

Vertebrate pigmentation: from underlying genes to adaptive function

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Animal coloration is a powerful model for studying the genetic mechanisms that determine phenotype. Genetic crosses of laboratory mice have provided extensive information about the patterns of inheritance and pleiotropic effects of loci involved in pigmentation. Recently, the study of pigmentation genes and their functions has extended into wild populations, providing additional evidence that pigment gene function is largely conserved across disparate vertebrate taxa and can influence adaptive coloration, often in predictable ways. These new and integrative studies, along with those using a genetic approach to understand color perception, raise some important questions. Most notably, how does selection shape both phenotypic and genetic variation, and how can we use this information to further understand the phenotypic diversity generated by evolutionary processes?

Genotypes and phenotypes

A fundamental pursuit in the field of evolutionary genetics is to determine the underlying molecular mechanisms that lead to natural variation in morphology, physiology and behavior (an individual's 'phenotype'). Understanding the link between genotype and phenotype can elucidate mechanisms that shape phenotypic variation within populations and how these affect patterns of evolutionary change. For example, knowing the underlying genetics of traits can reveal the type of evolutionary change affecting phenotypic variation [1] as well as the strength and timing of selection [2]. Thus, identifying the mechanisms that shape variation in morphology and behavior can offer important insights into the process of population divergence and speciation.

The study of mammalian pigmentation has long served as a model system to learn about molecular, cellular and developmental processes [3]. As a result, over 150 genes that affect animal color and patterning have been identified [4–7]. Although most of these genes were first identified in laboratory mice (genus *Mus*), they have more recently been examined in domestic and natural populations [8–14], and are thereby relevant to understanding the underlying molecular basis of adaptation in the wild. The dissection of the genetic architecture responsible for

color variation in nature affords opportunities to ask questions about (i) how selection on specific parts of the genome influences phenotype (mechanism) and, in turn, (ii) how selection on the phenotype itself (function) affects the genomic regions known to underlie various aspects of pigmentation. Still, these are early days in understanding the connections between the mechanistic and functional basis of animal coloration. In this review, we build on what is already known about the genetic basis and developmental mechanisms generating the diversity of pigmentation and color patterns in vertebrates [4–7], and highlight the importance of making new, explicit links between selection on both genotype and its associated phenotype to gain a comprehensive view of how the interaction and feedback of genetic and phenotypic variation are simultaneously shaped by evolutionary processes.

Adaptive function of coloration

In animals, coloration, via both pigmentation and nanostructure, has many functions. For example, it is often used for intraspecific communication (e.g. ornamental color used for mate choice and intrasexual competition [6,15–18]) and interspecific interactions (e.g. aposematic and cryptic coloration used for predator avoidance [6,15,19]). In many rodent species, coat color (i.e. pelage) closely matches the local substrate to minimize detection by visually hunting predators [2,20,21]. Moreover, many colors and pigments can have other adaptive functions such as photoprotection [6,22,23], structural support [23], microbial resistance [24] and thermoregulation [6,23,25]. Because animal color is often likely to be influenced both by genetic and environmental (e.g. nutritional status, maternal effects, disease state) factors it is instructive to isolate the genetic component of color traits to (i) predict the amount of selection required for an evolutionary response in these traits, (ii) determine the degree to which parental phenotype predicts offspring phenotype, or how heritable the trait is and (iii) better understand the proximate mechanisms driving or constraining evolutionary processes. A crucial consideration for the function of coloration traits with putative signaling roles is the visual perception of the receiver (Box 1). Indeed, measurable phenotypic differences are only biologically meaningful if the phenotypic change is detectable by the receiver.

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Box 1. Studying the genetic basis of avian color perception

Physical measures of coloration based on reflectance spectrometry have revolutionized the field of color research compared with earlier work that relied on human-assessed metrics [80]. Yet, when the putative function of color diversity is signaling, it is also crucial to identify what color differences are perceptible to the intended receiver [81]. To do so, measures of light reflectance must be filtered through the sensory range and perceptual thresholds of the recipient [82]. Sensory neurophysiology and behavioral psychophysics can identify both the range and the error in perceiving and responding to color differences, but these methodologies are not always suitable for large evolutionary studies or even for species-specific studies on subjects that are intolerant of captivity [83].

In birds, the functional interpretation of diverse avian plumage and egg coloration has benefited from large-scale comparative approaches. For example, recent studies have focused on the most variable component of avian color sensitivity – the violet or UV receptor sensitivity of the opsin gene [84]. Specifically, DNA sequencing of an individual's short-wavelength opsin receptor (SWS1) can provide information about its function. Functional differences can be measured based on the known peak absorbance of opsin types previously isolated or, in the case of novel sequences, via *in vitro* mutagenesis and functional tests of light absorbance [85]. Using non-invasive genetic means to characterize visual perception communication is especially relevant for understanding the functional and ecological context of avian color variation. For example, the frequent mismatch between the UV sensitivity of hosts and their violet-sensitive egg (mimetic) brood parasites, or between tetrachromatic avian prey and their dichromatic mammalian predators, enables the evolution of private communication channels protected from the risks of “eavesdropping” [86].

Similar approaches that combine knowledge of opsin protein sequences and their respective functions have broad applications for many vertebrate color vision studies. For example, within a sympatric species flock of Lake Victoria cichlids, expressed opsin sequences and *in vitro* predictions of their respective peak sensitivities tightly correlate with water depth range (which modulates illumination spectra), carotenoid-based male polychromatism and behavioral measures of female choice [30]. Ultimately, we would aim to alter opsin genes and measure the resultant changes in visual perception. Recently, progress toward this goal was made when viral delivery of the human version of red-sensitive opsin led otherwise dichromatic male squirrel monkeys to “catch up” with trichromatic female conspecifics’ abilities in color discrimination [87].

Pigmentation genes involved in melanin-based coloration

For melanin-based coloration, an impressive number of pigmentation genes have been identified, cloned and sequenced in laboratory mice [4]. These genes are scattered throughout the genome and are involved in a variety of cellular processes [4]. Despite the large number of potential targets, only a handful of genes have been identified as major contributors to color variation in a wide array of animal taxa. Of these, the melanocortin-1 receptor (*MC1R*) and agouti signaling protein (*ASIP*), both important in melanin synthesis [4], are among the most widely studied pigmentation genes in wild populations of mammals, birds, reptiles and fish [13,25–27] (Fig. 1). The majority of these studies have concentrated on uncovering the genetic basis of intraspecific differences between populations with discrete polymorphisms (e.g. light and dark colored mice) [2,11,12,14,25,28]. The wealth of knowledge about the molecular mechanisms underlying melanin-based coloration is unmatched relative to current information regarding carotenoid-based or structural coloration. It is worth

noting, however, that structural coloration is probably influenced by melanin pigmentation genes because in birds, reptiles and fish the underlying basis of structural colors often involves melanin pigments [29]. By contrast, carotenoid coloration is likely to be under less genetic control than melanin-based coloration because these molecules are derived from diet [22], rather than being synthesized endogenously [23]. Consequently, there is still much to learn about the proximate mechanisms that control the dazzling array of colorful phenotypes that are often the target of both natural and sexual selection, and are also known to play a role in defining boundaries among populations and species [14,30].

Melanocortin-1 receptor

MC1R is a seven-transmembrane domain G-protein-coupled receptor (GPCR) found primarily in melanocytes that acts as a switch to control the type of melanin synthesized for deposition in tissues [31]. In mammals and birds, the ratio of eumelanin and pheomelanin largely determines an animal's overall color: darker (black to brown) phenotypes result from the increased deposition of eumelanin, whereas lighter (red to yellow) phenotypes result from the increased deposition of pheomelanin [23,32,33]. Although melanocyte-stimulating hormone (α -MSH)-mediated *MC1R* activation induces eumelanin production, *ASIP* antagonizes *MC1R* and triggers pheomelanin production. Lizards and fish, by contrast, do not produce pheomelanin [25], and in these taxa *MC1R* is likely to affect eumelanin density rather than melanin type.

MC1R is highly conserved among vertebrates and has a relatively simple genetic structure (single 1 kb exon), which has facilitated its identification in a diversity of taxa. As a result, dozens of studies now show a link between variation in *MC1R* and pigmentation in numerous vertebrates [4–7,34] (but see [21,25,35] for examples where melanin color does not associate with *MC1R* variants). The majority of these studies have statistically associated a single nucleotide polymorphism (SNP), and the resulting amino acid change, with a discrete color polymorphism. Known mutations are largely interspersed throughout the protein-coding sequence, yet distinct mutations in closely related species as well as identical mutations at homologous positions in diverse taxa can lead to the same or similar phenotypes (Table S1; Online Supplementary Material). For example, the Arg⁶⁵Cys substitution contributes to pale coloration in beach mice (*Peromyscus polionotus*) that inhabit Florida's sandy coast [28]; the identical mutation is found in woolly mammoths (*Mammuthus primigenius*) [36] (Online Supplementary Material). *In vitro* assays (Box 2) undertaken in both species demonstrate that this single mutation causes a decrease in receptor signaling by reducing ligand binding [28], suggesting that like beach mice mammoths might have also varied in coat color [36]. Importantly, as shown here, statistical associations between *MC1R* mutations and color should be functionally verified because sometimes even mutations strongly associated with color variation have no measurable effect on receptor function [21,37] (Box 2). For *MC1R* this can be achieved via cell-based pharmacology assays [36,38], although transgenic assays remain the gold standard.

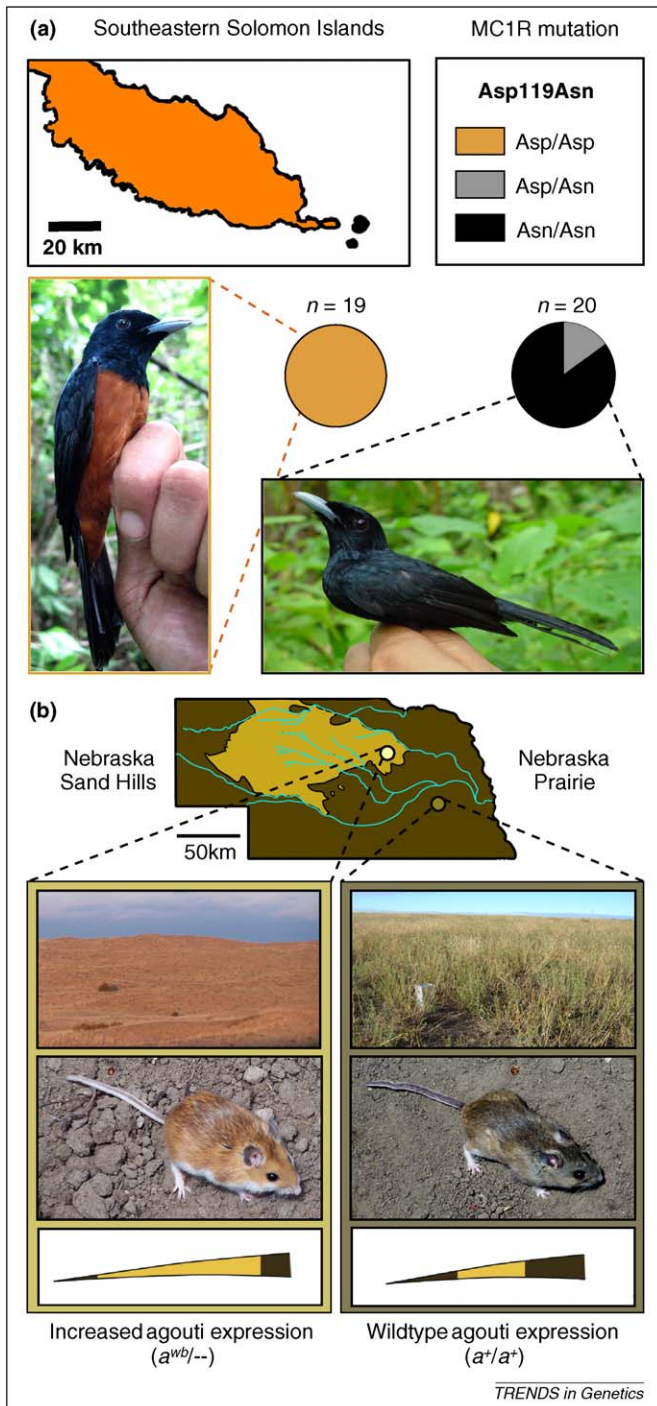


Figure 1. Illustration shows the association between mutations in pigmentation genes and color variation in natural populations of vertebrates. Mutations in *MC1R* and *ASIP* can have large effects on vertebrate coloration, which can be important in the origin of new species or local adaptation within species. (a) *Monarcha castaneiventris* flycatchers show distinct variation in plumage color throughout the Solomon Islands and might represent the early stages of species formation [14]. Distribution, plumage color and *MC1R* genotype frequency (pie charts) of the chestnut-bellied and melanic flycatchers of the southeastern Solomon Islands are shown. Ranges of the two subspecies are given: orange, chestnut-bellied form (*M. c. megarhynchus*; Makira Island) and black, melanic form (*M. c. ugiensis*; Santa Ana and Santa Catalina). A single *MC1R* amino acid substitution is perfectly associated with the color variation important for species recognition, linking this mutation with the early stages of speciation. (b) Driven by selection for crypsis from visual predators, deer mice (*Peromyscus maniculatus*) have evolved pelage to match their local substrate [2]. The location, habitat and hair banding pattern of deer mice living on and off Nebraska's Sand Hills are shown. Both mice are pictured on a dark soil background: yellow, *P. m. luteus* and brown, *P. m. bairdii*. *Cis*-acting mutation(s) at the *Agouti* locus are associated with changes in *Asip* expression and width of the subapical pheomelanin hair band, leading to overall

Box 2. Establishing causal relationships between mutation and phenotypic change

MC1R can be expressed *in vitro* and assayed for membrane integration, ligand binding and cyclic AMP activation [5]. A recent study in lizards highlights both the importance of functional studies and ways in which different functional mechanisms can produce similar changes in color. Three lizard species (*Sceloporus undulatus*, *Aspidoscelis inornata* and *Holbrookia maculata*) colonized the 8000 year-old White Sands in New Mexico, and each has evolved a similar blanched phenotype relative to their darker ancestors that inhabit the surrounding desert. All three species each have a single coding mutation in *MC1R* that is statistically correlated with phenotype [25], but when functionally assayed *MC1R* alleles from each species produces different results [37]. In *H. maculata*, there was no measurable difference in receptor activity; in *A. inornata*, the mutation resulted in lowered signaling potential; and in *S. undulatus*, the derived mutation decreased the efficiency by which *MC1R* integrated into the melanocyte membrane. Thus, it is clear that different *MC1R* variants can result in similar phenotypes but through different functional mechanisms [37].

MC1R mutations can also be functionally verified using other methods. For example, mutations identified in Mexican tetra populations have been assessed using the model organism zebrafish. Gross et al. [26] first demonstrated that knocking out *MC1R* in zebrafish resulted in a qualitatively lighter phenotype. Next, using Mexican tetra RNA transcripts from both surface and cave populations, they showed that the surface transcript rescued the ancestral phenotype, whereas the transcript from the cave populations did not. A causative link between *OCA2* variants and pigmentation differences between surface and cave populations of Mexican tetra was established using similar phenotype rescue experiments [55]. These heterologous experiments (either cell culture-based or *in vivo* assays) provide convincing evidence that the mutations found in the respective pigmentation genes indeed cause the observed phenotypic changes.

The number of studies that have implicated *MC1R* amino acid changes in color evolution, as well as the diversity of organisms in which these changes have been identified, is intriguing and raises the question of why *MC1R* repeatedly seems to affect vertebrate coloration. Potential answers to this question include the minimal pleiotropic effects of *MC1R*, large mutational target size, high mutation rate and ascertainment bias due to its simple and conserved structure [5,6,39].

Agouti signaling protein

ASIP is a paracrine signaling protein antagonist of *MC1R* that causes melanocytes to switch from producing eumelanin to pheomelanin. Multiple *ASIP* mutations are associated with color change [27,40–42]; however, compared with *MC1R* the number of examples from wild populations is far fewer and the types of molecular changes associated with color are different. Whereas all known *MC1R* mutations occur within the coding region, the genetic changes in *ASIP* occur in both the coding [40] and regulatory regions [27,41,43]. Although *ASIP* has been primarily studied in mammals, it seems to affect color in a variety of species including wild rodents [2,12,27], domestic horses (*Equus ferus*) [44], domestic cats (*Felis domesticus*) [45] and foxes (*Vulpes vulpes*) [42]. *ASIP* has also been studied in fish [43,46] and birds [41,43]. To our knowledge, however, agouti-like sequences have not been reported in reptiles.

differences in coat color brightness and ultimately survival. The dominant agouti wideband (a^{wb}) and recessive wild type (a^+) phenotypes/alleles are pictured.

Mutations in *ASIP* associated with color differences typically affect *ASIP* expression. For example, variation in *ASIP* mRNA expression levels are often highly correlated with pigmentation [12,27]. The increased expression, including experimental overexpression, of *ASIP* increases pheomelanin production because of its antagonistic effect on *MC1R*. In rodents, this can lead to an increased pheomelanin band on individual hairs as seen in mice inhabiting the light-colored substrate of Nebraska's Sand Hills [2] (Fig. 1) or, at the other extreme, a completely blonde mouse [4]. By contrast, loss-of-function mutations tend to cause the exclusive production of eumelanin and a melanic coat-color phenotype [27]. Although mutations in *ASIP* can affect melanin production and are associated with coloration, functional studies in wild populations remain largely absent.

Unlike *MC1R*, *ASIP* has well-described pleiotropic effects. In lab mice, the classic obese *yellow* mutant is the result of *Asip* overexpression in hair follicles that leads to a light color, whereas the misexpression of *Asip* in the brain, where it interacts with the melanocortin-4 receptor (*MC4R*), causes a refeeding behavior and ultimately obesity [47]. Moreover, this *yellow* mutation, when homozygous, is lethal [41]. In Japanese quail (*Coturnix japonica*), the *yellow* mutation, which also causes *ASIP* upregulation, resides in a similar genomic position to the *lethal yellow* mutation in mouse [41]. As in mice, when homozygous, the Japanese quail mutation is lethal, whereas heterozygotes have wheat-straw yellow-colored feathers [48]. Nadeau et al. [41] argue that these similarities suggest that the *ASIP* expression pattern and function is conserved across vertebrates [49,50]. Along with its complex gene structure and challenges associated with identifying regulatory mutations, its pleiotropic effects might explain why few associations between color and genetic variation at *ASIP* have been reported.

In addition to their independent effects, there are well-characterized epistatic interactions between *MC1R* and *ASIP*. In laboratory mice, *Mc1r* is epistatic to *Asip*; for example, dominant mutations in *Mc1r* that lead to a constitutively active receptor are not inhibited by *Asip* [51]. However, in foxes *ASIP* can counteract a constitutively active *MC1R* [42]. Another unique interaction has been found in beach mice. *Mc1r* mutations that lead to a lighter coloration are only visible when a mutation leading to increased *Asip* expression is also present [12]. These studies highlight that the phenotypic effects of both *MC1R* and *ASIP* mutations can be highly dependent on the genetic background in which they arise and, more generally, that interaction effects are allele- (not gene-) specific and thereby likely to vary among populations and species.

Other pigmentation genes

A growing number of recent studies in both domestic and wild animals have shown that several pigmentation genes originally identified in laboratory mice also play important roles in determining color variation in domestic and natural populations of vertebrates. For example, sequence variants of tyrosinase-related protein 1 (*TYRP1*), which codes for a melanogenic enzyme involved in the production of eumelanin [52], have been associated with color vari-

ation in several domestic animals including dogs, cats and cattle, as well as lab populations of Japanese quail [41]. Additionally, a single SNP in *TYRP1* is associated with a color polymorphism in wild Soay sheep (*Ovis aries*) [53], and transcript variants might explain color variation between wild populations of *Ficedula* flycatchers (Laura Buggiotti, PhD Thesis, University of Turku, 2007). Moreover, tyrosinase (*Tyr*) knockouts cause albinism in lab mice [54], whereas albinism in cave dwelling Mexican tetra (*Astyanax mexicanus*) is associated with multiple, independently derived polymorphisms in ocular albinism type 2 (*OCA2*) [55], a gene known to determine iris color in humans [56]. Melanism in the gray wolf (*Canis lupus*) showed no association with *MC1R* or *ASIP* mutations, but rather with a different member of the melanocortin pathway, the *K* locus [8]. A novel allele at the *K* locus seems to have been introduced via introgression from domestic dogs because the same 3 bp deletion is associated with dark coats in dogs, coyotes and wolves [8]. Finally, a recent and intriguing study implicates *cis*-regulatory changes in a highly conserved developmental gene, *Pax7*, in the orange-blotch (OB) phenotype in cichlid fish of Lake Malawi [57]. This OB allele might be the target of sexually antagonist selection, that is, it provides a camouflaging phenotype to females but disrupts species-specific male coloration important in mate selection.

Two additional studies have demonstrated that pigmentation genes identified in fish might also influence human skin color. First, differences in mRNA expression levels of the Kit ligand (*KITLG*) are associated with changes in gill and skin coloration in stickleback fish (*Gasterosteus aculeatus*) [58]. *KITLG* controls the proliferation, migration, differentiation and survival of Kit receptor-expressing melanocytes and, therefore, melanin patterning [59]. The same study implicates a *cis*-regulatory change in *KITLG* in human skin coloration because patterns of nucleotide polymorphism are consistent with selection in human populations with different skin phenotypes [58]. Second, the *golden* mutation in zebrafish (*Danio rerio*) has been linked to a diminished number, size and density of melanosomes, and ultimately to a mutation in *SLC24a5*, a putative potassium-dependent sodium/calcium exchanger [60]. In humans, an ancestral *SLC24a5* allele predominates in African and east Asian populations, but a derived allele, defined by a coding mutation, is nearly fixed in European populations; the derived allele is also associated with a light skin color in admixed populations [60]. Because mice show little variation in skin color, fish or other taxa with a known variability of epidermal pigmentation [58,60,61] are more promising models for studying human skin pigmentation.

One striking observation is that many of these pigmentation genes affect the production of eumelanin, or the switch between the production of eumelanin and that of pheomelanin (as with *MC1R* and *ASIP*). By comparison, we know very little about genes that are involved strictly in the synthesis of pheomelanin, or in other steps of melanogenesis, e.g. the ways in which pigment density or concentration are controlled. We expect that future studies that genetically dissect the mechanisms that control different aspects of the melanin pathway will be especially useful for

understanding the proximate mechanisms responsible for more subtle variation in color, for example, continuous variation within species that could be an important target of local adaptation and mate choice.

Pigmentation genes involved in non-melanin-based coloration

In addition to melanin pigments, animal coloration can involve the nanostructure of the tissue, carotenoid pigments and a handful of other pigments (e.g. pterins found in parrots and lizards [62,63]). To date there is very little known about the genetic mechanisms that underlie coloration caused by structure or non-melanin pigments. A recent study of the domestic chicken (*Gallus gallus domesticus*) showed that variation in expression levels of beta-carotene dioxygenase 2 (*BCD02*), a gene involved in cleaving β -carotene to create colorless apocarotenoids, is strongly associated with yellow versus white skin [61]. With few exceptions, most of what is known about the genetic basis of carotenoid-based traits comes from studies of heritability rather than specific genes. Some heritability estimates of carotenoid-based plumage suggest strong genetic effects (e.g. $h^2 = 0.84$ in house finches [64] and fish [65]). However, often these studies fail to control for environmental influences on color, because the brightness and hue of carotenoid-based traits are tightly linked to the availability of dietary carotenoids (Box 3) and can be condition-dependent. Accordingly, most studies of carotenoid-based coloration report low heritability, as demonstrated by a study in blue tits (*Cyanistes caeruleus*) [66]. Yet, there are many steps along the biosynthetic pathway leading to tissue deposition where genetic variation could have an effect (e.g. absorption from food, transport, sequestration, esterification) [22]. Consequently, identifying individual genes, or classes of genes, that affect carotenoid-based coloration seems to be a daunting task, but one that should produce high rewards.

Similar to carotenoid-based coloration, insights into the genetic basis of structural coloration have been mostly limited to heritability estimates and condition dependence in birds and fish [66–69]. Structural colors, excluding white, have a base layer of pigment to absorb light and prevent incoherent scattering by the underlying tissue [29]; in birds, this pigment layer is usually melanin; however, there are also examples of carotenoid pigment base layers [29]. Consequently, the same genes that affect the underlying pigments probably affect structural color traits. Yet, to our knowledge, no study has explored the role of known melanin pigmentation genes on structurally-based color traits (but see [14]). Because the nanostructure of the tissue (e.g. feathers, skin, hair) determines how light scatters within the tissue [29], the developmental mechanisms that control the nanostructure [70] are also potential targets of selection, and might be a treasure trove for genetic influences on structural color.

Linking mechanism and function

Using model organisms, we have gained great insight into the underlying genetic basis of pigmentation, specifically melanin-based pigmentation. With advancing technology, it is now possible to study the molecular mechanisms of

Box 3. Environmental influences on color

Color is not a physical phenomenon; rather it is the perceptual image formed by the sensory filters and cognitive architecture of the observer. As such, color, like many other perceptual phenomena, is extremely malleable and dependent on environmental context [88]. Take, for example, the rainforest dwelling eclectus parrot (*Eclectus roratus*) whose bright crimson and navy females sharply contrast with the duller monochromatic green males when judged in captivity by the human eye, leading this species to be classed as an example of “reversed sexual dimorphism” [89]. However, in nature, males are less conspicuous against the background foliage than females when viewed by their avian predators, which probably confers a selective advantage because males forage and provide for females almost exclusively during their prolonged breeding season. In turn, both females and males benefit from being more conspicuous against tree trunks than foraging males when viewed by the parrot visual system; females display to other females in competition for scarce nesting cavities and males display at cavities to females for mate attraction [90]. Therefore, the environmental context of perceived coloration can be dependent on both the micro- and macrohabitat in which individuals display, or on the circadian and seasonal variation in ambient light sources and filters (e.g. cloud coverage, substrate color, canopy structure and foliage coloration), including natural or anthropogenic change in visibility and turbidity [20,91].

In addition, temporal and geographic differences in the local availability of chemical and energetic resources necessary for the collection, transport, biosynthesis, incorporation and behavioral display of pigmentation patterns can also result in a variation of color displays and physical function. For example, long-term pedigree data reveal that eggshell maculation patterns are inherited through female sex-specific genetic elements in great tits (*Parus major*) breeding in Wytham Woods near Oxford [92]. However, in nearby populations the reduced availability of environmental calcium is correlated with increased density of the protoporphyrin-containing speckles concentrated in the thinner zones of the eggshell matrix, probably serving to increase the structural strength of the eggs in calcium-poor habitats [93]. Thus, environmental factors clearly can influence the appearance of eggshells. Finally, rapid, physiological modulation of an individual’s coloration for crypsis, mimicry or sexual display – like the incredible ability of cuttlefish to change from cryptic to showy coloration in the blink of an eye – illustrates the potential scope of diverse adaptive functions of dynamic feedback between coloration, sociality and the environment [94,95].

pigmentation in non-model, and even wild, systems. Indeed, these studies have demonstrated a highly conserved function of many of these genes across species. These recent genotype–phenotype associations also can inform our understanding of the evolutionary process leading to adaptive coloration. For example, we would like to know (i) how many genes affect pigment variation in natural populations; (ii) how often are the same genes involved in convergent phenotypes; (iii) how does the strength of selection affect color variation; and (iv) can we detect evidence of selection in patterns of nucleotide variation in pigmentation genes? We are just now beginning to understand the genetics underlying adaptive changes in coloration and color vision (Box 1), and in cases when these differences influence reproductive isolation, we also might be able to make inferences about the genetics of speciation.

To address these questions, we need a deep understanding, at the molecular, genetic and developmental level, of how changes in pigmentation genes and their interactions produce changes in color phenotype. Studies that have

Table 1. Pigmentation genes associated with color variation in wild populations of vertebrates.

GENE	DERIVED PHENOTYPE	CLASS	KEY REFS
<i>MC1R</i> ^A	Darker skin, plumage, coat Lighter Skin, coat	Actinopterygii, Aves, Mammalia Mammalia, Reptilia	[11,13,20,26,36] [25,28,38,96]
<i>ASIP</i> ^A	Darker coat Lighter coat	Mammalia Mammalia	[27,42] [2]
<i>TYRP1</i> ^A	Darker plumage Lighter coat	Aves Mammalia	Laura Buggiotti, PhD Thesis, University of Turku, 2007 [53]
<i>OCA2</i>	Lighter skin	Actinopterygii	[55]
<i>K locus</i> ^B	Darker coat	Mammalia	[8]
<i>KITLG</i>	Lighter gills, skin	Actinopterygii	[58]
<i>SLC24a5</i>	Lighter skin	Actinopterygii	[60]
<i>Pax7</i>	Dark skin blotches	Actinopterygii	[57]

^AMutations/genetic variants have been identified in this gene that associate with parallel phenotypic changes in lab populations and/or domestic animals.

^BMutations/genetic variants have **not** been identified or shown to have an effect on human skin, hair or eye color [97].

reported perfect associations between *MC1R* variants and coloration [5,9,10,14] provide convincing evidence that, in some cases, single genes can be responsible for phenotypic change, especially in cases where no intermediate phenotypes are found and Mendelian inheritance is clear. However, few studies have explored the role of more than one pigmentation gene in determining phenotype (but see [12]). Interestingly, most studies that have explicitly looked at more than one gene have found that interactions between genes affect phenotype [12,41,42]. Consequently, it remains difficult to determine precisely how many genes underlie color change.

Current evidence from pigmentation genetics in laboratory, domesticated and wild populations shows that many genes are involved in pigmentation (Online Supplementary Material). There are many examples in which the same genes (e.g. *MC1R* and, to a lesser extent, *ASIP*) are repeated targets of evolutionary change. By contrast, different pigmentation genes and/or different functional mechanisms in the same gene [13,14,71] can produce very similar phenotypes even among populations within a species [28,36] (Table 1). This suggests some genetic and developmental constraints and, at the same time, flexibility in the underlying mechanisms of adaptation.

Color traits are often the target of selection because even small changes in color can often have large implications for an organism's ability to survive or reproduce in the wild [18,72]. Although field observations and experiments can provide estimates of the strength of selection [10,73], the identification of genes underlying adaptive traits allows us to estimate selection at the genetic level. For example, assuming a model of migration–selection balance at equilibrium [74], selection coefficients can be estimated directly based on estimates of effective population size and migration rate from (neutral) genetic data. This approach was used to estimate strong selection against ‘mismatched’ mice – the selection against the ancestral light color morph on novel dark soil habitat as well as the derived dark morph on light habitat [10]. In addition, selection coefficients can be estimated using more sophisticated population–genetic approaches based on patterns of nucleotide variation [75–77]. For example, several methods take advantage of linkage disequilibrium (LD), or

the association among mutations from independent loci, to detect a signature of selection. The extent of LD should increase in regions under strong directional selection, and so genomic regions surrounding the target of selection initially will have high LD and low polymorphism (i.e. selective sweep) [78,79]. This method was used effectively in detecting and estimating the strength of selection on *Asip* allelic variants in *Peromyscus maniculatus* [2]. For more in-depth discussions of the various analytical techniques developed to detect selection at the molecular level several recent reviews are available on this topic [75–77].

Thus, it is clear that identifying the genetic basis of phenotypic traits can provide insight into the evolutionary process. Owing to the relative success of linking genotype to phenotype for pigmentation traits, much of this progress has come from the study of color variation in vertebrates. Future work, which will involve studies in diverse taxa and unique color variants, including brilliant colors, more complex color patterns and continuous color variation, will only increase our growing knowledge of the molecular basis of organismal phenotypic diversity. In addition to identifying informative genetic mutations underlying adaptive coloration in wild populations of vertebrates, future studies should quantify selection at the phenotypic and molecular levels to make progress toward understanding the evolutionary processes leading to phenotypic change.

Concluding remarks

Data on *MC1R* and *ASIP* have accumulated at a rapid rate, and offer some of the first direct links between ecologically relevant phenotypes and their underlying genotypes. Yet, there is much work to be done, even with these genes. First, we emphasize the need for careful functional assays not only to demonstrate empirically the causal links between genotype and phenotype, but also to provide a more detailed understanding of how mutations produce phenotypic variation (e.g. mechanism). Second, using population genetic approaches and/or experimental field studies we can also document selection at both the genetic and phenotypic levels (e.g. function). Of course, from a comparative perspective, future work will expand the scope of chemical, structural and genetic analyses to understand the mechanisms generating the awe-inspiring array of animal color-

Box 4. Outstanding questions

Here, we offer questions at the interface of pigmentation and vision genetics and their ecological and evolutionary context.

- How often do species with variation in color also show variation in color perception?
- Are genes underlying the coloration of fur, skin, scales, feathers and eggshells linked to (either physically or statistically), and coevolving with, genes underlying a perceptual bias in color vision (e.g. opsin genes)?
- Which evolved first: genes responsible for changes in pigmentation or those related to visual perception? Can we use phylogenetic comparative methods to date the evolutionary origins of variation in pigmentation and perception?
- Which genes (pigmentation or opsin) are less constrained for local adaptation (e.g. via coevolutionary arms races with predators or as light environment changes)?
- How often are genotypes underlying within-population and among-population color polymorphism (fur, skin, scales and feathers) maintained by non-random mating patterns?
- Is variation in pigmentation or opsin genes more often associated with color differences among closely related populations than between species?

ation, not only color variation controlled by well-characterized melanin-related genes, but also from the brilliant plumages, scales, skin and other pigmented tissues of a wide range of animals. New discoveries of genes regulating these colorful pigments and structures lie on the horizon, with even greater implications for understanding patterns of biodiversity because, in many cases, differences in these colors are more clearly involved in delimiting species boundaries and are associated with communication signals (Box 4). We suggest that explorations across a fully integrated spectrum of genes related both to the patterns and colors of pigments and to the perception of these phenotypes within an ecological and evolutionary context will lead to a deeper understanding of the processes responsible for the evolution of the spectacular diversity of animal coloration we see in nature.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tig.2010.02.002](https://doi.org/10.1016/j.tig.2010.02.002).

References

- 1 Barrett, R.D.H. *et al.* (2008) Natural selection on a major armor gene in threespine stickleback. *Science* 322, 255–257
- 2 Linnen, C.R. *et al.* (2009) On the origin and spread of an adaptive allele in deer mice. *Science* 325, 1095–1098
- 3 Silvers, W.K. (1979) *The coat colors of mice: a model for mammalian gene action and interaction*, Springer-Verlag
- 4 Hoekstra, H.E. (2006) Genetics, development and evolution of adaptive pigmentation in vertebrates. *Heredity* 97, 222–234
- 5 Mundy, N.I. (2005) A window on the genetics of evolution: *MC1R* and plumage coloration in birds. *Proc. Biol. Sci.* 272, 1633–1640
- 6 Protas, M.E. and Patel, N.H. (2008) Evolution of coloration patterns. *Annu. Rev. Cell Dev. Biol.* 24, 425–446
- 7 Roulin, A. (2004) The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biological Reviews* 79, 815–848
- 8 Anderson, T.M. *et al.* (2009) Molecular and evolutionary history of melanism in North American gray wolves. *Science* 323, 1339–1343
- 9 Doucet, S.M. *et al.* (2004) Concordant evolution of plumage colour, feather microstructure and a melanocortin receptor gene between mainland and island populations of a fairy-wren. *Proc. Biol. Sci.* 271, 1663–1670
- 10 Hoekstra, H.E. *et al.* (2004) Ecological genetics of adaptive color polymorphism in pocket mice: geographic variation in selected and neutral genes. *Evolution* 58, 1329–1341
- 11 Mundy, N.I. *et al.* (2004) Conserved genetic basis of a quantitative plumage trait involved in mate choice. *Science* 303, 1870–1873
- 12 Steiner, C.C. *et al.* (2007) Adaptive variation in beach mice produced by two interacting pigmentation genes. *PLoS Biology* 5, e219 [10.1371/journal.pbio.0050219](https://doi.org/10.1371/journal.pbio.0050219). (<http://www.plosbiology.org>)
- 13 Theron, E. *et al.* (2001) The molecular basis of an avian plumage polymorphism in the wild: a *melanocortin-1-receptor* point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola*. *Curr. Biol.* 11, 550–557
- 14 Uy, J.A.C. *et al.* (2009) Difference in plumage color used in species recognition between incipient species is linked to a single amino acid substitution in the melanocortin-1 receptor. *Am. Nat.* 174, 244–254
- 15 Amundsen, T. and Pärn, H. (2006) Female coloration: review of functional and nonfunctional hypotheses. In *Bird Coloration: Function and Evolution* (Hill, G.E. and McGraw, K.J., eds), pp. 280–345, Harvard University Press
- 16 Hill, G.E. (2006) Female mate choice for ornamental coloration. In *Bird Coloration: Function and Evolution* (Hill, G.E. and McGraw, K.J., eds), pp. 137–200, Harvard University Press
- 17 Senar, J.C. (2006) Color displays as intrasexual signals of aggression and dominance. In *Bird Coloration: Function and Evolution* (Hill, G.E. and McGraw, K.J., eds), pp. 87–136, Harvard University Press
- 18 Safran, R.J. and McGraw, K.J. (2004) Plumage coloration, not length or symmetry of tail-streamers, is a sexually selected trait in North American barn swallows. *Behavioral Ecology* 15, 455–461
- 19 Slagsvold, T. *et al.* (1995) Predation favours cryptic coloration in breeding male pied flycatchers. *Animal Behaviour* 50, 1109–1121
- 20 Nachman, M.W. *et al.* (2003) The genetic basis of adaptive melanism in pocket mice. *Proc. Natl. Acad. Sci. USA* 100, 5268–5273
- 21 Steiner, C.C. *et al.* (2009) The genetic basis of phenotypic convergence in beach mice: similar pigment patterns but different genes. *Mol. Biol. Evol.* 26, 35–45
- 22 McGraw, K.J. (2006) Mechanisms of carotenoid-based coloration. In *Bird Coloration: Mechanisms and Measurements* (Hill, G.E. and McGraw, K.J., eds), Harvard University Press
- 23 McGraw, K.J. (2006) Mechanisms of melanin-based coloration. In *Bird Coloration: Mechanisms and Measurements* (Hill, G.E. and McGraw, K.J., eds), pp. 243–294, Harvard University Press
- 24 Goldstein, G. *et al.* (2004) Bacterial degradation of black and white feathers. *The Auk* 121, 656–659
- 25 Rosenblum, E.B. *et al.* (2004) Adaptive reptile color variation and the evolution of the *Mc1r* gene. *Evolution* 58, 1794–1808
- 26 Gross, J.B. *et al.* (2009) A novel role for *Mc1r* in the parallel evolution of depigmentation in independent populations of the cavefish *Astyanax mexicanus*. *PLoS Genetics* 5, e1000326 [10.1371/journal.pgen.1000326](https://doi.org/10.1371/journal.pgen.1000326) (<http://www.plosgenetics.org>)
- 27 Kingsley, E.P. *et al.* (2009) Melanism in *Peromyscus* is caused by independent mutation in *Agouti*. *PLoS One* 4, e6435 [10.1371/journal.pone.0006435](https://doi.org/10.1371/journal.pone.0006435) (<http://www.plosone.org>)
- 28 Hoekstra, H.E. *et al.* (2006) A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science* 313, 101–104
- 29 Prum, R.O. (2006) Anatomy, physics, and evolution of structural colors. In *Bird Coloration: Mechanisms and Measurements* (Hill, G.E. and McGraw, K.J., eds), Harvard University Press
- 30 Seehausen, O. *et al.* (2008) Speciation through sensory drive in cichlid fish. *Nature* 455, 620–626

- 31 Mountjoy, K.G. *et al.* (1992) The cloning of a family of genes that encode the melanocortin receptors. *Science* 257, 1248–1251
- 32 McGraw, K.J. *et al.* (2004) European barn swallows use melanin pigments to color their feathers brown. *Behavioral Ecology* 15, 889–891
- 33 McGraw, K.J. *et al.* (2005) How feather colour reflects its melanin content. *Functional Ecology* 19, 816–821
- 34 Mills, M. and Patterson, L. (2009) Not just black and white: pigment pattern development and evolution in vertebrates. *Semin. Cell Dev. Biol.* 20, 72–81
- 35 Cheviron, Z.A. *et al.* (2006) Sequence variation in the coding region of the melanocortin-1 receptor gene (*MC1R*) is not associated with plumage variation in the blue-crowned manakin (*Lepidothrix coronata*). *Proc. Biol. Sci.* 273, 1613–1618
- 36 Römpler, H. *et al.* (2006) Nuclear gene indicates coat-color polymorphism in mammoths. *Science* 313, 62
- 37 Rosenblum, E.B. *et al.* (2010) Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proc. Natl. Acad. Sci. USA* 107, 2113–2117
- 38 Lalueza-Fox, C. *et al.* (2007) A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. *Science* 318, 1453–1455
- 39 Fang, M. *et al.* (2009) Contrasting mode of evolution at a coat color locus in wild and domestic pigs. *PLoS Genetics* 5, e1000341
- 40 Mundy, N.I. and Kelly, J. (2006) Investigation of the role of the agouti signaling protein gene (*ASIP*) in coat color evolution in primates. *Mamm. Genome* 17, 1205–1213
- 41 Nadeau, N. *et al.* (2008) Characterization of Japanese quail yellow as a genomic deletion upstream of the avian homolog of the mammalian *ASIP* (*agouti*) gene. *Genetics* 178, 777–786
- 42 Våge, D.I. *et al.* (1997) A non-epistatic interaction of *agouti* and *extension* in the fox, *Vulpes vulpes*. *Nat. Genet.* 15, 311–315
- 43 Klovin, J. and Schiöth, H.B. (2005) Agouti-related proteins (AGRP) and agouti-signaling peptide (ASIP) in fish and chicken. *Ann. N Y Acad. Sci.* 1040, 363–367
- 44 Ludwig, A. *et al.* (2009) Coat color variation at the beginning of horse domestication. *Science* 324, 485
- 45 Eizirik, E. *et al.* (2003) Molecular genetics and evolution of melanism in the cat family. *Curr. Biol.* 13, 448–453
- 46 Cerdá-Reverter, J.M. *et al.* (2005) Gene structure of the goldfish agouti-signaling protein: a putative role in the dorsal-ventral pigment pattern of fish. *Endocrinology* 146, 1597–1610
- 47 Fan, W. *et al.* (1997) Role of melanocortinergic neurons in feeding and the *agouti* obesity syndrome. *Nature* 385, 165–168
- 48 Minvielle, F. *et al.* (2007) Effects of the dominant lethal yellow mutation on reproduction, growth, feed consumption, body temperature, and body composition in Japanese quail. *Poult. Sci.* 86, 1646–1650
- 49 Jackson, I.J. (1997) Homologous pigmentation mutations in human, mouse and other model organisms. *Hum. Mol. Genet.* 6, 1613–1624
- 50 Jackson, P.J. *et al.* (2006) Structural and molecular evolutionary analysis of Agouti and Agouti-related proteins. *Chem. Biol.* 13, 1297–1305
- 51 Ollmann, M.M. *et al.* (1998) Interaction of Agouti protein with the melanocortin 1 receptor *in vitro* and *in vivo*. *Genes Devel.* 12, 316–330
- 52 Zdarsky, E. *et al.* (1990) The molecular basis of brown, an old mouse mutation, and of an induced revertant to wild type. *Genetics* 126, 443–449
- 53 Gratten, J. *et al.* (2007) Compelling evidence that a single nucleotide substitution in *TYRP1* is responsible for coat-colour polymorphism in a free-living population of Soay sheep. *Proc. Biol. Sci.* 274, 619–626
- 54 Kwon, B.S. *et al.* (1989) Isolation, chromosomal mapping, and expression of the mouse tyrosinase gene. *J. Invest. Dermatol.* 93, 589–594
- 55 Protas, M.E. *et al.* (2006) Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. *Nat. Genet.* 38, 107–111
- 56 Sturm, R.A. and Frudakis, T.N. (2004) Eye colour: portals into pigmentation genes and ancestry. *Trends Genet.* 20, 327–332
- 57 Roberts, R.B. *et al.* (2009) Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes. *Science* 326, 998–1001
- 58 Miller, C.T. *et al.* (2007) *cis*-regulatory changes in *Kit Ligand* expression and parallel evolution of pigmentation in sticklebacks and humans. *Cell* 131, 1179–1189
- 59 Wehrle-Haller, B. (2003) The role of Kit-ligand in melanocyte development and epidermal homeostasis. *Pigment Cell Res.* 16, 287–296
- 60 Lamason, R.L. *et al.* (2005) *SLC24A5*, a putative cation exchanger, affects pigmentation in zebrafish and humans. *Science* 310, 1782–1786
- 61 Eriksson, J. *et al.* (2008) Identification of the *yellow skin* gene reveals a hybrid origin of the domestic chicken. *PLoS Genetics* 4, e1000010
10.1371/journal.pgen.1000010: 10.1371/journal.pgen.1000010 (<http://www.plosgenetics.org>)
- 62 McGraw, K.J. (2006) Mechanics of uncommon colors: pterins, porphyrins, and psittacofulvins. In *Bird Coloration: Mechanisms and Measurements* (Hill, G.E. and McGraw, K.J., eds), pp. 354–398, Harvard University Press
- 63 Steffen, J.E. and McGraw, K.J. (2007) Contributions of pterin and carotenoid pigments to dewlap coloration in two anole species. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 146, 42–46
- 64 Hill, G.E. (1991) Plumage coloration is a sexually selected indicator of male quality. *Nature* 350, 337–339
- 65 Hughes, K.A. *et al.* (2005) Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*). *Journal of Evolutionary Biology* 18, 35–45
- 66 Hadfield, J.D. and Owens, I.P.F. (2006) Strong environmental determination of a carotenoid-based plumage trait is not mediated by carotenoid availability. *Journal of Evolutionary Biology* 19, 1104–1114
- 67 Siefferman, L. and Hill, G.E. (2005) Evidence for sexual selection on structural plumage coloration in female eastern bluebirds (*Sialia sialis*). *Evolution* 59, 1819–1828
- 68 Basolo, A.L. (2006) Genetic linkage and color polymorphism in the southern platyfish (*Xiphophorus maculatus*): a model system for studies of color pattern evolution. *Zebrafish* 3, 65–83
- 69 Kelsh, R.N. (2004) Genetics and evolution of pigment patterns in fish. *Pigment Cell Res.* 17, 326–336
- 70 Prum, R.O. *et al.* (2009) Development of colour-producing β -keratin nanostructures in avian feather barbs. *J. R. Soc. Interface* 6, S253–S265
- 71 Hoekstra, H.E. and Nachman, M.W. (2003) Different genes underlie adaptive melanism in different populations of rock pocket mice. *Mol. Ecol.* 12, 1185–1194
- 72 Endler, J.A. (1991) Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Res.* 31, 587–608
- 73 Grant, B.R. (1985) Selection on bill characters in a population of Darwin's finches: *Geospiza conirostris* on Isla Genovesa, Galápagos. *Evolution* 39, 523–532
- 74 Haldane, J.B.S. (1930) A mathematical theory of artificial and natural selection. Part VI. Isolation. *Proc. Camb. Phil. Soc.* 26, 220–230
- 75 Jensen, J.D. *et al.* (2007) Approaches for identifying targets of positive selection. *Trends Genet.* 23, 568–577
- 76 Nielsen, R. (2005) Molecular signatures of natural selection. *Annu. Rev. Genet.* 39, 197–218
- 77 Biswas, S. and Akey, J.M. (2006) Genomic insights into positive selection. *Trends Genet.* 22, 437–446
- 78 Kim, Y. and Stephan, W. (2002) Detecting a local signature of genetic hitchhiking along a recombining chromosome. *Genetics* 160, 765–777
- 79 Meiklejohn, C.D. *et al.* (2004) Identification of a locus under complex positive selection in *Drosophila simulans* by haplotype mapping and composite-likelihood estimation. *Genetics* 168, 265–279
- 80 Cuthill, I.C. *et al.* (1999) Plumage reflectance and the objective assessment of avian sexual dichromatism. *Am. Nat.* 160, 183–200
- 81 Cassey, P. *et al.* (2009) Are avian eggshell colours effective intraspecific communication signals in the Muscicapoidea? A perceptual modelling approach. *Ibis* 151, 689–698
- 82 Endler, J.A. *et al.* (2005) Animal visual systems and the evolution of color patterns: sensory processing illuminates signal evolution. *Evolution* 59, 1795–1818
- 83 Osorio, D. and Vorobyev, M. (2008) A review of the evolution of animal colour vision and visual communication signals. *Vision Res.* 48, 2042–2051
- 84 Shi, Y. and Yokoyama, S. (2003) Molecular analysis of the evolutionary significance of ultraviolet vision in vertebrates. *Proc. Natl. Acad. Sci. USA* 100, 8308–8313
- 85 Ödeen, A. *et al.* (2009) Assessing the use of genomic DNA as a predictor of the maximum absorbance wavelength of avian SWS1 opsin visual pigments. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 195, 167–173

- 86 Göth, A. and Evans, C.S. (2004) Social responses without early experience: Australian brush-turkey chicks use specific visual cues to aggregate with conspecifics. *J. Exp. Biol.* 207, 2199–2208
- 87 Mancuso, K. *et al.* (2009) Gene therapy for red-green colour blindness in adult primates. *Nature* 461, 784–787
- 88 Endler, J.A. and Thery, M. (1996) Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. *Am. Nat.* 148, 421–452
- 89 Heinsohn, R. (2008) The ecological basis of unusual sex roles in reverse-dichromatic eclectus parrots. *Animal Behaviour* 76, 97–103
- 90 Heinsohn, R. *et al.* (2005) Extreme reversed sexual dichromatism in a bird without sex role reversal. *Science* 309, 617–619
- 91 Endler, J.A. (1993) The color of light in forests and its implications. *Ecological Monographs* 63, 1–27
- 92 Gosler, A.G. *et al.* (2000) Inheritance and variation in eggshell patterning in the great tit *Parus major*. *Proc. Biol. Sci.* 267, 2469–2473
- 93 Gosler, A. *et al.* (2005) Why are birds' eggs speckled? *Ecology Letters* 8, 1105–1113
- 94 Safran, R.J. *et al.* (2008) Sexual signal exaggeration affects physiological state in male barn swallows. *Curr. Biol.* 18, R461–R462
- 95 Rubenstein, D.R. and Hauber, M.E. (2008) Dynamic feedback between phenotype and physiology in sexually selected traits. *Trends Ecol. Evol.* 23, 655–658
- 96 Ritland, K. *et al.* (2001) Inheritance and population structure of the white-phased “Kermode” black bear. *Curr. Biol.* 11, 1468–1472
- 97 Sturm, R.A. (2009) Molecular genetics of human pigmentation diversity. *Hum. Mol. Genet.* 18, R9–R17