.

In birds and mammals, blue-colored skin is caused by thick arrays of collagen fibers in the dermis [59,60]. These fibers are “quasi-ordered”: closely but not perfectly aligned with one another. The precise color produced is dependent on the diameter of fibers in the array, with smaller-diameter fibers reflecting shorter-wavelength light. The colors produced can therefore range from ultraviolet to (rarely) yellow or orange, although blue is most common. The collagen array is often underlain by melanin, which blocks reflective scatter from tissue beneath the array and absorbs wavelengths of light other than those reflected by the collagen. These color-producing collagen arrays have evolved repeatedly in both mammals and birds [59,60], but neither their development nor their evolution have been studied.

In birds' feathers, a matrix of air bubbles in keratin known as the “spongy layer” in the barbs produces non-iridescent colors dependent on the scale of the quasi-ordered matrix, similar to the scale-dependence of structural skin color [61]. Without underlying melanin, the scatter produced by this matrix appears pale [62], a condition that in some species has been exaggerated in the evolution of bright white feathers from structurally pigmented ones [61]. Iridescence, the color of which changes depending on the angle from which it is viewed, is produced by myriad patterns of alternating layers of keratin, air and melanin in barbules (subdivisions off the barbs) ([63] and references therein). This is the most common type of structural color in birds [61], and it may be particularly important as a source of UV reflectance.

Blue coloration in fish, amphibians and reptiles is due primarily to the presence of iridophores underlain by melanophores [58,64]. Iridophores and leucophores are both described as reflective or shiny pigment cells, with iridophores giving an iridescent shine and leucophores appearing white [10,51] or cream [65]. The appearance of both cell types is due to the presence of guanine and hypoxanthine crystals or platelets within the cells: longer, more horizontally arranged platelets in the iridophores; shorter, more vertically arranged platelets in leucophores [51]. These distinctions are certainly not always used: zebrafish have been occasionally reported to possess two types of iridophores, either gold and silver [66] or ‘S’ and ‘L’ for the short and long platelets in they contain [67,68], and these types may correspond to what other authors describe as iridophores and leucophores. White chromatophores have also been reported in the fins of zebrafish [66] and these may be leucophores [65]. Medaka have both iridophores and leucophores, and the broad array of mutants in which only one cell type or the other is affected offer an exciting opportunity to study the biology of these still-mysterious chromatophores [65].

2.4. Green and physiological color change

Combinations of structural blue color and pteridines or carotenoids can produce green skin or feathers [61,64,69] or purple feathers [48]. In a number of amphibians and reptiles, greens produced in this way can change to other colors like brown in anolis lizards or tree frogs [58,70]. Transitions between these colors are made possible by a three-dimensional arrangement of chromatophores known as a dermal chromatophore unit [71]. In amphibians and reptiles that undergo these significant physiological color changes, the dermal chromatophore unit is stereotypically made up of a single representative each (from top to bottom in the skin) of xanthophore, iridophore, and melanophore. Shifts between colors are caused by dispersion and aggregation of the chromatosomes in each layer of cells such that different wavelengths of light are absorbed or reflected. Changes in chameleon color and patterning are similarly caused by shifts in different layers of chromatophores.

While only a few fish undergo such striking physiological color changes (e.g. [72]), many exhibit lightening and darkening associated with changes in environmental conditions and stress. As mentioned previously, physiological color change in teleosts is controlled in part by the hormone pair MSH and MCH [10,35]. The mechanisms of MSH and MCH signaling in melanosome movement appear to be similar to the melanin stimulating roles played by the corresponding molecules in mammals. Physiological color change in amphibians is slightly different than in teleosts. At high levels, MSH causes melanosome dispersion as in teleosts, but low levels of MSH alone (rather than correspondingly high levels of MCH) are sufficient to drive melanosome aggregation [35]. In addition to responding to these hormones and others, chromatophores are innervated, allowing more rapid response to stimuli than would be possible through hormone signaling alone, and they are even able to respond to light stimulus directly (for review, see [10]).

3. Patterns

Changing the type of pigment produced by melanocytes can lead to dramatic differences in coloration over the entire body of an animal, but many of the most striking pigment patterns found in nature are the result of regional differences in pigmentation, such as splotches of orange on a guppy, the stripes of a zebra, or the white head and tail of a bald eagle. Most research on pigment patterning to date has focused on pigmentation rather than on patterning: how various genes affect the number of pigment cells, or their migration, but not specifically on how the complex patterns are established or maintained. In this section, we cover a few well-studied patterns (Fig. 1, Table 1) and highlight examples of patterns that may be useful for future study (Fig. 2).

3.1. Dorsal/Ventral Patterning

A contrasting dark dorsum and light ventrum is one of the most common pigment patterns found among vertebrates. This arrangement is a classic form of crypsis: for land animals, the “countershading” keeps overhead illumination from casting dark shadows on their undersides (e.g. [73]); for fish, a dark dorsum blends with deeper water, while a light ventrum blends with the light surface. In mice, this marked difference in dorsal-ventral pigmentation is caused by variation in the activity of two distinct *agouti* transcripts [21,22].

A “hair-cycle specific” transcript is expressed throughout the body during the mid-portion of hair growth [22,74]. This transcript is required for the characteristic pheomelanin band of agouti hairs. A second “ventral-specific” transcript is expressed throughout the hair cycle, but only on the ventral side of the body [74]. Mice expressing both “hair-cycle specific” and “ventral-specific” *agouti* transcripts develop a dorsum with agouti-banded hairs and a yellow or cream belly. Expression of “ventral-specific” *agouti* begins during mammalian embryogenesis suggesting the genes involved in the establishment and/or maintenance of ventral identity regulate expression of this transcript [22]. Analysis of the mouse *droopy ear* (de^H) mutant provides some insight into the mechanisms that might regulate *agouti*. *droopy ear* mutants show a shift in the dorsal-ventral pigmentation boundary, resulting from dorsal expansion of “ventral-specific” *agouti* expression [75]. Cloning of *droopy ear* revealed a large deletion in *Tbx15*, a T-box transcription factor that cues the establishment of dorsal dermis [75]. The effects of *Tbx15* on pigment patterning, however, are likely indirect, resulting from alterations in positional identity of the dermis rather than through direct regulation of *agouti* transcripts.

The combination of a dark dorsum and light ventrum is also common in poikilotherms, and it appears the mechanisms that generate this pattern are similar to those in mammals. In amphibians, a ventral specific factor, melanization inhibiting factor (MIF), represses differentiation and melanization of melanocytes *in vitro* [76,77]. MIF is highly expressed in unmelanized ventral skin and not only suppresses melanization, but also appears to promote iridophore localization in this region [78,79]. Similar to MIF, an *agouti* homolog expressed mainly in the golfish ventrum blocks melanization in cell culture [80]. Whether MIF is an amphibian homolog of *agouti* or a distinct molecule with similar function remains unknown. Regardless of MIF's identity, the expression of a ventral-specific inhibitor of melanization seems like a common mechanism responsible for generating a light ventral side in poikilotherms.

Additionally, other genes or pathways may determine how far pigmentation extends ventrally. Recently, Miller and colleagues showed that differences in gill and ventral skin pigmentation between marine and freshwater three-spine stickleback result from differences in *kit-ligand* expression [81]. In mice and zebrafish, kit-ligand (also known as Steel Factor, Mast Cell Growth Factor) is required for melanocyte migration and survival (see Spots section below) [82]. *kit-ligand* expression is reduced in freshwater three-spine stickleback populations with lighter gill and ventral skin pigmentation [81]. It will be interesting to see if reduced *kit-ligand* expression is always found in lightly pigmented populations of three-spine stickleback and to determine whether changes in *kit-ligand* expression are responsible for naturally-occurring variation in pigmentation patterns in other vertebrates.

3.2. Stripes and Bars

Stripes may be repeated across the body, like those of a tiger, or found only in discrete regions, like the rings on a raccoon tail. Tabby cats display one of the most familiar mammalian stripe patterns. Tabby stripes are visible only when functional *agouti* is expressed and result from agouti banded hairs (light stripes) alternating with completely eumelanic hairs (dark stripes) [7]. How the *tabby* locus interacts with *agouti* to achieve this

pattern is unknown, but its identification will no doubt shed light on the development of striped patterns in other mammalian species. Thus far, identifying the genes responsible for mammalian stripe formation has been difficult because only one mutation has been found to cause stripes in lab mice [83]. Despite the diversity of colors produced by birds, feather patterning resulting in bars, stripes or spots is controlled exclusively by melanin production [47]. Studies in Japanese quail suggest that local cues in the feather papillae act on melanocyte precursors to control their differentiation [84,85]. Differences in the timing or distribution of these differentiation cues could lead to differences in pigment patterns among different species of birds.

Though bird and mammalian models of stripe formation are lacking, good fish and amphibian models exist: adult zebrafish and larval salamanders (*Ambystoma tigrinum tigrinum* and others) develop horizontal stripes of melanophores separated by interstripes of xanthophores. Microsurgical experiments on salamanders demonstrated that chromatophore migration is influenced by the tissue over which they travel, and that the lateral line in particular is both directly and indirectly responsible for the melanophore-free interstripe region [86,87], although this is not true for all salamander species ([86,88] and references therein). Signals from the underlying tissue affect chromatophore location in embryonic zebrafish [89], but so far a role for such signals has not been demonstrated in adult zebrafish. Studies of stripe formation in adult zebrafish have instead focused on interactions between chromatophores themselves. In mutants lacking either all xanthophores (*csf1r* [90]) or all melanophores (*nacre* [91]), the remaining chromatophores fail to organize into stripes. When the missing cell type is added by cell transplantation, stripe formation is restored in the vicinity of the donor cells [92,93].

A recent *in silico* study modeled pattern formation in zebrafish by varying attractive or repulsive forces of both homotypic (melanophore-melanophore or xanthophore-xanthophore) and heterotypic (melanophore-xanthophore) interactions to examine the conditions that might generate patterns like those observed in wild-type or mutant *D. rerio* [94]. While previous descriptions of melanophore and xanthophore behaviors during teleost and amphibian pattern formation implied a repulsive action of xanthophores on melanophores [87,89], this model suggests that stripe formation requires both heterotypic attraction and homotypic repulsion. There are difficulties with applying these model conditions directly to stripe formation in *D. rerio*, but the suggestion that homotypic repulsive interactions may be involved in the formation of stripes is one worth investigating.

Experiments with temperature-sensitive alleles of *D. rerio csf1r* also suggest that the timing of stripe-formation signals may affect stripe orientation. When fish raised at restrictive temperatures are shifted to permissive temperature later in development, melanophores organize into stripes on the flank and fins, but in the caudal and anal fins, the orientation of these stripes is seemingly random [92]. Several danios and salamanders exhibit vertical stripes or bars, but whether these bars result from differences in the timing of the same stripe forming signals or from different signals remains unclear [95,96]. East African chiclid species present an opportunity to explore differences in stripe forming mechanisms between vertical bars and horizontal stripes. For several decades, two species of cichlids have been distinguished by the orientation of two dark facial stripes: *Neolamprologus pulcher* has two

curved vertical bars on the operculum, while *N. brichardi* exhibits one vertical bar on the operculum and one horizontal stripe connecting the eye to the operculum bar. A recent phylogenetic study revealed that these species are not monophyletic groups, and that *N. brichardi* T-shaped markings evolved repeatedly from the ancestral *N. pulcher* bar pattern [97]. These phenotypes provide an excellent opportunity to investigate the genetic basis of stripe/bar orientation, and further to determine whether the same mechanism has been utilized repeatedly to reorient the anterior stripe in the "*brichardi*" phenotype.

3.3 Spots

Spots can be regular and repeated, like those found on a cheetah, or broad and irregular, like the black and white pattern of dairy cows. Although regular spotting patterns are not found not in lab mice, several mouse mutants do show irregular white spotting patterns [98]. These mutants develop patches of white hair and skin that are completely devoid of melanocytes. Two white spotting mutants, *piebald-lethal* and *Dominant spotting* result from mutations in the seven transmembrane G-protein coupled receptor *Ednrb* and in the receptor tyrosine kinase *Kit*, respectively [99,100,101]. *piebald-lethal* homozygotes are almost completely white and develop megacolon due to defects of the enteric ganglia [99]. These mice lack most melanocyte precursors, suggesting that early Endrb activity is required for the development of these cells [102]. Melanocyte precursors also require Ednrb for dorsolateral migration from the neural crest [103]. *Dominant spotting* mutants develop completely white coats and are sterile, anemic, and lack mature mast cells. Mice heterozygous at this loci display diluted coats with characteristic white spots on the forehead and belly [98,104]. Kit signaling is required for melanocyte precursor migration and survival and later is required for their entry into developing hair follicles [105,106,107,108].

The pleiotropic effects of *Kit* and *Ednrb* make mutations in the coding regions of these genes unlikely candidates for spotting patterns in wild populations, but studies in domestic species reveal several instances in which spotted or completely white patterns are associated with *Kit* or *Ednrb* mutations. In domestic pigs, white coat color is dominantly inherited and is caused by the combined effects of two mutations in *Kit*: a duplication of the coding region of *Kit* including some, but not all of the regulatory regions; and a splice mutation in one copy of *Kit* resulting in skipping of exon 17 [109,110,111]. These mutations are not associated with defects in fertility or viability, though a reduction in white blood cells suggests a mild effect on hematompoesis [110]. Pigs with an extra copy of *Kit*, but no splice mutation develop a patched pattern of white spots interspersed with colored spots [109,110].

Spotting patterns in horses ranging from small regions of the body to nearly complete depigmentation and are often associated with *Kit* mutations. Exon skipping in *Kit* is associated with a Sabino spotting pattern in horses, resulting in irregular white patches on the face and feet in heterozygotes and a completely white coat in homozygotes [112]. This mutation does not completely eliminate wildtype *Kit* transcript, possibly explaining the lack of reported health defects in these horses. Distinct mutations in *Kit* coding sequence are also associated with depigmentation and dominant white coat color in Franches-Montagnes, Camarillo White Horse and Arabian horse breeds [113]. Additionally, frame overo white markings in American Paint Horses are associated with a dominant mutation in *Ednrb*

[114,115]. Similar to mice, horses homozygous for this allele are completely white, but die shortly after birth due to an absence of enteric ganglia.

The roles of *kit* and *ednrb* are conserved in zebrafish melanophore development, but show some intriguing differences compared to mammals. Whereas mouse *Kit* and *Ednrb* mutants lack nearly all melanocytes, zebrafish, *kit* and *ednrb1* mutants each develop roughly half the normal number of adult melanophores [116,117]. Double mutants lack all adult body melanophores, demonstrating that *kit* and *ednrb* are required by genetically distinct subsets of adult melanophores [66]. Interestingly, several species of *Danio* develop spotted patterns similar to zebrafish *ednrb1* mutants. These species also develop fewer melanophores than *D. rerio*, and fail to complement zebrafish *ednrb1* mutants, making changes in *ednrb1* activity a good candidate for species differences in pigment patterning [118] (LBP and DM Parichy, unpublished data).

While *Ednrb* and *Kit* mutants demonstrate how differences in melanoblast migration and differentiation can effect pigment pattern formation, interactions among pigment cell types are also likely to play a large role in spotted pattern development. Several zebrafish mutants may provide insight into the types of cell interactions that form spots. Like *ednrb* or *kit* mutants, *leopard* mutants fail to develop a subset of adult melanophores [66]. Pigment patterns of various *leopard* alleles range from broken stripes to spots to widely dispersed melanophores and are caused by mutations in connexin41.8, a gap junction component [119]. Cell transplants suggest that connexin41.8 promotes both homotypic attractions among melanophores and among xanthophores, as well as repulsive and attractive heterotypic interaction between melanophores and xanthophores [93].

Yellow spots on the anal fins of male haplochromine cichlids act as egg-dummies, and are an important part of the mating system of many species. Salzburger and collaborators report that *csf1ra* is expressed in sites of xanthophore spot formation on the anal fin of a wide variety of species, but that in one basal riverine haplochromine csf1ra RNA is expressed in pearly spots on the dorsal fin but not in the yellow tissue surrounding the spots [120]. This result indicates that we still have much to learn about the genes that control pigment cell development and pattern formation.

4. Seasonal and Life Cycle Alterations to Colors and Patterns

Changing ecological pressures throughout the ontogeny of an organism can necessitate corresponding changes in the organism's appearance. Animals that experience drastic seasonal ecosystem change, such as snowy winter, change their pigmentation to match: snowshoe hare, artic fox, snowy owl and ptarmigan are well-known examples [20]. Other animals exhibit specific coloration during the mating season: for example, three-spine stickleback males exhibit nuptial coloration that ranges from bright red to black depending on the species or morph [121].

Permanent changes in color or patterning are common as well. In a number of mammals this change is a relatively simple one: puma cubs and white-tailed deer fawns have spots, while older individuals of their species do not. Some fish species, such as wrasse and stoplight parrotfish, undergo a female-to-male transition and exhibit associated color changes

[122,123]. Many teleosts and amphibians undergo metamorphosis, and the coloration or patterning changes associated with this can be dramatic [86,96]. Hormones like testosterone or the steroid androgen 11KT are known to be involved in these changes (e.g. [123]) but the mechanisms connecting hormone level and pigment cells have not been studied directly. In addition, while the behaviors of particular populations of cells during metamorphosis or any other coloration change may be known, the signals underlying these behaviors are still largely mysterious. To what extent is the pattern established by the pigment cells themselves, or by a prepattern set in the underlying tissue? Is patterning attributable to direct cell-cell contact or to secreted signaling molecules? Because the changes taking place are so significant, metamorphosis is an excellent time to study these questions of cell migration and behavior, cell-cell interactions, and pattern formation [57,124,125].

5. Evolution of Coloration, and Other Areas for Future Study

The study of animal coloration provides a framework in which to integrate tremendously divergent research interests. Pigment cells can be used to study a wide range of cellular and molecular topics: specification and differentiation of neural crest derivatives; cell migration; pigment synthesis; intracellular organelle transport; cell signaling; and pattern formation – not to mention the many diseases and syndromes associated with the pigmentary system [126].

At the same time, the importance of coloration and color patterning to the ecology of organisms cannot be overstated: predation avoidance and mate attraction in particular have been studied extensively as possible ecological explanations for specific colorations. Guppies provide a classic example of the tradeoffs between mate attraction (sexual selection) and predation avoidance (natural selection). In a classic series of experiments, Endler demonstrated that orange spots on male guppies made them more attractive to potential mates, but also made them easier targets for predators. Therefore, high predation pressure resulted in duller-colored guppies, while lower predation pressure allowed sexual selection to drive the evolution of guppies with larger, brighter orange spots [12,127]. A recent study examined an interesting variation on this tradeoff: sexual selection for increased visibility vs. the incidence of melanoma in male swordtails (*Xiphophorus cortezi*). In populations with relatively low frequency of the melanoma-causing *Xmrk* allele, females strongly preferred a male with a large melanophore spot on its caudal fin over a male without this spot. The role of *Xmrk* in enlarging this spot could explain why this oncogene has been maintained in these populations. In a population with much higher frequency of the *Xmrk* genotype and higher incidence of malignant melanomas, females still show a strong preference for increased melanization, but actually prefer males without the caudal fin spot, indicating an adaptation away from the pleiotropic effects of *Xmrk* [128].

The role of pigmentation and pigment patterns in evolution give us the opportunity to tie molecular, cellular, and ecological studies together in the same organisms. Doing so requires adding two more layers of complexity: the visual systems of the organisms involved; and the ecosystem in which the relevant inter- or intraspecific interactions take place. The importance of UV signals in particular (e.g. [129,130]) demonstrate the folly of relying on human perception of an animal's coloration or patterning in determining which components

are important [131]. Any analysis of how a particular pigment mutation or adaptation affects a species or group of species must take into account how conspecifics and predators perceive the change, as well as the environment in which the change is viewed [132,133]. So far, these different fields have remained quite separate from one another, but connecting them to each other offers an exciting future direction for research. One very recent paper ties together some of these previously disparate fields [134]. This is by no means the first study to cross boundaries in this way, but it is a particularly elegant example. Seehausen and collaborators focus on the sympatric speciation of haplochromine cichlids by evaluating the evolution of an opsin, correlations of light penetration gradients with depth and turbidity, male nuptial coloration, and female mating preference [134]. The results – that the strength of preference of females with red-biased vision for red males, and of females with blue-biased vision for blue males, is dependent on both the absolute environmental conditions experienced by each population and by the rate of variation in those conditions – show the power of approaching a complicated problem like cichlid speciation from so many angles at once [135]. With increases in the availability of sequenced genomes and an increasing array of pigment pattern mutants to learn from, such integrative studies should become more common and even more informative – a tremendously exciting prospect.

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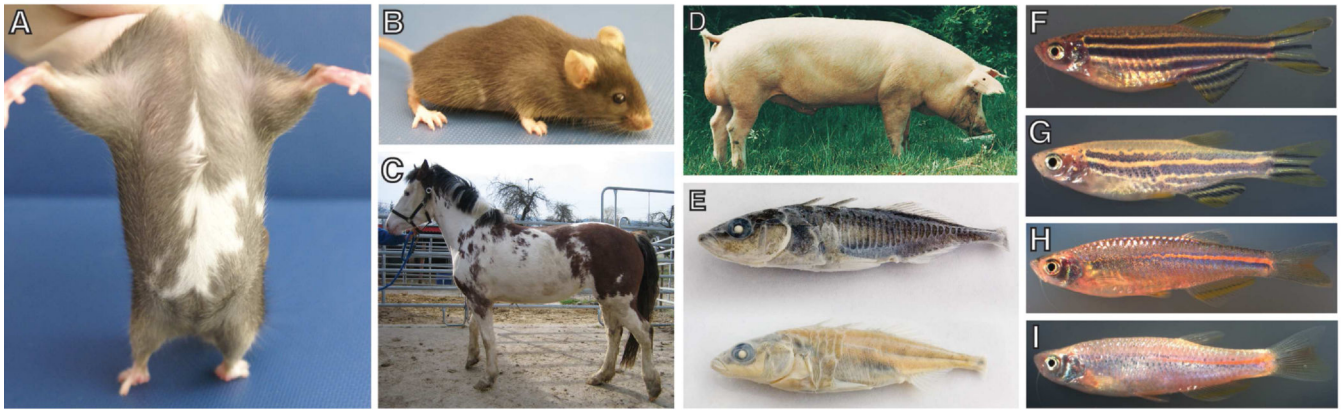

Figure 1.

Pigment patterns caused by KIT pathway mutations.

A, B. Heterozygous kit mice are mostly brown with irregular belly and sometimes forehead spots.

C. Franches-Montagnes horse with p.Y717X mutation in KIT

D. Dominant white Swedish Landrace pig

E. Marine (above) and freshwater (below) threespine sticklebacks (*Gasterosteus aculeatus*) with higher and lower levels of kit ligand expression

F, G: Wild-type and homozygous kit-mutant *Danio rerio*

H, I: Wild-type and homozygous kit-mutant *Danio albolineatus*

Photo credits: A, B: A Incao; C: B Haase; D: L Andersson; E: F Chan, C Miller, D Kingsley; F, G: LBP; H, I: MGM.

Figure 2.

Examples of interesting pigment patterns for which the genetic and developmental bases remain unknown.

A. Tabby stripes on a domestic shorthair cat

B, C. Cryptic dorsum and brilliant ventrum of a male western fence lizard, *Sceloporus occidentalis*

D. Brilliant coloration and patterning in a poison dart frog, *Oophaga pumilio*

E. Spots on *Danio kyathit*, a close relative of the zebrafish, *Danio rerio*

E. Irregular orange and black spots on a male guppy, *Poecilia reticulata*

F. Stripe-like patches on a salamander, *Ambystoma tigrinum*

Photo credits: A: DM Parichy; B, C: A Leache; D: D Gonzalez; E: LBP; F: A Price; F: HB Shaffer.

Table 1

Mutations and polymorphisms affecting pigmentation and patterning.
D = Dorsal; V = Ventral; ( ) = allele

Color or Pattern	Gene	Common name (Species)	Phenotype	Reference
Melanism	Mc1r	chicken (*Gallus gallus*)	black (E)	[30, 31]
		sheep (*Ovis aries*)	black	[27]
		pig (*Sus sp*)	black	[26]
		bananaquit (*Coereba flaveola*)	black	[28]
		jaguarundi (*Herpailurus yaguarondi*)	black	[23]
		artic skua (*Stercorarius parasiticus*)	gray (intermediate), black	[29]
		lesser snow geese (*Anser c. caerulescens*)	blue variant	[29]
		jaguar (*Panthera onca*)	black	[23]
		rock pocket mouse (*Chaetodipus intermedius*)	black	[18]
		mouse (*Mus musculus*)	black (Eso)	[98]
	Agouti	horse (*Equus caballus*)	black	[24]
		domestic cat (*Felis catus*)	black	[23]
		mouse (*Mus musculus*)	black (a or ae)	[98]
		Japanese quail (*Coturnix japonica*)	blackish-brown (rb)	[25]
	B-defensin	domestic dog (*Canis familiarus*)	black	[33, 34]
Albinism	Tyrosinase	human (*Homo sapiens*)	albino	[38]
	Oca2	mexican tetra (*Astyanax sp*)	albino	[19]
		human (*Homo sapiens*)	albino	[38]
Yellow/Red	Mc1r	Kermode Bear (*Ursus americanus*)	very pale yellow	[44]
		pig (*Sus sp*)	red	[26]
		horse (*Equus caballus*)	chesnut	[43]
		cow (*Bos taurus*)	red	[42]
		chicken (*Gallus gallus*)	uniformly red-yellow	[30]
		Golden Retriever, Irish Setter, Yellow Labrador Retriever (*Canis familiarus*)	yellow or red coat	[40, 41]
		mouse (*Mus musculus*)	yellow (e)	[98]
		beach mouse (*Peromyscus polionotus*)	lighter pigmentation	[45]
	Agouti	Japanese quail (*Coturnix japonica*)	wheat-straw yellow plumage (Y)	[39]
		mouse (*Mus musculus*)	yellow (Ay or Avy)	[39]
Light Ventrum	Agouti	mouse (*Mus musculus*)	agouti hairs D and V (A)	[98]
			agouti hairs D, yellow hairs V (Aw)	
			black hairs D, yellow hairs V (at)	
	Steel	three-spine stickleback (*Gasterosteus aculeatus*)	light ventral skin and gills	[81]
Stripe/Bar	Csf1r	zebrafish (*Danio rerio*)	no xanthophores, fewer melanophores	[90]
	Mitfa	zebrafish (*Danio rerio*)	no melanophores	[91]

Color or Pattern	Gene	Common name (Species)	Phenotype	Reference
Spotting/Dominant White	Kit	horse (*Equus caballus*)	Sabino1 spotting, white coat	[112]
			white spotting, dominant white	[113]
		pig (*Sus sp*)	spots, dominant white	[109, 110]
		mouse (*Mus musculus*)	white forehead, belly spots, dominant white (W, Wv)	[100, 101]
		zebrafish (*Danio rerio*)	reduced melanophores in stripes	[116]
	Ednrb	horse (*Equus caballus*)	frame overo white markings, lethal white foal syndrome	[114, 115]
		mouse (*Mus musculus*)	white spots, fully white coat (s, sl)	[99]
		zebrafish (*Danio rerio*)	reduced melanophores, spots	[117]