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SHORT REVIEW

Genetics, development and evolution of adaptive pigmentation in vertebrates

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The study of pigmentation has played an important role in the intersection of evolution, genetics, and developmental biology. Pigmentation's utility as a visible phenotypic marker has resulted in over 100 years of intense study of coat color mutations in laboratory mice, thereby creating an impressive list of candidate genes and an understanding of the developmental mechanisms responsible for the phenotypic effects. Variation in color and pigment patterning has also served as the focus of many classic studies of naturally occurring phenotypic variation in a wide variety of vertebrates, providing some of the most compelling cases for parallel and convergent evolution. Thus, the pigmentation model system holds much promise for understanding the nature of adaptation by linking genetic changes to variation in fitness-related traits. Here, I first discuss the historical role of pigmentation in genetics, development and evolutionary biology. I then discuss recent empirically based studies in vertebrates, which rely on these historical foundations to make connections between genotype and phenotype for ecologically important pigmentation traits. These studies provide insight into the evolutionary process by uncovering the genetic basis of adaptive traits and addressing such long-standing questions in evolutionary biology as (1) are adaptive changes predominantly caused by mutations in regulatory regions or coding regions? (2) is adaptation driven by the fixation of dominant mutations? and (3) to what extent are parallel phenotypic changes caused by similar genetic changes? It is clear that coloration has much to teach us about the molecular basis of organismal diversity, adaptation and the evolutionary process.
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Introduction

Understanding the generation and maintenance of phenotypic diversity requires the integration of genetics, development and evolutionary biology in an ecological context. Historically, biologists have used two parallel approaches to study evolutionary change, one working at the level of genotype and a second working at the level of phenotype (Lewontin, 1974). For example, population geneticists have focused on temporal and spatial changes in allele and genotype frequencies, whereas organismal biologists have studied how individuals differ in phenotypic traits across natural environments. However, research linking genotype and phenotype (ie, identifying the molecular changes responsible for phenotypic adaptation and the developmental mechanisms by which genotypes encode phenotypic traits (eg, Carroll *et al*, 2001; Brakefield *et al*, 2003)) is necessary to truly understand the processes responsible for generating both genetic and organismal diversity.

The pigmentation system is a particularly promising phenotype in which to explore connections between genotype and phenotype for ecologically important traits. Coat color mutations in laboratory mice have served as a premier model for studying gene action in a variety of biological processes (Silvers, 1979), leading to a wealth of information about genes involved in pigmentation and their developmental interactions. Because melanin-based pigmentation biology is highly conserved across vertebrates, a deep understanding of mouse coat color genetics translates easily and directly into testable hypotheses for studying the molecular basis of pigmentation variation in natural vertebrate populations (Bennett and Lamoreux, 2003). Most important, color quality and/or color patterns frequently exhibit dramatic variation both within and between species in a way that can be quantified (Endler, 1990) and is conspicuously affected by natural selection (Caro, 2005). In particular, selective forces such as crypsis, aposematism, thermoregulation, and sexual signaling drive variation in both pigmentation and color pattern (Thayer, 1909; Cott, 1940). Thus, pigmentation phenotypes in natural populations present an ideal opportunity for studying the genetic basis of phenotypic diversity and evolutionary change.

Here, I first discuss how the rich history of pigmentation biology in the fields of genetics, development and evolution provide the essential background information to address fundamental questions in evolutionary developmental biology. Then I provide empirical examples in which the link between genotype and phenotype has been successfully made for adaptive pigmentation in natural populations. Throughout, I focus primarily on studies of mice and melanin-based pigmentation, from which the most data are available, but draw on studies of other vertebrates and pigment types whenever possible. Together, these studies provide exciting insight into how

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adaptation proceeds at the molecular level and also shed light on the evolutionary process in general.

History of pigmentation biology

The study of pigmentation has played a critical role in the fields of genetics, development and evolution. Beginning in 18th century China and Japan, so-called mouse fanciers collected, maintained and bred together unusual morphs of wild mice (Morse, 1978); in doing so, these novice geneticists generated mice with a large diversity of color variation, much of which is represented today in laboratory mice. With the development of diverse mouse strains, pigmentation phenotypes were readily available for study, and much of our knowledge of the pigmentation process has subsequently come from studies of these laboratory mice. Correspondingly, observations of coat color variation, first in the laboratory and later in the field, have played an essential role in the understanding of many fundamental biological processes (Table 1).

Genetics: the pigmentation loci

In the early 1900s as Mendel's principles were rediscovered, coat color differences were used to test several fundamental theories of genetics, and 50 years later, mutations of the same genes provided the first estimates of mammalian mutation rates. As early as 1903 (before the term 'genetics' was even coined), independent experiments by Lucien Cuénot (1904) and William Castle and Allen (1903) first demonstrated Mendelian inheritance in mammals by documenting segregation patterns of albino phenotypes in genetic crosses. Then, in 1915, Haldane *et al* (1915) published the first genetic linkage study in vertebrates, establishing linkage between the *pink-eyed dilution* locus and the *albino* locus in the mouse. Parallel studies of pigmentation genetics in other vertebrate taxa provided additional early examples of genetic linkage, perhaps most notably in guppies (Winge, 1927).

Around the same time, Sewall Wright, under the tutelage of Castle, developed key experiments to demonstrate epistasis and pleiotropy using coat color variation in hooded rats – animals that were mostly white except for a pigmented area on the head and neck. In these experiments, the size of the pigmented area or 'hood' was selected to be small in some lines and large in others. Castle initially thought these size differences reflected different alleles of the major gene responsible for hooding; however, Wright showed that so-called modifier genes were responsible for variation in hood size, providing the first experimental demonstration of epistasis (Little, 1917). Wright's work with pigmentation in guinea pigs also revealed the importance of interactions within and among gene systems. Wright demonstrated that apparently unrelated or pleiotropic phenotypes can have a single underlying developmental mechanism, suggesting how adaptive change in one trait might easily dictate nonadaptive change in other traits. These classic experiments using coat color phenotypes clearly influenced the fields of quantitative and population genetics.

In the 1950s and 1960s, mouse coat color genetics became increasingly used as a test system for studying induced and spontaneous mutation. Arguably, the earliest and most comprehensive estimates of mammalian mutation rates relied directly on visible phenotypes, including a number of coat color loci (eg, *agouti* (*a*), *brown* (*b*), *albino* (*c*), *dilute* (*d*), *leaden* (*ln*), *pink-eyed dilution* (*p*) and *piebald-spotting* (*s*)). First, a student of Wright, William Russell created a *T-stock* mouse packed with seven recessive, viable, radiation-induced mutations, six of which were coat color mutations (Russell, 1951). By examining large numbers of progeny ($>85\,000$) from crosses of *T-stock* mice to mutagenized animals, Russell was able to estimate the rate of heritable gene mutations, an approach later termed 'the specific locus test'. These experiments revealed a 10 times higher mutation rate in mammals relative to earlier estimates in *Drosophila* as well as a high degree of variation in mutability among loci (reviewed in Davis and Justice, 1998). This work was followed by a series of studies of *spontaneous* mutation rates in the 1960s, when large production colonies were carefully examined for spontaneous coat color variants; more than 100 new genes were identified (Schlager and

Table 1 Timeline of some milestones and fundamental discoveries using pigmentation phenotypes

Date	*Milestone*	*Representative reference*
1700s	Establishment of laboratory mouse strains with 'fancy' coat color patterns	Morse (1978)
1902–1904	Demonstration of Mendelian inheritance in vertebrates using the albino locus	Castle and Allen (1903), Cuenot (1904)
1915	Establishment of genetic linkage in mammals using two pigmentation loci	Haldane *et al* (1915)
1917	Seminal papers on coat color genetics in laboratory animals	Wright (1917a, b, c, d)
1920s	Natural history studies linking vertebrate pigmentation to environmental variation	Sumner (1921, 1929a, b), Benson (1933), Dice and Blossom (1937)
1948	First mathematical treatment of clinal variation based on adaptive pigmentation traits	Haldane (1948)
1950s	First estimate of radiation-induced mammalian mutation rates at six coat color loci	Russell (1951), Russell and Major (1957)
1960s	Estimates of spontaneous mammalian mutation rates using coat color phenotypes	Schlager and Dickie (1966, 1969)
1986	Cloning of the first pigmentation gene	Shibahara *et al* (1986)
2000s	Linking mutations in pigmentation genes to adaptive phenotypic variation in the wild	

Dickie, 1966, 1967). Together, these studies highlight how pigmentation loci have provided a foundation for many fundamental concepts in genetics.

As a result of the tremendous utility of pigmentation phenotypes, a sizeable list of genetic loci with well-characterized phenotypes in mice has been accumulated (Table 2). The first pigmentation gene to be cloned, *tyrosinase-related-protein-1* (*Tyrp1*), was initially thought to be the gene responsible for the *albino* mouse mutant, but albinism was later mapped, cloned, sequenced and

Table 2 Pigmentation genes that have been cloned and sequenced in laboratory mouse

Gene	*Classic mutant*	*Chrom*[a]	*Function*[b]
A disintergrin and metalloproteinase domain 17 (Adam17)		12	a
Adamts20	Belted (bt)	15	a
Ectodysplasin-A (Eda)	Tabby (ta)	X	a
Endothelin 3 ligand (Edn3)	Lethal spotting (ls)	2	a
Endothelin receptor B (Ednrb)	Piebald spotting (s)	14	a
Epidermal growth factor receptor (Egfr)	Darkskin5 (dsk5)	11	a
Fibroblast growth factor receptor 2 (Fgfr2)		7	a
Inhibitor of kappaB kinase gamma (Ikbkg)		X	a
C-kit receptor (Kit)	Dominant white-spotting (W)	5	a
Ligand for c-kit receptor (Kitl)	Steel (Sl)	10	a
Keratin complex 2, gene 17 (Krt2–17)	Dark skin 2 (dsk2)	15	a
LIM homeodomain protein 1 (Lmx1a)	Dreher (dr)	1	a
Mucolipin 3 (Mcoln3)	Variant-waddler (Va)	3	a
Microphthalmia transcription factor (Mitf)	Microphthalmia (mi)	6	a
Pax-3 transcription factor (Pax3)	Splotch (Sp)	1	a
Sideroflexin (Sfxn1)	Flexed tail	13	a
Neural crest transcription factor (Snai2)	White spotting	16	a
Sry-box-containing gene 10 (Sox10)	Dominant megacolon (Dom)	15	a
Sry-box-containing gene 18 (Sox18)	Ragged (ra)	2	a
Transcription factor AP-2 alpha (Tcfap2A)		13	a
T-box gene (tbx15)	Droopy ear (de)	3	a
Tyrosinase-related protein 2 (Tyrp2/Dct)	Slaty (slt)	14	b
Glycoprotein (Gpnmb)	Iris pigment dispersion (ipd)	6	b
Membrane-associated transporter protein (Matp)	Underwhite (uw)	15	b
Member of RAS oncogene family (Rab38)	Chocolate (cht)	7	b
Silver protein (Pmel17)	Silver (si)	10	b
Solute carrier family 24 member 5 (Slc24a5)	Golden (gol)	2	b
Tyrosinase (Tyr)	Albino (c)	7	b
Tyrosinase-related protein 1 (Tyrp1)	Brown (b)	4	b
Beta 3 subunit of adaptor protein 3 (Ap3b1)	Pearl (pe)	13	c
Bloc1s3	Reduced pigmentation (rp)	7	c
Delta subunit of adaptor protein 2 (Ap3d)	Mocha (mh)	10	c
Dtnbp1	Sandy (sdy)	13	c
Cno	Cappuccino (cno)	5	c
Hermansky-Pudlak syndrome gene 1 (Hps1)	Pale ear (ep)	19	c
Hermansky-Pudlak syndrome gene 3 (Hps3)	Cocoa (coa)	3	c
Hermansky-Pudlak syndrome gene 4 (Hps4)	Light ear (le)	5	c
Hermansky-Pudlak syndrome gene 5 (Hps5)	Ruby-eye 2 (ru2)	7	c
Hermansky-Pudlak syndrome gene 6 (Hps6)	Ruby eye (ru)	19	c
Lysosomal trafficking regulator (Lyst)	Beige (bg)	13	c
Muted (Mu)	Muted (mu)	13	c
Ocular albinism type 1 (Oca1)	Oca1 (oca1)	X	c
Ocular albinism type 2 (Oca2)	Pink-eyed dilution (p)	7	c
Pallidin (Pldn)	Pallid (pa)	2	c
Rab geranylgeranyl transferase (Rabgtta)	Gunmetal (gm)	14	c
Vacuolar protein sorting 33a (Vps33a)	Buff (bf)	5	c
Melanophilin (Mlph)	Leaden (ln)	1	d
Myosin type Va (Myo5a)	Dilute (d)	9	d
Myosin type 7a (Myo7a)	Shaker-1 (sh-1)	7	d
RAS-associated protein (Rab27a)	Ashen (ash)	9	d
Agouti signaling protein (Asip)	Nonagouti (a)	2	e
Attractin (Atrn)	Mahogany (mg)	2	e
Gamma gluyltranspeptidase 1 (Ggt1)	Ggt1	10	e
Gl	Grey lethal (gl)	10	e
Melanocortin-1 receptor (Mc1r)	Extension (e)	8	e
E3 ubiquitin ligase (Mgrn1)	Mahoganoid (md)	16	e
Proopiomelanocortin (Pomc1)		12	e
Solute carrier family 7 member 11 (Slc7a11)	Subtle grey (sut)	3	e
ATPase (Atp7a)	Mottled (mo)	X	f

[a]*Mus musculus* chromosome position.
[b]Functional classes: a, melanocyte development; b, components of melanosomes; c, melanosome construction; d, melanosome transport; e, melanin synthesis and switching; f, systemic effects. Modified from Oetting WS, Bennett DC. Mouse Coat Color Genes. International Federation of Pigment Cell Societies. URL: http://www.cbc.umn.edu/ifpcs/micemut.htm (references therein).

correctly attributed to the *tyrosinase* locus (Jackson, 1988; Kwon *et al*, 1989). Since then, nearly 100 genes affecting pigmentation have been cloned in mice, but almost an equal number have yet to be identified (Bennett and Lamoreux, 2003), and new loci are accumulating as a result of chemical mutagenesis programs (Mouse Genome Database). In addition, complementary vertebrate systems, such as zebrafish, are providing additional pigmentation genes (Haffter *et al*, 1996; Odenthal *et al*, 1996; Kelsh *et al*, 2004), one of which (*Slc24a5*) has recently been linked to variation in human skin coloration (Lamason *et al*, 2006). Together, these loci provide excellent candidates for studying adaptive variation in natural populations of vertebrates.

Development: the pigmentation pathway

The characterization of pigmentation loci has provided considerable insight into fundamental developmental processes and a detailed understanding of the pigmentation pathway. In vertebrates, melanin-based pigmentation is the culmination of a complex process including the inception, migration and regulation of melanocytes (reviewed in Jackson, 1994). Based on studies in mammals, changes in melanocyte development and regulation ultimately lead to two primary ways in which variation in pigmentation phenotype is generated: (1) altering the spatial distribution of pigmentation across the body or (2) altering the density or distribution of pigmentation along individual hairs. Both of these strategies can have profound effects on overall appearance, but likely have a distinct genetic basis and are manifested in different parts of the developmental pathway. While there has been a growing interest in understanding how pigmentation patterns are generated, the most careful dissection of the pigmentation pathway has focused on regulation of melanocytes.

Pigmentation patterning

Pigmentation patterning has long captured the interest of biologists, largely because of the tremendous diversity in color pattern among animals, from butterfly wing spots to zebra stripes. In vertebrates, several mechanisms may contribute to regional variation in melanin type and density. During vertebrate embryogenesis, neural crest cells arise along the dorsal neural tube, and some differentiate into melanoblasts (precursors of melanocytes), which migrate ventrally along the body. Melanoblasts typically enter the epidermis, where some remain, while others localize to the hair follicles and differentiate into melanocytes. These melanocytes produce pigment (melanocyte regulation is discussed in detail below), and, once pigment is produced, it is packaged into melanosomes and transferred to keratinocytes of developing hair (or epidermal cells). Therefore, genes involved in patterning likely act early in development and are involved in melanocyte differentiation, development and migration.

While several genes essential for proper melanocyte development and dispersal have been identified (Table 2, Baxter *et al*, 2004), little is known about their spatial and temporal control. It has been postulated that developmental timing plays an important role in generating regular patterns; for example, subtle differences in the timing of melanocyte differentiation could be responsible for variation in the number of stripes among zebra species (Bard, 1977). However, the genes regulating these differences in timing remain unknown. The question of which genes are responsible for generating regional differences in pigmentation is a difficult one, largely because patterning variation in genetically tractable laboratory mouse strains is lacking and therefore not available for study. In mice, most progress in understanding patterning has been made in uncovering genes which determine differences in dorsal–ventral pigmentation.

Most vertebrates have a distinct boundary between dorsal and ventral pigmentation, typically characterized by a light colored ventrum and a darker dorsum (Figure 1). In mice, allelic variation at the *agouti* locus is largely responsible for dorsoventral differences in pigment type (Bultman *et al*, 1992; Miller *et al*, 1993; Millar *et al*, 1995). Careful dissection of the *agouti* regulatory region has revealed two major transcript initiation sites, one for a ventral-specific transcript, which is likely responsive to positional cues established in the embryo, and a second 'hair cycle-specific' transcript involved in switching between alternative types of melanins (described below, Bultman *et al*, 1994; Vrieling *et al*, 1994). The developmental mechanisms responsible for melanocyte density and differentiation along the dorsoventral axis likely involve the interactions of many proteins. How additional proteins may interact with or spatially regulate Agouti represents a first step in understanding how more complex patterns are generated.

The recent cloning of the gene responsible for the classical *droopy ear* (*de*) mouse mutant provides a first glimpse into developmental mechanisms that may be responsible for ecologically relevant variation in the spatial distribution of pigment across the body (Figure 1). The droopy ear phenotype produces a lateral shift in the dorsal–ventral boundary by allowing expansion of the

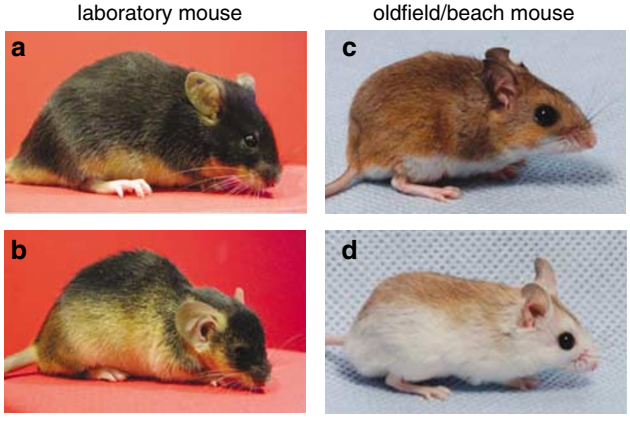

Figure 1 Variation in dorsoventral pattern in laboratory mice and natural populations of *P. polionotus*. A large deletion in the *transcription-box 15* (*Tbx15*) gene results in a lateral shift in the dorsal–ventral boundary (and modifications of craniofacial morphology) as shown in this laboratory mouse (**a** and **b**; on the Agouti *black and tan* genetic background) described by Candille *et al* (2004). A similar color pattern phenotype is observed in natural populations of beach mice relative to their darker mainland conspecific, the oldfield mouse (**c** and **d**). Spontaneous laboratory mutants can mimic naturally occurring phenotypic variation and provide candidate genes for adaptive traits.

ventral-specific Agouti transcript (Figure 1b, Candille *et al*, 2004). This shift in pigmentation is caused by a null allele of the spatially restricted T-box transcription factor (Tbx15), which likely acts as a developmental cue required to establish dorsal dermis (Candille *et al*, 2004). Importantly, several developmental genes, like *Tbx15*, may not directly participate in the pigmentation pathway, but rather indirectly affect pigmentation as a secondary consequence of its role in cellular differentiation. In either case, this result is particularly exciting because similar patterning phenotypes occur in nature (Figure 1c and d), highlighting the possible utility of candidate genes in studies of natural populations.

Additional research is needed to understand the genetic basis and developmental origins of color patterning. In particular, proteins with patterned expression in the dermis such as Agouti, Tbx15 or others like Msx1 (Houzelstein *et al*, 2000) that may form distinct boundaries/gradients in developing embryos are particularly relevant. Such proteins are strong candidates for spatial patterning of hair pigmentation in different body regions and may even be involved in the formation of more complex lateral stripes observed in many vertebrates, like some snakes, lizards and ground squirrels. Additional insights will likely come from the zebrafish model system, which shows ample variation in both striping and spotting (Parichy, 2003; Kelsh, 2004), and from emerging model systems, like *Peromyscus*, where the combination of natural variation in patterning, ability to breed in the lab and new genomic tools make it possible to identify genes, and even the molecular changes, contributing to adaptive mammalian patterning (Hoekstra *et al*, in press).

Whole-body changes in pigmentation

One aspect of mammalian pigmentation that has been a frequent substrate for natural variation is the regulation and distribution of pigment types produced by melanocytes. In mammals, there are two types of pigments: eumelanin, which is responsible for black to brown color, and pheomelanin, which is responsible for red to yellow color. In melanocytes, several genes are involved in the coordination of 'pigment type-switching' between the synthesis of eumelanin and pheomelanin (Barsh, 1996). This switch is controlled by the interaction of two primary genes: the *melanocortin-1 receptor* (*Mc1r*), which encodes a seven-transmembrane receptor expressed in melanocytes, and its ligand, *agouti*, whose protein product is secreted from nearby dermal papilla cells and acts to inhibit Mc1r signaling (Figure 2a). In the absence of Agouti protein, basal levels of Mc1r activity keep levels of intracellular cyclic AMP (cAMP) sufficiently high to activate the eumelanin synthetic pathway. However, in the presence of Agouti protein, Mc1r activity is inhibited, cAMP levels are reduced, and melanocytes stop producing eumelanin and start producing pheomelanin. The interaction of these two proteins therefore plays a critical role in determining which pigment type is deposited along individual hairs.

Additional genes are known to alter the density and distribution of melanosomes (pigment granules) found in melanocytes. Tyrosinase, for example, is the rate-limiting enzyme in melanogenesis. There are over 100 alleles of *tyrosinase* that have been characterized, ranging

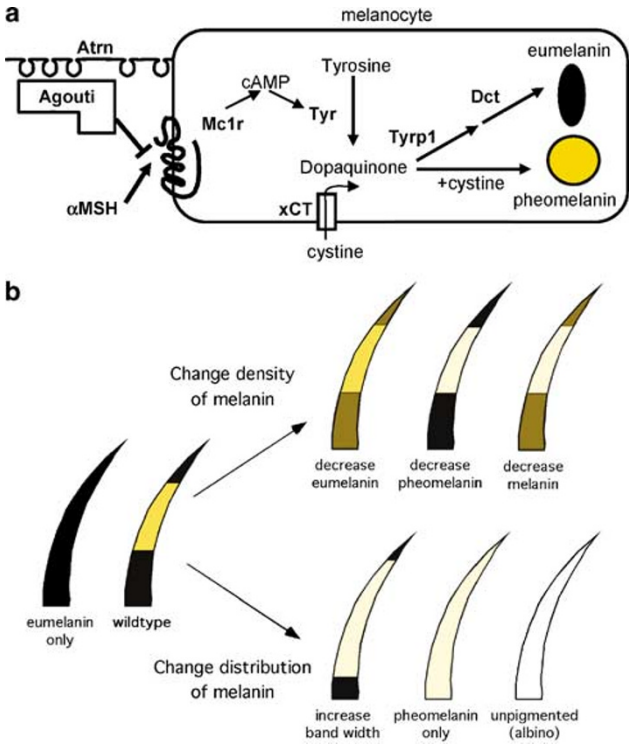

Figure 2 Genetic pathway regulating mammalian melanogenesis and phenotypic effects on individual hair pigment and pattern. (**a**) Circulating α-MSH (a derivative of POMC) activates Mc1r, a G-protein-coupled transmembrane receptor, and signals via cAMP. Intracellularly, tyrosine is oxidized to dopaquinone, a reaction catalyzed by the enzyme tyrosinase (Tyr). Cyclic AMP is thought to affect the enzymatic activity of tyrosinase as well as eumelanic-specific enzymes, tyrosinase-related protein 1 (Tyrp1) and dopachrome tautomerase (Dct). When all three of these enzymes function properly, eumelanin (brown to black pigment) is deposited in melanosomes. Agouti, the inverse agonist of Mc1r, binds to Mc1r with the aid of the extracellular protein Atrn to repress intracellular cAMP levels, resulting in the 'switch' to the production of pheomelanin (yellow to red pigment). The production of pheomelanin is dependent on the incorporation of cystine, whose uptake is at least partially regulated by xCT (the *Slc7a11* locus). (**b**) Overall coat color in mammals is determined by the density of melanin and the distribution of melanin (or melanin types) on individual hairs. Pigment on individual hairs ranges from fully pigmented with dark eumelanin to complete absence of pigment resulting in albino hairs. Typical wild-type hairs in mammals have a subterminal band of light-colored pheomelanin flanked by darker eumelanin, providing an overall brushed appearance.

from null alleles, resulting in the complete absence of pigmentation (albino), to alleles with reduced function that limit the production of melanin (Beermann *et al*, 2004). Other genes, such as *tyrosinase-related protein-1* (*Tyrp1*) and *dopachrome tautomerase* (*Dct* or *Tyrp2*), primarily regulate the eumelanin pathway. *Gamma-glutamyl transpeptidase-encoding protein* (*Ggt*) affects the production of pheomelanin, and more recently, a second gene specific to pheomelanogenesis was described, *Slc7a11* (Chintala *et al*, 2005). In addition, several genes, *Rab27a*, *Myo5a* and *Mlph*, are well studied as models for organelle transport because they coordinate the transport and distribution of melanosomes, both eumelanosomes and pheomelanosomes, in melanocytes (Nascimento *et al*, 2003). Mutations in these genes disrupt melanosome organization and can dilute overall coloration.

Clearly, many different molecular and developmental changes can affect the type, density and distribution of melanin on individual hairs and result in variation in overall pelage coloration (Figure 2b). Close examination of pigment and pattern on individual hairs can yield insight into the developmental changes and possible genes responsible for overall coloration. However, these candidates provide no guarantees – often changes in different genes can produce similar phenotypic effects.

Comparative pigmentation

Although the majority of our knowledge about the pigmentation pathway has been gathered in mammals in general, and laboratory mice in particular, the melanin pathway is highly conserved among vertebrates. However, modifications of this developmental pathway can generate dramatic variation in pigmenation among vertebrate taxa (Searle, 1968; Bagnara and Hadley, 1973). In mammals, melanocytes produce two types of pigment (eumelanin and pheomelanin), and the ratio of melanin types is largely responsible for variation in hair color. By comparison, variation in human skin color among ethnic groups has less to do with melanin ratio or even the number of melanocytes present, but instead is largely due to differences in melanosome size, number and density in the epidermis (Barsh, 2005). Birds, like mammals, produce both pigment types (for a review, see Mundy, 2005), but reptiles lack pheomelanin (Ito and Wakamatsu, 2003), suggesting that either reptiles have the lost the ability to produce pheomelanin or that mammals and birds independently have evolved the ability to produce pheomelanin. Given the similarities in the genetic and developmental mechanisms of pheomelanin production in birds and mammals (Boswell and Takeuchi, 2005), it seems unlikely that they have evolved independently, although this question warrants further investigation. Further, reptiles, amphibians and teleost fish can regulate body color by aggregation or concentration of melanin granules in melanocytes (also referred to as melanophores) via a mechanism controlled by the melanin-concentrating hormone (Kawauchi *et al*, 1983; Nery and Castrucci, 1997).

In addition to melanophores, amphibians, reptiles and teleosts have more diversity in chromatophores, including yellow/red carotenoid-containing xanthophores and reflective iridophores. Overall color and pattern result from the position and interaction among these differently colored chromatophores. Colors produced by xanthophore and iridiphore cells are particularly important for a number of classical traits involved in inter- and intra-specific communication, such as *Anolis* lizard dewlaps (Tokarz, 1995) and guppy spots (Endler, 1983). Studies in mice provide excellent candidate genes for melanin-based traits but provide little insight into the genetic mechanisms underlying non-melanin-based color traits. Recent work, primarily in zebrafish, has focused on understanding the genetic and developmental processes controlling these more colorful pigment cells (Kelsh, 2004) and will help identify the genetic basis of traits which rely on the interaction of multiple chromatophore cell types.

Evolution: natural variation in pigmentation

While pigmentation has served as a model for genetics and developmental biology, it has also played a prominent role in evolutionary biology. Naturally occurring color variation has served as a model for understanding local adaptation and ecologically mediated divergence and speciation. Such classic studies linking color variation to environmental heterogeneity span a broad taxonomic scale, from banding in *Cepea* snails (Sheppard, 1951) to melanism in peppered moths (Kettlewell, 1955) to patterning in water snakes (Camin and Ehrlich, 1958). Among the most comprehensive studies are those of cryptic coloration in rodents because early mammalogists documented variation in dorsal pigmentation, measured substrate color and later experimentally tested the adaptive significance of substrate-matching.

Starting in the early 1920s, classic work by museum-based natural historians linked dorsal coloration of diverse vertebrate species to environmental variation. In one seminal study, Dice and Blossom (1937) described the dramatic variation in coloration of vertebrates in the Tularosa Basin of New Mexico, where in less than 25 km, the substrate color ranges from nearly black basaltic lava to brilliant white gypsum dunes. In particular, the dorsal pelage of pocket mice (genus *Chaetodipus* and *Perognathus*) ranges from nearly pure black to nearly pure white, closely matching the substrate on which the mice were caught (Figure 3). Around the same time, Sumner conducted a parallel study on the sandy dunes of Florida's Gulf and Atlantic coasts, documenting the extremely pale phenotypes of mice relative to their darker inland counterparts (Figure 1c and d, Sumner, 1929a, b). However, unlike the pocket mice from New Mexico, most of the variation in these beach mice (genus *Peromyscus*) is reflected in patterning differences. Beach mice differ in a number of traits, such as the extent of tail striping and facial pigmentation, both between mainland and coastal populations and among coastal populations (Bowen, 1968). Genetic crossing experiments revealed that these patterning differences are heritable and likely controlled by just a few genes (Bowen and Dawson, 1977). Sumner's intensive sampling, documenting gradual change in pigmentation from coastal to mainland populations, later formed the basis for the first mathematical treatment of clinal variation and estimates of selection (Haldane, 1948). In addition, experimental studies of the adaptive significance of color variation show that substrate matching has a strong effect on predation rates by visual avian hunters (Dice, 1947; Kaufman, 1974). Adaptive substrate matching is not limited to mammals. Similar patterns of variation have been observed in many vertebrates, including lizards (Figure 3; Norris and Lowe, 1964), and corresponding selection experiments have been conducted (Luke, 1989). These early studies document ecologically relevant variation in pigmentation and elucidate the selective agents driving this variation.

These classic studies also provide striking examples of the independent evolution of similar phenotypes in similar habitats both within and among species. For example, rock pocket mice (*Chaetodipus intermedius*) have melanic morphs on geographically distant lava flows with little evidence of historical gene flow among them (Hoekstra *et al*, 2005). Similarly, beach mice (*Peromyscus*

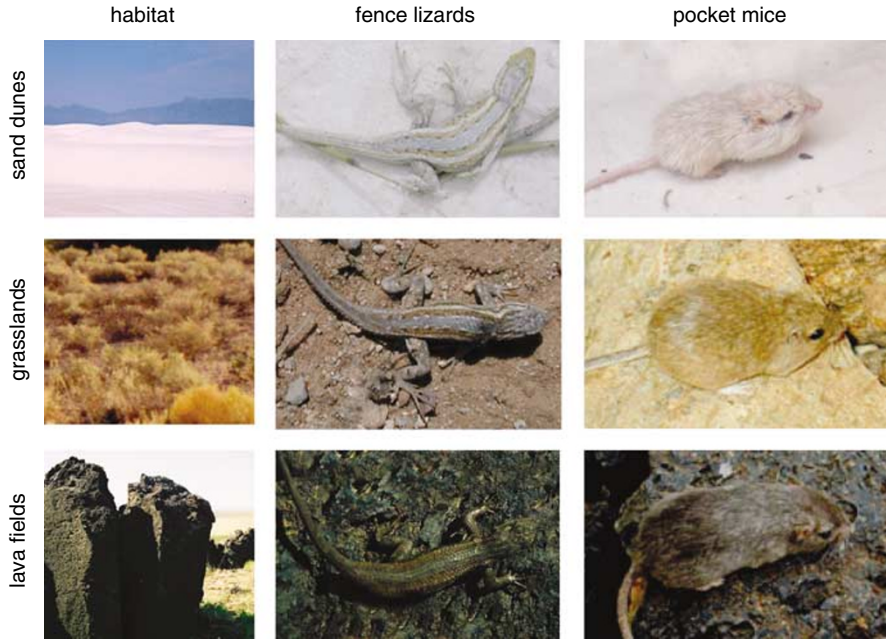

Figure 3 Convergent evolution of adaptive pigmentation in the Tularosa Basin of New Mexico. The Carrizozo lava field is separated from the gypsum sand dunes of White Sands by 25 km of desert grasslands. Western fence lizards, *Sceloporus undulatus*, rock pocket mice, *Chaetodipus intermedius* (melanic and wild-type morphs) and apache pocket mice, *Perognathus flavescens* (blanched morph) are pictured on the substrate where they were captured.

polionotus) have colonized both the Gulf and Atlantic coasts of Florida, and independently evolved similar light-colored dorsal coats (Bowen and Dawson, 1977; Hoekstra *et al*, in press). Independent convergence toward similar phenotypes is also observed among species. For example, three taxonomically diverse squamates have evolved blanched coloration in White Sands, New Mexico (Rosenblum, 2006). Observations of several different species of blanched and melanic animals ranging from lizards to pocket mice inhabiting the Tularosa Basin provide a dramatic example of convergent evolution over a broad taxonomic scale (Figure 3). Independent evolution of pigmentation and pattern has been observed in many vertebrate species, including poison frogs (Vences *et al*, 2003), orioles (Omland and Lanyon, 2000), cavefish (Strecker *et al*, 2003) and cichlid fish (Allender *et al*, 2003), and provides exciting opportunities to ask whether the same or different genes are responsible for convergent phenotypes.

Linking genotype to phenotype

The field of 'evo-devo' focuses on understanding the genetic and developmental mechanisms responsible for evolutionary changes in form among species. As a traditional model system, pigmentation provides (1) a plethora of candidate genes from studies in genetics, (2) an understanding of the role of these genes in pathways and networks from developmental biology and (3) ecologically relevant pigmentation phenotypes from studies in evolutionary biology. The integration of these resources makes pigmentation an ideal system in which to tackle several long-standing questions about the genetic and developmental basis of adaptive phenotypic variation.

One approach that has been successful in identifying the molecular basis underlying adaptive phenotypes is to focus on candidate genes. This approach is promising for studies of ecologically relevant color variation because of the wealth of knowledge of the genetic basis of color phenotype (eg, Table 2, Figure 2). Candidate gene approaches are necessarily limited in genomic scope, but can be especially useful in species that are not amenable to laboratory crosses or for which few genetic resources are available. For example, population-level association studies between nucleotide variation in candidate pigmentation genes and segregating color variation can provide statistical evidence that a particular gene is contributing to variation in color phenotype. However, such studies often are complicated by population structure, and require large sample sizes and functional assays to identify and verify the precise functionally relevant mutations. Despite these challenges, this approach has been especially successful for simple color polymorphisms segregating in natural populations of non-model organisms (eg, Ritland *et al*, 2001; Theron *et al*, 2001; Nachman *et al*, 2003; Mundy *et al*, 2004), although functional verification of the role of mutations in these genes is still needed. More recently, genetic and molecular tools have been developed for nontraditional species, which can be manipulated in the laboratory (eg oldfield mice (*Peromyscus polionotus*), threespine sticklebacks (*Gasterosteus aculeatus*), cavefish (*Astyanax fasciatus*)). In these systems, quantitative trait locus (QTL) mapping approaches provide a more comprehensive method for the identification of genetic regions underlying pigmentation variation and, when combined with candidate pigmentation genes, can provide an extremely powerful approach to make links between genes, phenotype and fitness.

Through both candidate gene and QTL approaches, a growing number of empirical studies have linked genetic variation to adaptive (nonpigmentation) traits (reviewed

in Peichel, 2005). These studies have contributed to our understanding of several questions about the process of evolutionary change: (1) are changes in coding or regulatory regions differentially responsible for adaptive morphology? (2) are adaptive mutations generally dominant or recessive? and (3) are the same genes responsible for similar adaptive phenotypes? Recent success in identifying the genetic basis of pigmentation variation in natural populations of vertebrates provides additional, and sometimes surprising, insight into these questions. Together, these studies suggest that color adaptation does not follow a simple evolutionary route: mutations in both coding and regulatory regions, which are often dominant but other times recessive, contribute to pigmentation diversity in nature, and furthermore, sometimes the same but sometimes different, genetic mechanisms are involved in parallel and convergent color evolution in vertebrate populations.

Insights into genetic mechanisms of adaptive evolution from pigmentation studies

Mutations in both coding regions and regulatory regions have been identified and linked to adaptive variation for a variety of traits in a number of systems, but the relative prevalence of coding *versus* regulatory mutations in generating morphological diversity remains unknown (Coyne, 2005; Carroll, 2005a). King and Wilson (1975) originally argued that phenotypic change may largely be driven by changes in gene regulation, citing the extreme similarities in protein sequences between humans and chimpanzees despite their dramatic differences in morphology, physiology and behavior. More recently, mutations in regulatory regions have been proposed as a mechanism to fine-tune phenotypes because mutations in specific *cis*-regulatory elements may alter the expression of a protein in particular tissues, while preserving expression in others (Stern, 2000; Carroll *et al*, 2001; Carroll, 2005b). A handful of studies have already identified such mutations in *cis*-regulatory elements (eg Belting *et al*, 1998; Wang and Chamberlin, 2002; Gompel *et al*, 2005).

Functional changes in the coding region of *Mc1r* represent some of the most striking exceptions to a growing consensus that most changes in morphology are governed by changes in gene regulatory regions. All known mutations in the *Mc1r* locus, many of which are adaptive, occur in the coding region, either as amino-acid changes or small deletions (Table 3, Majerus and Mundy, 2003). However, it is important to note that little is known about the regulatory mechanisms that govern the expression of Mc1r at the intracellular level (Rouzaud

Table 3 Pigmentation mutations segregating in natural populations of vertebrates

Species[a]	*Gene*	*Derived phenotype*[b]	*Dominance*	*Mutation*	*Adaptive significance*	*Reference*
Beach mice (*P. polionotus*)	*Mc1r*[c]	Pattern, pheomelanic	Variable	Coding	Crypsis	Hoekstra *et al* (in press)
Pocket mice (*C. intermedius*)	*Mc1r*	Eumelanic	Dominant	Coding	Crypsis	Nachman *et al* (2003)
Japanese wild mice (*Mus musculus molossinus*)	*Mc1r*	Pheomelanic	Recessive	Coding	?	Wada *et al* (1999)
Woolly mammoth (*Mammuthus primigenius*)	*Mc1r*[c]	?	?	Coding	?	Rompler *et al* (in press)
Black bears (*Ursus americanus*)	*Mc1r*	Pheomelanic	Recessive	Coding	?	Ritland *et al* (2001)
Jaguar (*Panthera onca*)	*Mc1r*	Eumelanic	Dominant	Coding	?	Eizirik *et al* (2003)
Jaguarundi (*Herpailurus yaguarondi*)	*Mc1r*	Eumelanic	Semi-dominant	Coding	?	Eizirik *et al* (2003)
Bananaquit (*Coereba flaveola*)	*Mc1r*	Eumelanic	Dominant	Coding	?	Theron *et al* (2001)
Arctic skua (*Stercorarius parasiticus*)	*Mc1r*	Eumelanic	Semidominant	Coding	Mate choice	Mundy *et al* (2004)
Lesser snow geese (*Anser c. caerulescens*)	*Mc1r*	Pattern; eumelanic	Semidominant	Coding	Mate choice	Mundy *et al* (2004)
Little striped whiptail (*Aspidoscelis inornata*)	*Mc1r*	Blanched	?	Coding	Crypsis	Rosenblum *et al* (2004)
Lesser earless lizard (*Holbrookia maculata*)	*Mc1r*	Blanched	?	Coding	Crypsis	Rosenblum *et al* (2004)
Deer mice (*P. maniculatus*)	*Agouti*	Pheomelanic	Dominant	?	Crypsis	Dodson (1982) and Hoekstra *et al* in preparation
Mexican tetra (cave fish; *A. fasciatus*)	*Oca2*[c]	Albino	Recessive	Coding	?	Protas *et al* (2006)
Pachón population				(deletion of exon 21)		
Molino population				(deletion of exon 24)		
Japonés population				(unidentified)		

[a]Studies in domesticated, farmed or captive populations were not included. *Mc1r* variation observed in fence lizards (Rosenblum *et al*, 2004) and fairy-wrens (Doucet *et al*, 2004) was not included because of the confounding effects of population structure in these association studies.
[b]Derived phenotypes (as reported in each study) are categorized as follows: pattern, change in distribution of pheomelanin and eumelanin across the body; pheomelanic, predominately or exclusively pheomelanin; eumelanic, exclusively or predominantly eumelanin; blanched, reduction in the amount of eumelanin (pheomelanin is not present in reptiles); albino, complete loss of melanin.
[c]Studies in which mutation(s) in pigmentation gene were functionally characterized and verified.

and Hearing, 2005), and that bias toward discovering these coding sequence changes may be due to the ease of assaying *Mc1r* across vertebrates (based on its conserved structure and single 1 kb exon). Nonetheless, it is certainly possible to change protein structure without measurable antagonistic effects on other traits as demonstrated by amino-acid change in Mc1r and deletions in Oca2 in albino cavefish (Protas *et al*, 2006). Like Mc1r, proteins that are specific to given cell or tissue type or occur late in developmental pathways may be particularly good candidates for adaptive coding region change.

However, variation in pigmentation certainly will not be caused *only* by changes in protein structure. For example, variation in pigmentation pattern is likely driven by changes in the spatial regulation of pigment rather than changes in the pigments themselves; however, this process may involve mutations in *cis*-regulatory regions of pigment genes or changes (either coding or regulatory) in upstream transcription factors. One promising area for future research focuses on Mc1r's agonist, Agouti, which is known to produce similar whole-body changes in coloration, but can also have more subtle effects on color as well as alter the spatial distribution of pigmentation. And by contrast, mutations in *Agouti*, unlike *Mc1r*, are likely to be regulatory. The vast majority of known mutations in the *Agouti* gene that cause subtle changes in pigmentation phenotypes in laboratory mice occur at the level of Agouti expression, whereas the complete abrogation of Agouti occurs through lesions in the *Agouti* coding region (Miltenberger *et al*, 2002). Because the upstream *cis*-regulatory region of *Agouti* is modular, that is, several well-characterized regulatory elements can independently affect Agouti's spatial expression, Agouti is relatively free of pleiotropic consequences (Figure 4). Association studies with *Agouti* will likely be more difficult than those with *Mc1r* given that the majority of alleles described in laboratory mice are caused by mutations embedded in the large, albeit well-characterized, regulatory region, which spans over 100 kb in mice. Thus, association studies in *Agouti* may only be feasible in cases of strong and recent selection and/or in low recombination rate regions, where one would expect the extent of linkage disequilibrium to be large. However, genetic crosses in deer mice, *Peromyscus maniculatus*, have already pointed to regulatory changes in *Agouti* as being responsible for adaptive coloration (Figure 4, Hoekstra *et al*, in preparation; Dice, 1941; McIntosh, 1956; Dodson, 1982). Therefore, as is the case for most adaptive traits, variation in adaptive pigmentation is likely caused by a combination of changes in both coding (eg, Mc1r) and noncoding DNA (eg, Agouti).

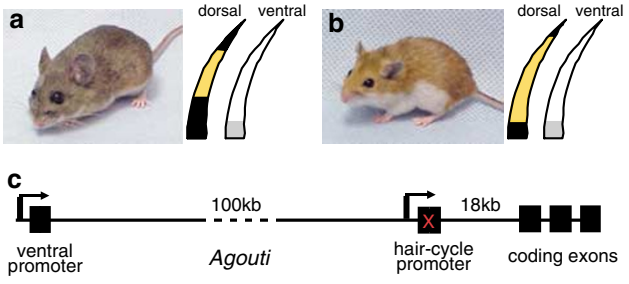

Figure 4 *Agouti* is a strong candidate gene for variation in vertebrate pigmentation and patterning in natural populations. (**a**) Deer mice (*P. maniculatus*) have banded dorsal hairs and light-colored ventral hairs. (**b**) Deer mice, which inhabit the light-colored Sand Hills in Nebraska, have dorsal hairs with a wider subterminal band, generating an overall golden color, better matched to the lighter sandy substrate they inhabit. (**c**) Mutations in *Agouti*'s dorsal promoter region provide a plausible mechanism for the observed phenotypic change in dorsal but not ventral pigmentation.

A second major question about the process of adaptation is the relative contribution of dominant *versus* recessive mutations to adaptive change. JBS Haldane (1924) suggested that adaptation is driven by the fixation of dominant mutations because of the bias against the establishment of recessives, termed 'Haldane's sieve' (although this prediction may not hold for deleterious mutations previously maintained at mutation-selection balance (Orr and Betancourt 2001)). Empirical data from pigmentation genes in natural populations can be used to address Haldane's prediction (Table 3). Melanism has evolved repeatedly in vertebrates, likely driven by both natural and sexual selection. In several cases, melanism has been linked to dominant or semidominant mutations in the *Mc1r* locus (Majerus and Mundy, 2003; Mundy, 2005), likely causing hyper or constitutive activation of Mc1r. Because of the nature of pigmentation-type switching, recessive null mutations in several other genes, such as *agouti*, *attractin* or *mahogunin*, can also result in similar melanic phenotypes in laboratory mice (Figure 2). Although additional genes which can cause melanism have not been surveyed to the extent of *Mc1r*, in part because of their complex genomic structure, mutations in these loci have yet to be linked to melanic forms in nature.

These few observations support the notion that dominant mutations often contribute to adaptive phenotypes. However, there are three notable exceptions (Table 3). First, in cavefish, multiple populations have lost pigmentation through recessive null alleles at the *Oca2* locus (Protas *et al*, 2006). Second, recessive alleles of *Mc1r* are likely responsible for pale coloration in black bears (Ritland *et al*, 2001) and Japanese wild mice (Wada *et al*, 1999). However, in all three of these cases, the adaptive significance of the color variation remains unclear. Third, blanched forms of two lizard species are associated with mutations in *Mc1r* (although breeding studies have not been conducted, based on Mc1r's function, we predict that these are null or reduced function alleles) (Rosenblum *et al*, 2004). In pigmentation studies, recessive null alleles often (although not always) result in lighter coloration (loss or reduction of pigmentation), whereas dominant mutations are often associated with darker color (gain of pigmentation). Thus, like for pigmentation traits, the contribution of dominant *versus* recessive alleles to evolutionary change may be confounded by whether the traits of interest represent loss of structures, where loss-of-function, recessive null alleles may prevail, or the gain of novel structures.

A third fundamental question about the adaptive process focuses on the role of developmental constraint in directing evolutionary change. It has been predicted that the same genes will often underlie parallel changes in closely related organisms because there are only a limited number of genetic changes free of antagonistic pleiotropic effects (Haldane, 1932; Gould and Lewontin, 1979; Maynard Smith *et al*, 1985). Studies of pigmentation variation are particularly appropriate for addressing this

question because of the many examples of convergent and parallel evolution in color (eg, Figure 3). While similar pigmentation phenotypes have evolved repeatedly among divergent taxa as well as within species, there are also hundreds of genes that encode different developmental mechanisms and are known to affect pigmentation (Table 2). Despite the wealth of genes involved in the vertebrate pigmentation pathway, one of the most striking examples of molecular convergence stems from studies of *Mc1r* in a wide variety of vertebrate taxa. Perhaps the most intriguing example involves mice and mammoths: a single amino-acid change ($Arg^{65}Cys$) contributes to adaptive light coloration in beach mice (Hoekstra *et al*, in press) and the identical amino-acid change at the homologous position is segregating in woolly mammoths (identified through ancient DNA studies), suggesting that mammoths may also have been polymorphic in color (Rompler *et al*, in press). In addition, *Mc1r* has been repeatedly co-opted in the evolution of melanic forms (Table 3). However, while *Mc1r* is thus far the only gene linked to melanism in natural populations of vertebrates, already several genes (including *Mc1r, agouti* and *Oca2*) have been linked to light-color or albino phenotypes, suggesting that there may be few ways to 'gain' pigmentation but many ways to 'lose' pigmentation.

So the question remains: why is *Mc1r* repeatedly co-opted for adaptation? Perhaps the most compelling argument is that *Mc1r* appears to be largely free from pleiotropic effects (Mundy, 2005); in other words, changes in *Mc1r* appear to be specific to pigmentation and, thus, free from developmental constraints. Mc1r is also part of a larger melanocortin gene family (Mc1r–Mc5r), whose members are specialized in their tissue expression and are involved in diverse pathways from pigmentation to energy homeostasis (Schioth, 2001). Such duplication events allow for fine-tuning of mutational effects analogous to modularity in *cis*-regulatory regions. In other words, specific amino-acid mutations in specific melanocortin receptors can alter protein function in some tissues while preserving function in other tissues or pathways. Additionally, there are many changes in *Mc1r* that produce large (beneficial) phenotypic effects. Therefore, despite its small target size (less than 1 kb), there are several possible mutations, even single amino-acid changes, that can produce a wide range of phenotypes from complete eumelanism (eg, pocket mice, bananaquits, jaguars) to complete pheomelanism (eg, black bears, Japanese wild mice) as well as intermediate patterning phenotypes (eg, beach mice, snow geese).

While mutations in *Mc1r* are associated with color variation in a wide variety of taxa, there are also several examples *within* species where some populations utilize *Mc1r* for color adaptation and other populations in similar selective environments use other genes. For example, while beach mice inhabiting the sandy dunes of the Gulf Coast of Florida have a single amino-acid change in *Mc1r*, which contributes to their light coloration relative to their mainland counterparts, beach mice on the Atlantic coast of Florida do not share this mutation in *Mc1r*, despite the similarity in adaptive color phenotype and selective environment (Hoekstra *et al*, in press). Similarly, lava-dwelling pocket mice in Arizona have a melanic phenotype caused by amino-acid mutations in *Mc1r*; however, geographically distant lava populations in New Mexico, which also harbor melanic mice, do not share these mutations or have any new mutations in *Mc1r* associated with melanism (Hoekstra and Nachman, 2003). It is also important to note that many melanic organisms are not associated with mutations in *Mc1r*, suggesting that other genes are responsible for their melanic coloration (MacDougall-Shackleton *et al*, 2003; Mundy and Kelly, 2003; Rosenblum *et al*, 2004). Understanding *why* sometimes the same gene is responsible for convergent evolution between divergent taxa and other times different genes are involved remains an interesting but largely unanswered question.

Understanding the nature of adaptive change in wild populations requires knowledge of multiple adaptive traits in taxonomically diverse systems. While pigmentation represents a particularly amenable phenotype in which to link phenotypic variation to genes in vertebrates (as is true for the invertebrate pigmentation system (True, 2003) and the anthocyanin pathway in plants (Holton and Cornish, 1995)), analysis of additional traits, including morphological, physiological and behavioral characters, is essential. The growing number of molecular markers and complete genome sequences from diverse organisms represents a major step toward developing additional non-model systems in which to identify the genetic basis of adaptation.

Conclusions

The prominent role of pigmentation biology in laying the conceptual foundations of genetics, development and evolutionary biology have since resulted in a wealth of knowledge about the genes, pathways and adaptive significance of pigmentation in vertebrates. This background has made it increasingly possible to identify the genetic basis of pigmentation variation in natural populations, identifying the genes, and in some cases the mutations, underlying adaptive traits. These recent advances have begun to shed additional light onto fundamental questions about the process of adaptation. However, future work that (1) continues to identify genes involved in pigmentation in laboratory mice and other organisms, (2) dissects the developmental mechanisms responsible for color patterning and (3) identifies the genes underlying ecologically relevant traits (including color and color pattern) in taxonomically diverse systems will contribute to a more comprehensive glimpse into the genetic and developmental mechanisms underlying organismal diversity.

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Review

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 $Arg^{65}Cys$ 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.

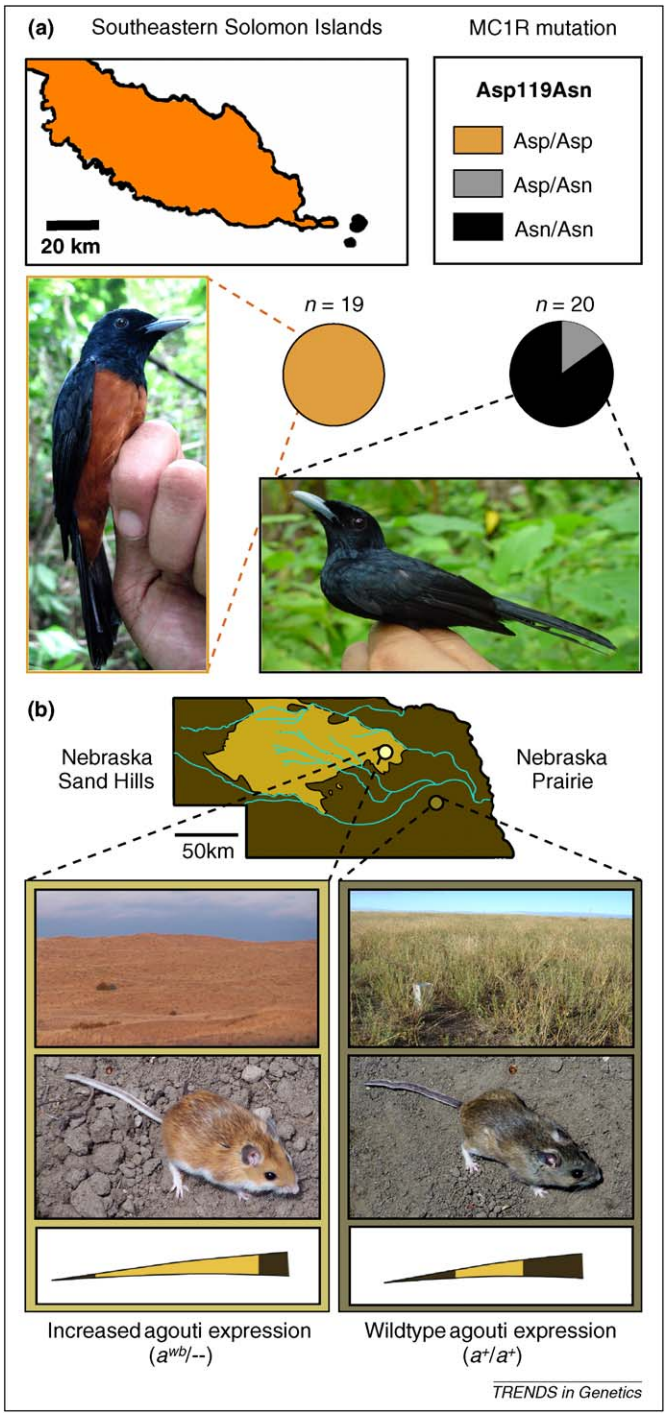

Figure 1. Illustration shows the association between mutations in pigmentation genes and color variation in natural populations of vertebrates. Mutations in *MC1R* and *ASIP* can have large effects on vertebrate coloration, which can be important in the origin of new species or local adaptation within 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 pheomelanic hair band, leading to overall differences in coat color brightness and ultimately survival. The dominant *agouti wideband* (a^{wb}) and recessive wild type (a^{+}) phenotypes/alleles are pictured.

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.

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 pheomelanic 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 variation 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 pigment 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 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

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].

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

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

[A]Mutations/genetic variants have been identified in this gene that associate with parallel phenotypic changes in lab populations and/or domestic animals.
[B]Mutations/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.

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Coloration in Mammals

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Abstract

Mammalian colors and color patterns are some of the most diverse and conspicuous traits found in nature and have been widely studied from genetic/developmental and evolutionary perspectives. In this review we first discuss the proximate causes underlying variation in pigment type (i.e., color) and pigment distribution (i.e., color pattern) and highlight both processes as having a distinct developmental basis. Then, using multiple examples, we discuss ultimate factors that have driven the evolution of coloration differences in mammals, which include background matching, intra- and interspecific signaling, and physiological influences. Throughout, we outline bridges between developmental and functional investigatory approaches that help broaden knowledge of mammals' memorable external appearances, and we point out areas for future interdisciplinary research.

The Diversity of Mammalian Color Patterns

Zebras became striped after 'standing half in the shade and half out of it, and what with the slippery-slidy shadows of the trees falling on them' [1].

Although children learn the striking colors of mammals from an early age, that (zebras *Equus* sp.) are striped, leopards (*Panthera pardus*) are spotted, and giant pandas (*Ailuropoda melanoleuca*) are black and white, they are still kept in the dark as to how and why these coloration patterns arise, shielded from science through fairy stories. Yet, we now know an increasing amount about the development and function of mammalian coloration. Here, we summarize genetic and developmental mechanisms underlying these phenotypes. Second, we review the functions of mammalian external appearances, a group in which crypsis predominates but where **signaling** (see Glossary) to conspecifics and to predators occasionally prevails. Finally, we suggest ways in which these approaches can be consolidated.

Genetics of Hair Pigmentation: Historical Perspective

Beginning in the 18th century China and Japan, so-called mouse fanciers collected, maintained, and bred together unusual color morphs of wild mice [2]; in doing so, these

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amateur geneticists generated mouse strains with distinct color variation. Thus, pigmentation phenotypes in mice, and later rats and guinea pigs, were readily available for study. Coat-color phenotypes were used as early as the 1900s to test several fundamental concepts in genetics – from Mendelian inheritance [3,4] to linkage [5], to demonstrations of epistasis and pleiotropy [6].

Because of the utility of pigment phenotypes, a sizeable list of genetic loci with well characterized phenotypes has since accumulated. The first pigmentation gene to be cloned, tyrosinase-related-protein-1 (*Tyrp1*), was initially thought to be the gene responsible for **albinism**, but was later mapped, cloned, sequenced and correctly attributed to the tyrosinase locus [7,8]. Since then, more than 100 genes affecting pigmentation have been cloned in mice and it is likely that many more remain to be identified [9] with ongoing chemical mutagenesis programs (Mouse Genome Database).

Pigmentation Regulation and Patterning

Color variation in mammals is primarily determined by two factors: (i) pigment regulation, altering the type, density and/or distribution of pigments along individual hairs; or (ii) pigment patterning, altering the spatial distribution of pigmentation across the body. Both of these processes can have profound effects on overall appearance, but likely have a distinct genetic basis and are manifested in different parts of the developmental pathway.

Pigment regulation: mammalian hair color results from a complex process involving the migration, differentiation, and regulation of melanocytes, the pigment producing cells in the epidermis [10]. Melanocytes can produce two types of melanin pigment, **eumelanin** and **pheomelanin**, through a process that is primarily determined by the interaction between *Agouti* and α-melanocyte stimulating hormone (MSH) with the melanocortin-1 receptor (*Mc1r*) [11]. When *Agouti* is not present, α-MSH will readily bind to *Mc1r*, causing intracellular cAMP to accumulate inside the cell. cAMP accumulation leads to the downstream activation of *Tyr* and the eventual production of eumelanin. When *Agouti* is present, it binds to *Mc1r* and causes a decrease in the production of intracellular cAMP, which eventually leads to downregulation of *Tyr* and causes melanocytes to switch from the production of eumelanin to pheomelanin [11]. Genomic changes in *Agouti/Mc1r* as well as in the genes involved in the downstream enzymatic reactions governing melanin synthesis underlie natural variation in mammalian coats [12].

Pigment patterning: in vertebrates, several mechanisms may contribute to regional variation in melanin type, density, and distribution. During embryogenesis, neural crest cells differentiate into melanoblasts (precursors of melanocytes) that migrate ventrally along the body axis. Melanoblasts typically enter the epidermis, where some remain (in certain species), while others localize to the hair follicles and differentiate into melanocytes. Once mature, these melanocytes can produce pigment, which is then packaged into melanosomes and transferred to keratinocytes of developing hair [13].

Pigment patterns can be classified as random and nonrandom, based on developmental origin and appearance [12]. Random patterns, such as those seen in some breeds of domestic

animals, arise from stochastic developmental events affecting melanocyte behavior. For example, the white-spotting phenotype commonly present in cats and dogs is caused by a failure of melanocyte migration. In contrast, nonrandom patterns, such as periodic stripes and spots, arise from spatially constrained developmental processes. Recent advances in genomics show how molecular mechanisms shape natural variation in mammalian pigment patterns. Although *Agouti* plays a central role in regulating melanocyte behavior during hair growth (see above), a different isoform of *Agouti* is expressed during development and can directly influence melanocyte maturation. In *Peromyscus* mice, differences in the position of the dorsal/ventral pigmentation boundary, seen in two locally camouflaged populations, are explained by changes in the spatial expression domain of *Agouti* that occur during embryonic development [14]. This example illustrates how the same gene, through the production of different isoforms, can influence pigmentation patterning (ventral-specific expression during development) and pigment distribution within a single hair (switch from eumelanin to phaeomelanin production). In African striped mice, *Rhabdomys pumilio*, which have a coat comprised of dark and light longitudinal stripes (Figure 1A), the transcription factor ALX3 is expressed at high levels in the light stripes where it acts to repress melanocyte differentiation and pigment production [15]. Kaelin and coworkers [16] used a combination of association and gene expression studies to show that periodic patterns (spots and stripes) in felids are established in two stages: first, when skin is developing, the gene *Taqpep* is involved in setting a prepattern that is later implemented during hair growth by the gene *Edn3*; a secreted factor that is expressed at high levels in the black spots of cheetahs (*Acinonyx jubatus*), where it promotes melanocyte differentiation and proliferation. These three examples demonstrate how specific genes, by being expressed in a spatially restricted fashion, can act to establish color differences along the body. Remarkably, spatially restricted gene expression patterns can also be seen at much smaller scales, such as within a single hair follicle: in Dun horses, *Equus ferus*, the transcription factor TBX3 is expressed only in the posterior half of hair follicles, resulting in radially asymmetric deposition of pigment in hair follicles [17].

Introgression as a Source of Color Variation

Although differences in color between and within individuals can be generated via changes in the coding sequence of genes or the regulatory region of genes controlling pigment synthesis or pigment patterning, it is becoming more evident through the use of genomic and molecular approaches that factors other than mutations, such as **introgression**, play a major role in promoting color variation in mammals. For instance, melanism in North American gray wolves, *Canis lupus*, is caused by a mutation in the *K* locus, which encodes a β-defensin protein that acts as an alternative ligand for *Mc1r*. By analyzing the evolutionary history of the melanistic *K* locus, Anderson and colleagues [18] were able to determine that this mutation, which has risen to high frequency and displays signatures of positive selection, originated in domesticated dogs and was transferred to wolves through past hybridization events. Thus, a mutation originally selected in a domesticated animal has provided favorable genetic diversity in a natural population. In another example, southern populations of snowshoe hares, *Lepus americanus*, no longer moult from a brown to a white coat in winter, like their northern counterparts (Figure 1B), but remain brown all year. As

southern populations experience milder winters with less snow, brown coats allow them to better blend in with the environment and avoid predator attack. Using association mapping and population genetic analyses, Jones and colleagues [19] showed that this variation maps to a regulatory region in the gene *Agouti*, and that the brown winter coat allele was likely introgressed from black-tailed jackrabbits (*Lepus californicus*). Thus, introgression has provided snowshoe hares a key source of genetic variation to adapt to rapidly changing environments. In another recent study, McRobie and coworkers [20] found that melanism in gray (*Sciurus carolinensis*) and fox squirrels (*Sciurus niger*) is caused by the same amino acid change in *Mc1r*, and that this allele likely originated in fox squirrels and introgressed into gray squirrels. Each example illustrates the importance of introgression as a continuous source of genetic variation upon which natural selection can act.

Color Change in Mammals

Despite the fact that hair color is largely determined by the genotype of an individual, mammal **pelage** color is not necessarily fixed throughout the lifetime. Some changes are age-related: certain suids, felids, and artiodactyls, for instance, are born with spotted or striped coats that become uniform when offspring become mobile. In pigs and peccaries, variegated pelage is associated with litter size perhaps because neonate interactions attract predator attention [21], whereas in felids and artiodactyls young are sequestered in hidden locations. Some pinniped and primate species are born with natal coats, which neonates soon lose. Although we know that white seal pups match a snowy white arctic background, or are dark if born in caves or on predator-free islands [22], the functions of black, white, orange, or gray neonate pelage in primates remain elusive but include attraction of allomothers and reduction of infanticide by males. Hair color may also change in old age as in the silvery white of human hair. Second, a few mammals change color seasonally, such as ermines (*Mustela erminea*) and snowshoe hares, to match a white arctic background in winter, and later to match background soil in spring, a cycle that is being disrupted by climate change [23].

Turning from crypsis to intraspecific signals, mammalian pelage color may vary according to dominance, as in males from uni-male units turning dark blue–black in the greater kudu (*Tragelaphus strepsiceros*) or ashen in mountain gorillas (*Gorilla beringei*). Finally, skin color can change rapidly due to hormones and vasodilation, particularly in primates. For example, tumes-cent red genitalia may advertise fertility and possibly ability to raise offspring to independence in female baboons (*Papio cynocephalus*) [24], and may be used in mate choice in rhesus macaques (*Macaca mulatta*) [25]. Male genital hue or facial luminance can signal dominance, as in vervet monkeys (*Chlorocebus pygerythrus*) or mandrills (*Mandrillus sphinx*), respectively [26–28]. Even more rapid signaling is possible: changing blood supply can alter facial coloration in response to anger or stress. Blushing in humans may be such an example, although surprisingly little work has been conducted since Charles Darwin devoted a chapter to it in his 1872 book, the *Expressions of Emotions in Man and Animals* [29].

Color Vision

Knowledge of species' and individuals' differing spectral sensitivities might help us understand the role that external color appearances play in interspecific and intraspecific signaling. Mammals were originally thought to be **trichromats** based on their reptilian ancestry but evolved to be **dichromats** with maximal retinal sensitivities in short and medium wavelengths of the visual spectrum when they became nocturnal, so *a priori*, they might be expected to be dowdy. However, some primates and perhaps a few marsupial species secondarily became trichromats coincident with the advent of **diurnality** [30]. The evolution of routine trichromacy (see below) in African and Asian monkeys and apes [30] may have been driven by the benefits of picking out orange and red ripe fruits and edible young leaves from a background of mature green leaves [31–33] but the relationship between vision and foraging benefits is complex [34,35]. Reconstructions of the ancestral states under maximum parsimony indicate that trichromatic color vision evolved at the ancestor to extant tarsiidae–platyrrhine–catarrhine species before the evolution of red skin or pelage, suggesting that the presence of color vision may have been necessary for the evolution of red external appearance in primates [36] but the association is not strong [37].

Among catarrhine primates, both sexes discern hues in the red–green range and along the ancestral blue–yellow color axis [38]. This routine trichromacy is enabled by two distinct opsin genes on the X chromosome that code for mid-wavelength-sensitive (MWS, green) and long-wavelength-sensitive (LWS, red) cone photopigments, and a third autosomal opsin gene coding for short-wavelength-sensitive (SWS, blue) pigments. In contrast, certain lemurs and most primate species from the Americas possess polymorphic trichromacy due to allelic variation of a single mid- to long-wavelength-sensitive opsin gene (M/LWS). In these polymorphic species, some females and all males are red–green color blind, and only females that are M/ LWS heterozygotes are capable of trichromatic vision [38–40]. Howler monkeys (*Alouatta* sp.), however, are an exception and have independently evolved routine trichromacy similar to catarrhines; interestingly, they are heavily reliant on young red leaves [41].

Whether trichromatic species are more colorful than dichromats is little researched but at present the association seems weak [37]. A related proposal with stronger support is that medium- and long-wave cone maximum sensitivities for trichromats are optimized for discriminating variations in blood oxygen saturation so that trichromacy may also be linked to primate facial and sexual skin coloration as found in Asian and African groups [28,42].

Evolutionary Drivers: Crypsis

Rodents and bats, constituting approximately one quarter and one fifth of mammals respectively, are often dull browns or grays probably to match their background to avoid detection. Research on rodent hair color has a long history with early North American mammologists such as Sumner, Benson, Dice, and Blossom describing coat color variation in US southwestern desert rodents [43]. For example, they showed that in the rock pocket mouse (*Chaetodipus intermedius*), coat color typically matches that of rubble on which the mice live; the dorsal pelage varies from a light, sandy color for populations found on some

granites to dark, nearly black for populations found on basalt lava flows. These pelage hues, in some cases stemming from *Mc1r* mutations [44], are likely selected for by owl predation [45,46] with patterns of migration across different substrates driven by stronger selection against light morphs on a dark background than against dark morphs on light background [47] (Figure 1C). Phenotype–environment matches also occur on much larger geographic scales [48], while interspecific comparisons of other small mammalian prey species similarly indicate pelage coloration matches different backgrounds [49]. Background matching is the form of protective coloration whose genetic basis is best understood.

Of course, it is not just prey that need to remain cryptic. Carnivores need to approach prey unnoticed; think of white polar bears (*Ursus maritimus*) approaching seals hauled out on ice. Other carnivores and perhaps pinniped and cetacean piscivores wear spotted, dappled, or uniform fur to approach their prey undetected; ocelots (*Leopardus pardalis*) and tigers (*Panthera tigris*) on land [50], gray seals (*Halichoerus grypus*) [22], and Atlantic spotted dolphins (*Stenella frontalis*) [51] at sea are all examples of what used to be called aggressive coloration in Victorian times.

Beyond background matching, many mammalian prey are countershaded (dark on top, lighter underneath). This pattern is common in both terrestrial and aquatic animals although the underlying mechanisms are hotly debated and include crypsis, protection from UV light, and reducing abrasion [52]. In some mammals, the dark to light boundary on the animal's lateral surface precisely counteracts the shadow cast by the animal's own body [53] perhaps rendering it 2D and thus more difficult to detect than a 3D object. However, there must be other influences on dorsal pigmentary darkening: the white ventrum of murids hugging the ground is unlikely to have evolved for the same reasons as a gerenuk (*Litocranius walleri*) with its long legs. Other mechanisms of remaining cryptic through disruptive coloration, masquerade or transparency have yet to be identified in mammals.

Evolutionary Drivers: Signaling

Many aspects of coloration in birds are driven by male–male competition and female choice but these evolutionary drivers seem to have less force in mammals, except perhaps in African and Asian primates where ornamental skin patches are more common [54] and in gibbon species where males and females differ in pelage color. Sexual differentiation in fur color is also found in a tiny handful of other species such as the male ribbon seal's (*Histriophoca fasciata*) red inflatable nostril, the mouflon's (*Ovis orientalis*) blond beard, and various dark male bovids and pinnipeds but has received minimal study [55]. The best worked example is the black manes of lions (*Panthera leo*) that are attractive to females but intimidating to other males [56], a male ornament kept in check by overheating.

Color patches are used in *interspecific* signaling; the most famous being aposematic coloration in mustelids that advertises noxious anal secretions [57]. However, contrasting color patches can be used to thwart predators in other ways. Black flank stripes of gazelles amplify pursuit deterrent stotting behavior [58], and conspicuous ear tips of lagomorphs may deter pursuit [59]. Some color regions such as white heads in cetaceans may function to drive fish into shoals for easier capture [51]. Complex primate face colors may signal

species' identity so as to avoid hybridization in sympatric primate congeners [60,61] (Figure 1D). It is challenging for researchers to explain the absence of sexual dichromatism in mammals where mate choice is common, or the anachronistic distribution of color patches used in signaling to other species [62].

Perhaps the take home message is that there is no single evolutionary cause of conspicuous pelage in mammals [63]. Black and white coats of zebras are not a form of warning coloration (Figure 1E): instead a growing number of studies indicate that stripes thwart attack by disease-carrying biting flies [64–66]. In giant pandas, comparative work indicates white fur blends in with background snow, black legs with deep forest shade, but black eyes spots and ears help in individual recognition and signal aggressive intent [67]. Blackbuck (*Antilope cervicapra*) are sharply countershaded (black above, white below) to counteract dark shadow cast by the glaring tropical sun [53], while the functional significance of black and white coats of colobus species, Malayan tapirs (*Tapirus indicus*) and orcas (*Orcinus orca*) are still enigmatic but likely to differ from each other.

Evolutionary Drivers: Physiology

From first principles, we assume that external coloration has inevitable consequences for heat load; lighter coats reflect heat and are found in deserts, for instance. However, we still know little about the relative contributions that hair color, density, length, and structure contribute to temperature regulation in mammals. Like several other groups, mammals are darker in the tropics (Gloger's rule) but the melanin that produces darker color has other consequences including being relatively impervious to abrasion, antimicrobial benefits, preventing UV-caused mutagenesis, and absorbing heat, so that the underlying mechanisms for Gloger's rule may be multifaceted [68,69]. In an important study, Hetem and coworkers [70] implanted thermodevices in three differently colored springbok (*Antidorcas marsupialis*); a species that is normally light brown in color although black and white morphs exist. Daily maximum temperatures of the black morph were significantly higher than normal or white morphs in spring due to absorbing more solar radiation. Daily minimum temperatures in the winter were significantly lower for the white than the black or normal morphs. Thus, stabilizing selection appears to act at different periods over the year. In winter, black morphs reduce energy expenditure but experience higher heat load in summer. White morphs live close to the energetic edge in winter. The normal springbok occupies a compromise position which may explain why few black and white morphs are found. Oddly, the relationship between hair color and thermoregulation is little explored in other mammals.

Turning to UV radiation protection, humans are polymorphic for skin color with people living at higher latitudes having lighter skin than those nearer the tropics where UV radiation is stronger. The underlying principles are well established and often used as an example of evolutionary trade-offs in teaching students about evolution. On the one hand, eumelanin prevents UV from causing oxidative damage to DNA which can result in skin cancer. Additionally, it prevents vitamin B folate photolysis. Folic acid is an essential nutrient needed in nucleotides and hence DNA synthesis, especially in maturing bone marrow and developing red blood cells. Exposure to excessive amounts of UV leads to folate deficiency

and this results in fetal abnormalities especially spina bifida and anencephalitis where the neural tube fails to close properly in the developing embryo. On the other hand, UV promotes the synthesis of Vitamin D3 in the skin. Vitamin D has many positive effects including promoting bone formation and mineralization and reducing cardiovascular disease, diabetes, multiple sclerosis, and inflammatory bowel disease. While there is sufficient UV at lower latitudes for people to synthesize vitamin D, even with melanized skin, there is not enough UV at higher latitudes so people must reduce melanin synthesis and consequently have lighter skin. Humans living in intermediate latitudes (in the Mediterranean) with high annual variance in UV show facultative tanning in which melanization starts slowly allowing vitamin D production to occur in spring and winter, but prevention of vitamin B folate photolysis in hot summer months [71,72].

In addition to the physiological impacts of differences in color described above, it is worth noting that the physiology of an organism can have a large impact on color production, mediated by hormones. For example, melatonin and prolactin play a key role in regulating seasonal moulting in various animals: when days get shorter, specialized photosensitive ganglion cells in the eye retina convey a signal that ultimately reaches the pineal gland, which controls secretion of melatonin. Melatonin is produced at night at rates that are inversely proportional to day length. During the winter, as days get shorter, higher levels of melatonin inhibit the production of prolactin, leading to the production of white winter fur [73].

Concluding Remarks and Future Perspectives

At present, the study of mammalian coloration is divided into two camps – developmental and evolutionary – that, with notable exceptions [14,74–76], interact infrequently (hence our attempt here). Developmental biologists concentrate on cellular mechanisms and morphology, whereas evolutionary ecologists focus on behavioral and ecological variables to explain macroevolutionary patterns. Moreover, the former relies on model systems of a handful of species whereas the latter often focuses on many species in a clade. We see three ways to bridge this divide (see Outstanding Questions). First, we need to test the ecological significance of phenotypes being studied at the molecular level. Already experimental and observational studies show that different selective backgrounds drive population gene frequencies [76]; that the relative strengths of selection on alleles that code for pelage background matching differ according to habitat [77]; and that changing ecology can precede changes in genetic variation [78]. However, although we understand that the terminal differentiation of ventral melanocytes is delayed in mice that are countershaded as adults [14], we do not understand the adaptive significance of countershading in rodents. Similarly, we understand the genetic underpinnings (*ALX3*) of striping in striped mice species [15] but not its adaptive significance. Moreover, we have yet to make genetic modifications in known pigmentation/patterning genes and then test their effects on survival and reproduction in the wild. This is now within our grasp since we have the capability to manipulate genomes of a diversity of species [79,80].

Second but conversely, we need to understand the molecular and developmental basis of phenotypes that have already been shown to be adaptive. We can already do this for

melanin in humans and some rodents in fire climax populations [81,82]. For example, the *MC1RΔ24* allele is associated with melanism in both gray squirrels and fox squirrels [20]. However, there are other advantages of melanism for species living in shady or humid dark environments [83,84] (Figure 1F) that could be explored genetically. We are still a long way from knowing the molecular underpinnings of say aposematism or primate skin and hair color.

Third, we need to combine evolutionary and developmental approaches to understand convergence. For instance, the repeated appearance of pigment patterns and color morphs is key to evo-devo biology and we need to map developmental mechanisms underlying pelage coloration onto a phylogenetic tree to confirm that these mechanisms are conserved across the mammalian clade. And while we are getting a good handle on simple whole body changes in color from both genetic/developmental and ecological standpoints, we do not know if the rules governing mechanisms and functions are congruent across species. Once we can marry these disciplines the study of mammalian coloration will take a large step towards reaching maturity [85].

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Glossary

Albinism	a genetic condition that reduces the amount of melanin formed in the skin, hair, and/or eyes
Dichromat	possesses two independent channels for conveying color information, derived from the two different types of cone cells in the eye
Diurnality	being active during the day
Eumelanin	the other type of melanin produced by melanocytes in mammals. Eumelanin is largely responsible for black/brown hair
Introgression	transfer of genetic material between hybridizing species
Pelage	the fur, hair, or wool of a mammal
Pheomelanin	one of the two types of melanin produced by melanocytes in mammals. Pheomelanin is largely responsible for red/yellow hair
Signals	are acts or structures produced by signalers, which have evolved for the purpose of conveying information to recipients, such that the information elicits a response in recipients resulting in fitness consequences that, on average, are positive for both the signaler and recipient

Trichromat	possesses three independent channels for conveying color information, derived from the three different types of cone cells in the eye

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Outstanding Questions

Can developmental biologists take advantage of recent advances in genomic and molecular approaches to study the mechanisms underlying natural variation in mammalian pigment patterns?

Can we incorporate mechanistic studies of color/color pattern formation into a comparative phylogenetic context so that we can understand whether convergent phenotypes have arisen through similar molecular mechanisms?

What factors constrain the evolution of conspicuous skin coloration in primates?

Why do some primates have distinctive natal coat coloration?

Why does conspicuous coloration evolve in mammals that are not aposematic?

Why is sexual dichromatism so rare in mammals?

What are the mechanisms underlying Gloger's rule in mammals?

Highlights

Mammalian external appearances are well known and their developmental and adaptive significance are under active study.

Pigment regulation and pigment patterning determine how hair color develops.

Mammalian hair and skin color can change during an individual's lifetime.

Most mammals have dichromatic vision but some monkeys and apes are trichromatic which may influence pelage coloration involved in signaling.

Most mammals, both prey and predators, are assumed to match their background. Nonetheless, diverse and anachronistic color patches are used in interspecific signaling, such as the aposematism seen in black and white mephitids and porcupines.

Coloration has consequences for heat management, and also for UV protection, most famously skin coloration in humans.

We attempt to integrate developmental and evolutionary approaches to explain the origins of mammalian coloration.

Figure 1. Selected Species of Mammals That Are Currently Being Used to Further Understanding of Developmental[a] and Evolutionary[b] Mechanisms Driving Coloration in Mammals.

Top left: African striped grass mouse *Lemniscomys pumilio*[a]; top right: snowshoe hare *Lepus americanus*[a,b]; middle left: oldfield mouse *Peromyscus polionotus* [a,b]; middle right: Preuss's guenon *Cercopithecus preussi*[b]; bottom left: plains zebra *Equus quagga*[b]; bottom right: melanistic gray squirrel *Sciurus carolinensis*[a] (photographs from WikiMedia).

The Adaptive Significance of Coloration in Mammals

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The Adaptive Significance of Coloration in Mammals

TIM CARO

Coloration is a diagnostic tool for identifying mammals, but inquiry into its function has lain dormant for almost a century. Recently, the topic has been revived and modern phylogenetic methods have been applied to large data sets, allowing researchers to assess, for the first time, the relative importance of three classic hypotheses for the function of coloration in mammals: concealment, communication, and regulation of physiological processes. Camouflage appears to be the single most important evolutionary force in explaining overall coloration in mammals, whereas patches of colored fur are used for intraspecific signaling. Sexual selection is associated with flamboyant ornamentation in a minority of primates and other restricted mammalian taxa, but to a far lesser extent than in birds. Interspecific signaling among mammals includes aposematic coloration, exaggeration of signals to deter pursuit, and lures for misdirecting predatory attack. Physiological causes of coloration, including melanism, are evident but poorly researched. The relative importance of evolutionary forces responsible for external coloration varies greatly between vertebrate taxa, but the reasons for this variation are not yet understood.

Keywords: comparative method, color, functional hypotheses, mammals, signals

One of the first things children learn about nature is that certain large mammals have characteristic fur colors: The giraffe is reticulated (i.e., its reddish-brown coat is divided by a network of fine white lines into large geometric shapes), whereas the skunk and giant panda are black and white (figure 1a). When children ask why, adults recite reasons that were formulated more than a century ago, when naturalists speculated about the survival value of pelage and skin colors that they saw in specimens brought back from collecting expeditions (Wallace 1889, Poulton 1890). Parents' dated or incomplete answers (camouflage, advertisement, or "I don't know") stem not from their own ignorance but, sadly, from the fact that the field has advanced so little in 100 years. Naturalists' anecdotes about mammalian coloration were never put to experimental test, and the generality of these ideas—most of them formulated on the basis of only one or a handful of species—remained unexplored until very recently, except for one monumental treatise (Cott 1940). Now, however, we are in a better position to answer children's awkward questions with a modicum of authority.

The most salient point about the evolution of animal coloration is that different species and different parts of the body are subject to different selective pressures (Hingston 1933, Cott 1940). Classically, these can be divided into concealment, communication, and regulation of physiological processes.

My purpose here is to review new evidence for each of these evolutionary pressures that may have helped to form skin and pelage coloration in mammals and to attempt to assess their relative frequency in nature.

Concealment

Animals can remain concealed when their overall coloration (box 1) resembles or matches the natural background of their environment (Endler 1978). This phenomenon, also known as *general color resemblance,* includes crypsis (a type of camouflage), in which overall body color resembles the general color of the habitat, or pattern blending, in which color patterns on the body match patterns of light and dark in the environment. Background matching may change seasonally (termed *variable background matching*) or with age. Concealment may also be achieved through disruptive coloration (also termed *obliterative shading*) by contrasting colors or irregular marks that break up the body's outline (Merilaita 1998). Finally, animals may attain concealment if they have a lighter ventral surface, because this may counteract the sun's effects—lightening the dorsum and shading the ventrum—when it shines from above (Thayer 1909, Kiltie 1988).

Uniform coloration. There is overwhelming evidence of mammals' pelage coloration matching their backgrounds, both between and within species. Across species, at least five different coat colors appear to match the typical background on which they are found among carnivores, artiodactyls, and

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Figure 1. Striking examples of mammalian coloration: (a) striped skunk (photograph: © 1989 Jeff Wilcox, used with permission), (b) Burchell's zebra (photograph: Tim Caro), (c) ermine (photograph from the collection of the Museum of Wildlife and Fish Biology, University of California–Davis, used with permission), (d) tiger (photograph: Tim Caro), (e) beisa oryx (photograph: Tim Caro), and (f) vervet monkey (photograph: © 1987 Lynne Isbell, used with permission).

Box 1. The measurement of color.

The artist Albert Munsell developed a system for measuring color. He divided hue into 10 classes, red, yellow, green, blue, purple, and their intermediates; he then divided saturation, also known as chroma or intensity, into 6 uniform steps from 0 to 5; finally, he divided tone, or brightness, into 10 intervals ranging from black (0) to white (10). These scores can be measured using a reflectance spectrophotometer, or they can be compared to color chips in a standard reference collection. This has become standard practice in avian studies (Hill 2002), but it is rarely used for mammals (but see Sumner and Mollon 2003). Instead, color is still scored subjectively, classifying first the overall coloration of the coat as patterned or uniform (usually ignoring variation, e.g., lumping black, dark brown, dark gray-black, and brown-black under "dark") and then the markings on specific body parts, usually extremities, such as ears, tails and legs. Markings are defined as an area of color contrasting with the rest of the body or with the nearest area of the body. Thus, a white tail tip on a white animal would not be recorded as such, but a white tail tip on a black animal or one with a black tail would constitute a marking (Ortolani and Caro 1996).

Unfortunately, for most taxa, it is difficult to relate coloration or markings to crypsis (camouflage) or conspicuousness because animals that are easy to notice close up may be difficult to see a long way off; zebras are highly conspicuous nearby but surprisingly difficult to see at a distance (figure 1b). Second, the contrast between an animal and its background depends on ambient illumination and spectral reflectance to the background; thus, an animal may be cryptic at one time of day but not later on (Burtt 1981), or against one background but not another (Endler 1990). Third, an animal may be conspicuous to humans but not to nonprimate animals, because primates have three types of color-sensitive retinal cones, whereas carnivore predators possess only two; or they may be cryptic to humans but conspicuous to birds, which have four types of cones, the additional one of which is sensitive to ultraviolet light. Most mammalian studies throw caution to the wind and assume that the human visible spectrum is representative of all the visible spectra possessed by conspecifics and predators in an animal's environment.

lagomorphs, the three orders in which statistically and phylogenetically controlled comparisons have been made to date (table 1). Thus, species that are white or become white in winter are found in arctic and tundra biomes (figure 1c), pale species in desert and open environments, red and gray species in rocky habitats, and dark species in closed environments and in dense or tropical forests. Unfortunately, these robust associations do not make a clear-cut case for concealment, because coats of different color have differing thermoregulatory properties. White fur might scatter solar radiation toward the skin and hence be expected in cold climes; pale fur that reflects light might be expected in very hot environments such as deserts; and dark fur might be expected in the tropics, because it enhances water evaporation more readily than cool surfaces (Gloger 1833) or because it protects against ultraviolet radiation.

The same findings pertain within species. Individual desert rodents with paler coats are found on pale soils, and those with darker coats are found on blackened lava beds (Belk and Smith 1996), but again, vigorous argument has raged over whether the close match signifies camouflage or thermoregulation, with experimental studies on predation by owls eventually tipping the consensus in favor of protective concealment (Kaufman 1974). In a handful of species, individuals are polymorphic for coat color (see box 2).

Pattern blending. Less equivocal evidence of background matching that acts as concealment comes from pattern blending. A coat with the appearance of dappled light, for example, might be expected in a diurnal, solitary species that lives in forests, where crypsis is a likely mechanism by which an animal could escape notice. This has been confirmed in artiodactyls (table 2); in particular, there is a very tight association between young having spotted coats and young being sequestered during the first week after birth (hider species; figure 2). Among carnivores, spotted species tend to be arboreal and to live in closed habitats, whereas striped species are found in grasslands, supporting the hunters' old adage that tigers are striped to hide in tall reeds and grasses (figure 1d).

Disruptive coloration. It is difficult to marshal convincing evidence for disruptive coloration in mammals. Numerous artiodactyls have prominent black side bands and leg markings that could function to break up the body's outline; but although these markings are found in species that are diurnal and live in open country and in desert habitats, few associations between potentially disruptive coloration and these behavioral and ecological variables stand after controlling for phylogeny (Stoner et al. 2003a). Black-and-white species such as giant anteaters, tapirs, and giant pandas, obvious candidates for disruptive coloration, will require difficult experimental approaches; being found in orders with so few other black-and-white species, they defy comparative analyses.

Self-shadow concealment. Countershading is widespread in mammals, and one function may be to aid in concealment by reducing shadow in well-lit environments. For example,

Table 1. Summary of significance tests showing relationships between the overall uniform coloration of different mammals (artiodactyls, carnivores, and lagomorphs) and types of habitat.

Group	Habitat associated with animal color				
	White	Pale	Red	Gray	Dark
Artiodactyla	Arctic*[a], tundra†[a]	Open environment*, desert*	NS	Rocky*	Tropics†, closed environment*, dense forest*
Cervids	Arctic†[a], tundra†[a]	NS	NS	NS	Dense forest*
Bovids	Arctic†[a], tundra†[a]	Open environment*, desert*	Not rocky*	Rocky*	Tropics†, closed environment*, dense forest*, swamp*
Carnivora	Arctic†[b]	Desert†	NT	NT	Tropical forest†
Canids	Arctic†[b]	Desert†	NT	NT	Tropical forest†
Ursids	NS	NA	NT	NT	Tropical forest†
Procyonids	NA	NA	NT	NT	NS
Mustelids	Arctic†[b]	NA	NT	NT	NS
Viverrids	NA	NA	NT	NT	NS
Herpestids	NA	NA	NT	NT	Tropical forest†
Hyaenids	NA	NA	NT	NT	NA
Felids	NA	NS	NT	NT	NS
Lagomorpha	Arctic*[a], tundra*[a]	Open environment†, desert†	Rocky†	Rocky*	Forest/woodland*

Asterisk (*), significant results of nonparametric chi-square or Fisher exact probability tests; †, significant results of phylogenetically controlled comparisons using MacClade and Maddison's concentrated changes tests (Maddison 1990).

NA, not applicable (no species showing that type of coloration); NS, not significant (no significant association found between coloration and habitat); NT, not tested.

a. Includes only species that turn white in winter, not species that remain white all year.

b. Includes species that turn white in winter and species that remain white all year.

Source: Ortolani and Caro 1996, Ortolani 1999, Stoner et al. 2003a, 2003b.

Table 2. Summary of significance tests showing relationships between the coat patterns of mammals (artiodactyls and carnivores) and ecological and behavioral variables.

Group	Ecological and behavioral variables associated with coat pattern	
	Spots	Stripes
Artiodactyla	Solitary†[a], hiders†[a], dense forest habitat*[a]	Solitary*[a], hiders*[a], dense forest*[a] and light forest†[b] habitat
Cervids	Diurnal†[b], not solitary*[b], hiders*[a], grassland/bushland habitat*[a]	NS
Bovids	Solitary*[a, b], hiders*[a], light forest habitat†[a, b]	Hiders*[a], light forest habitat†[a, b]
Carnivora	Arboreal†, ungulate prey†, closed environment†	Arboreal*, terrestrial†, grassland habitat†
Canids	NA	NA
Ursids	NA	NA
Procyonids	NA	NA
Mustelids	NA	NA
Viverrids	Arboreal†	NA
Herpestids	NA	NA
Hyaenids	NA	NS
Felids	Arboreal†, forest habitat†	NS

Asterisk (*), significant results of nonparametric chi-square or Fisher exact probability tests; †, significant results of phylogenetically controlled comparisons using MacClade and Maddison's concentrated changes tests (Maddison 1990).

NA, not applicable (no species showing that type of coloration); NS, not significant (no significant association found between coat pattern and ecological or behavioral variables).

a, young; b, adults.

Source: Ortolani and Caro 1996, Ortolani 1999, Stoner et al. 2003a.

photographs of gray squirrels show that countershading removes brightness gradients, although not completely, and only when specimens are placed horizontally (Kiltie 1989a). Across species, countershaded bovids and artiodactyls are diurnal and live in desert environments, as might be predicted under this hypothesis (Stoner et al. 2003a); similarly, countershaded lagomorphs are diurnal and live in grassland habitats (Stoner et al. 2003b), although most of these latter associations collapse after controlling for shared ancestry. Unfortunately, countershading itself cannot be taken as evidence that selection has acted to reduce shadow. A dark dorsum may be a device to reduce ultraviolet radiation or to counteract dorsal abrasion (Kiltie 1988). Also, if pigmentation is costly, background matching is a sufficient explanation for countershading, as animals would be expected to refrain from producing melanin below. Consider naked mole rats, which have dark dorsa but pink ventral surfaces and very short legs; they are fossorial but occasionally disperse above ground at night. Under these circumstances, dark backs are more likely to match the background when viewed by aerial predators rather than to help in minimizing shadow, in thermoregulation, or in protecting against ultraviolet light (Braude et al. 2001).

Communication

Patches of color, rather than overall coloration, may also be used to communicate to conspecifics. Intraspecific signals may help animals maintain visual contact, as between mothers and young (Leyhausen 1979); may function as social releasers (Fox 1971), that is, as signals of subordination or devices to intimidate rivals (Ewer 1973); may warn conspecifics that predators are close (Alvarez et al. 1976); or may signal reproductive condition, dominance, health, or even genetic quality to potential mates (Pagel 1994). Interspecific signaling may include *aposematism,* in which prey advertise their noxiousness or pugnacity; lures that deflect predatory attack away from the body; or lures that prevent prey from recognizing that a predator is present.

Intraspecific communication. The second major evolutionary force thought to be responsible for coloration of particular body parts is communication between conspecifics, but, unfortunately, the meaning of many of these signals is still opaque. Systematic evidence from artiodactyls, carnivores, and lagomorphs ties markings on the face, ears, legs, tail, and rump to intraspecific signaling, because these markings are associated with conditions in which they are most visible (diurnal activity and open habitats) and are seen in gregarious species (table 3). Specifically, white or dark faces are seen in social ungulates, as are white patches on the ears in forest-living carnivores, dark ear patches in group-living lagomorphs, and conspicuous legs in diurnal desert and grassland ungulates (figure 1e). Conspicuous tail coloration in ungulates is strongly associated with being diurnal and living in groups, whereas carnivores exhibit black tail tips in grassland habitats. Finally, ungulates with white rumps inhabit open habitats and are gregarious. Interpretation is tricky, however. For example, the association between white spots on the backs of the ears and living in forests, or between black ears and living in grasslands (both of which are found in felids), might either serve to let young follow their mothers (Ewer 1973) or be used in intraspecific fights when the ears are twisted forward to face an opponent (Hingston 1933).

Coloration as communication has been advanced most thoroughly in primates. Primates are particularly colorful not only because they sport different pelage hues but because some exhibit brightly colored patches of blue and red skin. Interspecific and intraspecific variation in fur color in primates is well described but poorly understood, because primate coloring is quite labile even among closely related species (see below). Additionally, in a number of primates, infants have coats that range from flamboyant to deep black or white, whereas parental coats are often agouti. Attempts to generate and test predictions concerning the function of primate natal coats have met with great difficulty. Straightforward matching of species to behavioral and ecological variables lends weight to ideas of avoiding infanticide (Treves 1997),

Box 2. Melanism in mammals.

While most mammals show gradual variation in color across populations, some populations exhibit discontinuous variation and are either white or black. Albinism is caused by a single genetic mutation that is thought to have no adaptive significance, since albinos are removed from populations rapidly. Melanism (black or very dark brown pelage), however, may be found in 20% of individuals in some populations. Most famously, it is seen in certain individual predators living in tropical forests, such as black panthers (melanistic jaguars), but it also appears in burnt areas and urban landscapes. For instance, fox squirrels that inhabit fire-climax pine savannas in the southeastern United States have light, dark, and intermediately colored morphs. There, the percentage of black hair on the dorsum is positively correlated with frequency of lightning-caused wildfires, as well as climatic factors that influence fires (Kiltie 1989b). Intermediate and black-backed morphs matched their background better than light morphs—only briefly, however, for just the first two weeks after an area had burnt (Kiltie 1992)—calling antipredator benefits into question. Nonetheless, red-tailed hawks responded more slowly to intermediate-colored morphs than to dark or light morphs, which could be sufficient to maintain melanistic alleles in the population (Kiltie and Laine 1992). Given that melanism could also be important for temperature regulation in humid habitats, such as tropical forests, the functional advantages (if any) are unclear.

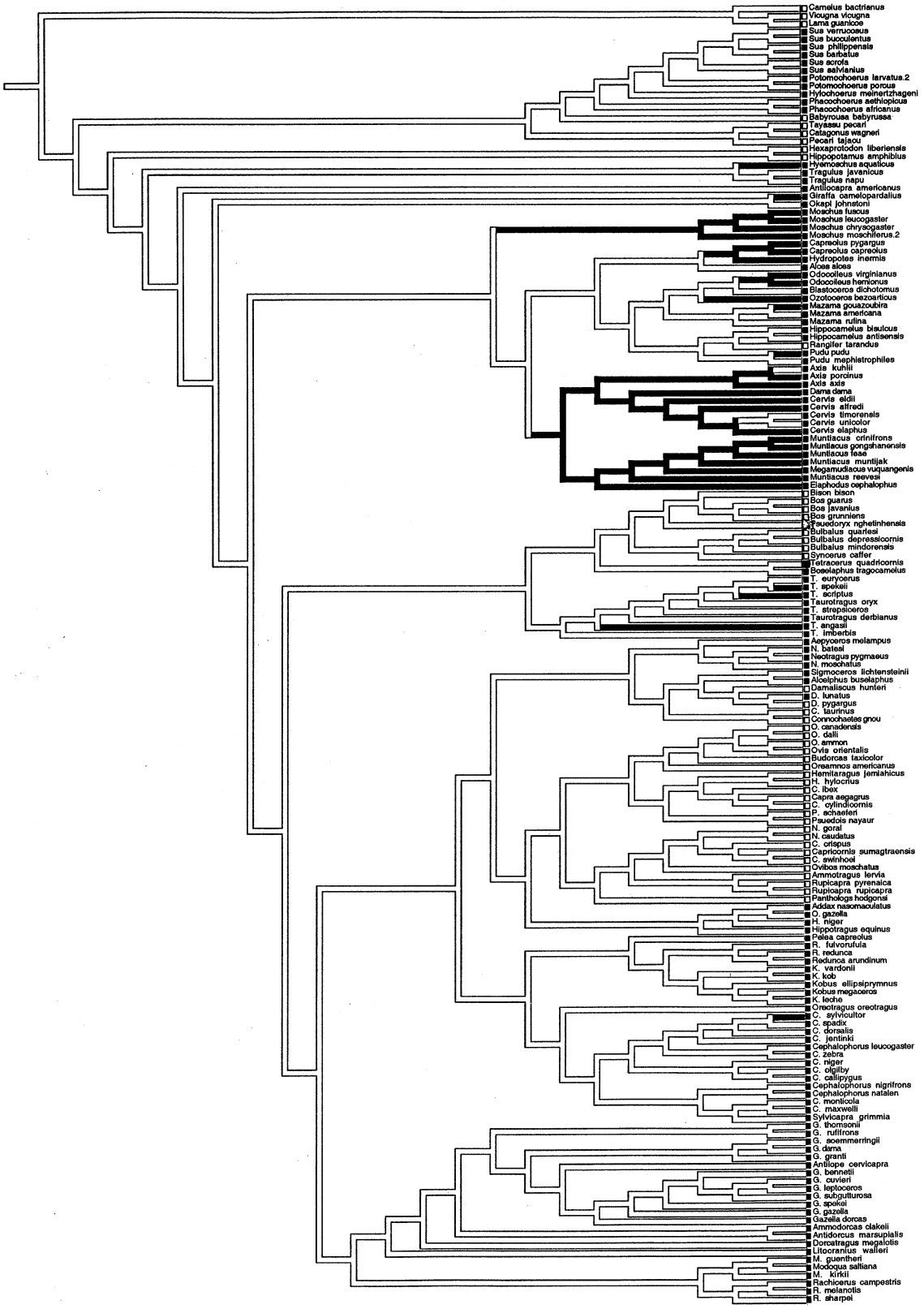

Figure 2. Association between spotted coats in young artiodactyls and hider species (species that sequester their young during the first weeks after birth). Black lines denote species with spotted coats, and white lines denote nonspotted species. Black boxes on the right denote species that are hiders; white boxes denote species that are not hiders. Source: Stoner and colleagues (2003a); © 2003 Oxford University Press, used with permission.

but tests employing phylogenetic controls fail to support these suppositions or other ideas about attracting the attentions of allomothers (individuals raising offspring that are not their own) (Ross and Regan 2000). Ideas that conspicuous coats might signal an animal's condition, or, conversely, that they are inconspicuous in natural settings, have yet to be explored, and at present the adaptive significance of natal coats in primates is an open question.

Sexual selection. In contrast to almost all other mammalian orders, primates display considerable sexual dichromatism (differences in the coloring of males and females), but the function of colorful patches on males (often associated with facial structures) or on females (usually sexual swellings) is poorly understood. In some species with polygynous or polygynandrous mating systems, males show colorful sexual skin that may be used in settling dominance relations, although there is debate over the means by which this occurs. Certainly, sexual skin plays some role in male–male displays. For example, the degree of scrotal "blueness" of male vervet monkeys (figure 1f) predicts dominance when unfamiliar males are paired (Gerald 2001). In species in which male sexual skin resembles that of females (e.g., hamadryas baboons), males may present to other males in order to mollify aggression (sociosexual mimicry; Wickler 1968), although much criticism has been leveled at this argument.

The adaptive significance of sexual skin coloration in female primates is perhaps marginally more straightforward. Sexual swellings around the buttocks and vulva are usually bright red and are found in 10% of primate species, having evolved three times in old-world monkeys and apes. Numerous hypotheses have been advanced for the function of these swellings, including advertising receptivity and, as a consequence, fostering male–male competition and confusing paternity (Nunn 1999). One recent analysis suggests that the size (length) of the swelling, at least in yellow baboons, is correlated with earlier age of reproduction, larger number of offspring born and surviving per annum, and higher proportion of offspring that survive; in other words, with female quality (Domb and Pagel 2001). Males compete over these high-quality females, grooming them more and suffering more aggression when they consort with them. While color per se was not measured in this study, bright color draws attention to the swollen area and therefore has all the features of an advertisement of female quality.

In contrast to birds (box 3), few other mammals show sexual dichromatism (although they do show considerable sexual dimorphism). Some male ungulates, such as eland, turn dark blue as adults or harem holders, but little more is known about this phenomenon. In lions, in contrast to other felids, females live in prides and males live in small coalitions. Unique among felids, male lions carry manes, some of which are black whereas others are sandy-colored like the rest of the coat. Black mane coloration is associated with higher food intake, with age, with testosterone concentrations, and with cooler environments. Dark-maned males are more likely to lead an approach toward playbacks of recorded male roars, and are more likely to survive wounding in fights that occur over access to prides. As a result, dark-maned males have longer reproductive life spans and higher offspring survival, possibly as a result of enhanced paternal protection against foreign infanticidal males. Unsurprisingly, lionesses prefer to mate with the darkest-maned male in their coalition. While dark manes indicate health and vigor, they are held in check by the disadvantages of overheating (West and Packer 2002).

Box 3. A brief history of research on coloration in birds.

Early discussions of bird coloration, including those between Darwin and Wallace (Blaisdell 1992), centered on species recognition, mate choice, and crypsis, but attention later turned to bright coloration in monomorphic species that was controversially viewed as advertising unprofitability or unpalatability (Baker and Parker 1979). Recent research has concentrated on explaining skin and feather coloration in the context of communication. Plumage badges, for example, are known to settle contests over food and breeding territories (Pryke and Andersson 2003), but most attention has focused on explaining sexual dichromatism, specifically, bright colors seen in males. In the last 15 years, a raft of studies have shown that females choose to mate with colorful males or with males sporting bright epaulets, head patches, or chest patches of feathers (Gustafsson et al. 1995). These variously correlate with male nest attentiveness, overwinter survival, or reproductive success, depending on the study; as a result of female preference, they consequently result in earlier laying dates and sometimes polygyny. In some species, the size of feather patches or the brightness and color of wattles and combs are associated with an absence of parasite load or with some other aspect of male condition. In particular, red or yellow feathers predict nutritional status, because they are carotenoid-based pigments that cannot be synthesized, only ingested (Hill et al. 2002), but even orange plumage produced by melanin pigments and blue-ultraviolet coloration produced by feather microstructure reflect male quality (Siefferman and Hill 2003). In short, colorful patches of feathers appear to be honest signals of male condition and are passed from fathers to sons. At this point it is not clear whether the extraordinary diversity of bird coloration patterns will eventually be interpreted principally in term of sexual selection, or whether this is a consequence of a sexual selection–driven research bias currently in vogue.

Interspecific communication. The most famous example of aposematism in the animal kingdom is the spotted skunk,

Table 3. Summary of significance tests showing relationships between the occurrence of contrasting patches on the coats of mammals (artiodactyls, carnivores, and lagomorphs) and ecological and behavioral variables.

	Variables associated with contrasting patches on different parts of the body						
Group	**Face (patch color/ appearance)**	**Throat (patch color/ appearance)**	**Ears (patch color/ appearance)**	**Legs (patch color/ appearance)**	**Tail (patch color/ appearance)**	**Side (patch color/ appearance)**	**Rump (white)**
Artiodactyla	Intermediate group size (dark†, white†), large group size (dark†), diurnal (white†), grassland habitat (conspicuous†)	NT	NT	Large group size (dark†), diurnal (white†, conspicuous†), desert habitat (dark†, conspicuous†), grassland habitat (conspicuous†)	Intermediate group size (dark*, conspicuous†), large group size (dark*, white†, conspicuous†), diurnal (dark*, conspicuous†)	Intermediate* or large† group size	Intermediate* or large* group size, diurnal†, open environment†
Cervids	Diurnal (dark*), grassland habitat (conspicuous†)	NT	NT	NS	NS	NS	Intermediate† or large* group size
Bovids	Intermediate group size (dark†), large group size (dark†, conspicuous*)	NT	NT	NS	Large group size (dark*, conspicuous*), diurnal (dark*, white*, conspicuous†)	NS	Intermediate* or large* group size, open environment†
Carnivora	NT	NS	Forest (white†)	NT	Nocturnal (ringed tail†), diurnal (black tail tip*), arboreal (ringed tail†), terrestrial (black tail tip†), closed environment (ringed tail†), grassland habitat (black tail tip†, white tail tip†), forest habitat (ringed tail†)	NS	NS
Canids	NT	NS	NS	NT	NS	NT	NT
Ursids	NT	NA	NS	NT	NA	NT	NT
Procyonids	NT	NA	Groups (conspicuous†)	NT	NA	NT	NT
Mustelids	NT	NS	Forest habitat (white†)	NT	Grassland (black tail tip*)	NT	NT
Viverrids	NT	Groups (white†)	NS	NT	NS	NT	NT
Herpestids	NT	NS	NS	NT	Grassland (black tail tip*)	NT	NT
Hyaenids	NT	NA	NS	NT	NS	NT	NT
Felids	NT	NS	Forest habitat (white†), grassland habitat (black†)	NT	NS	NT	NT
Lagomorpha	NT	NT	Groups (dark†), not diurnal (dark*), not closed environment (white*)	NT	Groups (white†), burrows (white†), no burrows (dark*), grassland habitat (white†)	NT	NT

Asterisk (*), significant results of nonparametric chi-square or Fisher exact probability tests; †, significant results of phylogenetically controlled comparisons using MacClade and Maddison's concentrated changes tests (Maddison 1990).

NA, not applicable (no species showing that type of coloration); NS, not significant (no significant association found between contrasting coat patches and ecological or behavioral variables); NT, not tested.

Source: Ortolani and Caro 1996, Ortolani 1999, Stoner et al. 2003a, 2003b.

which has contrasting black and white patches of fur on its body. Seven species of mustelid have black-and-white coats, and all produce noxious anal secretions, a highly significant association after controlling for phylogeny (figure 3). Similarly, light tails are associated with the production of these secretions in mustelids and herpestids, as are black undersides in the latter family. Black-and-white coloration may even warn of pugnacity, as suggested for the ratel (Estes 1991), and may advertise quills and spines, as seen in some species of porcupines and tenrecs.

More subtly, artiodactyls and lagomorphs use color patches to enhance pursuit-deterrent signals aimed at predators. These signals may inform an approaching predator that it has been detected (perception advertisement); they may also inform the predator of the prey's condition and hence its probability of escaping (quality advertisement; Caro 1995). For instance, when pursued by wild dogs, Thomson's gazelles stot vigorously (a stylized gait with legs held stiff and straight) and lift their tails, perhaps to flaunt their white rump patch (FitzGibbon and Fanshawe 1988). Pursuit-deterrent signals might therefore be expected to be directed at stalking predators, and it is interesting that both dark and white tails are seen in artiodactyls that are principally attacked by stalkers. By contrast, quality advertisement might be directed at coursing predators, and both white rumps and dark faces are associated with pursuit by coursers in bovids and artiodactyls (Stoner et al. 2003a). The size or brightness of color patches could be related to condition in ungulates, although this has never been tested.

Finally, patches of color may be used to attract heterospecifics' attention to particular areas of the body. Ortolani (1999) found that carnivores with white tail tips were species that preyed on bovids or small mammals, raising the intriguing possibility that rapid flicking of the tail tip may distract or lure prey, as occurs in some snakes. She noted also that white tail tips in carnivores (but not black tips) were associated with predation by raptors. In a singular experiment that has never been followed up, Powell (1982) trained three red-tailed hawks to attack various weasel models that were towed across an experimental arena. The hawks consistently missed attacking models with a black tail tip but struck those with a black mark on the body, suggesting that black tips may distract avian predators or draw them to a less vulnerable area of the body.

Physiological hypotheses

The final major class of hypotheses for coloration in mammals concerns a potpourri of physiological and physical functions that are involved in regulating body temperature (by reflecting or absorbing radiation, or by providing a surface that

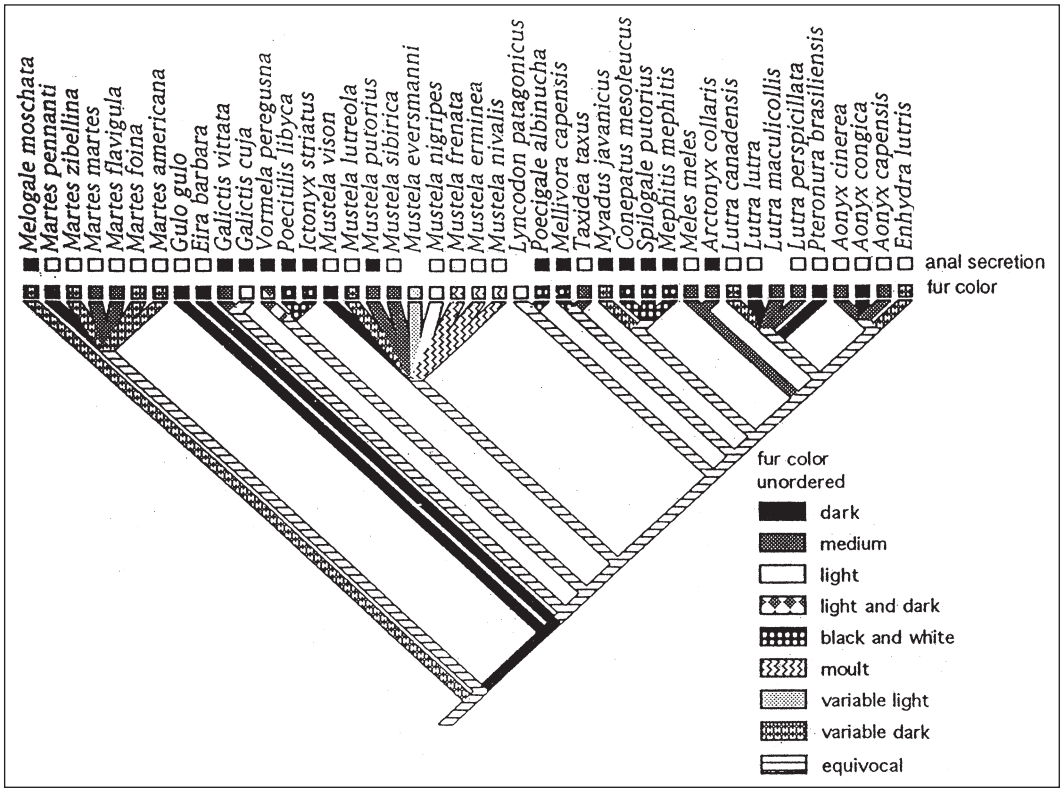

Figure 3. Phylogeny of the Mustelidae, showing the reconstructed evolution of fur color; equivocal branches denote ambiguities in character reconstruction. The row of boxes labeled "anal secretion" denotes whether the species possess a noxious anal sac secretion (black box) or not (white box); data for three species are missing for this character. Source: Ortolani and Caro (1996); © 1996 Cornell University Press, used with permission.

Table 4. Summary of significance tests showing the relationships between the coloration of mammals (artiodactyls, carnivores, and lagomorphs) and ecological and behavioral variables.

	Variables associated with mammal coloration				
Group	**Uniform coloration (color)**	**Face markings (color)**	**White rump**	**Dark eye markings (type of mark)**	**Dark tail**
Artiodactyla	Closed environment (dark*), tropics (dark†), dense forest (dark*)	Grassland (white†)	Desert*, grassland*	NT	NT
Cervids	Dense forest (dark*)	Open environment (white†)	Desert*	NT	NT
Bovids	Closed environment (dark*), tropics (dark†), dense forest (dark*), swamp (dark*)	Desert (white*), grassland (white*)	Desert*, grassland*	NT	NT
Carnivora	Tropical forest (dark†)	NT	NT	Open and closed environment (eye patch†), small body size (eye patch†), terrestrial (eye contour†), crepuscular (eye contour†, eye patch†), riparian (marking below eye†, eye patch†), grassland (eye contour†)	NS
Canids	Tropical forest (dark†)	NT	NT	Crepuscular (marking below eye†)	NT
Ursids	Tropical forest (dark†)	NT	NT	NS	NT
Procyonids	NS	NT	NT	NS	NT
Mustelids	NS	NT	NT	Diurnal (marking below eye†)	NT
Viverrids	NS	NT	NT	NS	NT
Herpestids	Tropical forest (dark†)	NT	NT	NS	NT
Hyaenids	NS	NT	NT	NS	NT
Felids	NS	NT	NT	NS	NT
Lagomorpha	Tropics (dark*)	NT	NT	NS	Arctic*, high latitude*

Asterisk (*), significant results of nonparametric chi-square or Fisher exact probability tests; †, significant results of phylogenetically controlled comparisons using MacClade and Maddison's concentrated changes tests (Maddison 1990).

NS, not significant (no significant association found between coloration and ecological or behavioral variables); NT, not tested.

Source: Ortolani and Caro 1996, Ortolani 1999, Stoner et al. 2003a, 2003b.

enhances or reduces evaporation) and reducing glare from the sun, although predictions regarding these functions vary according to whether skin or hair color is under discussion, and depend on physical properties of hair follicles (box 4; Walsberg 1983). While there is reasonably strong correlational evidence to bolster the idea of an adaptive physiological function for mammal coloration, its precise mechanisms remain hazy. The chief finding from phylogenetically controlled comparisons is that artiodactyls, carnivores, and perhaps lagomorphs obey Gloger's rule, in that dark overall pelage is associated with species that live in the tropics (table 4). Unfortunately, we do not know why dark fur is advantageous in tropical areas, particularly in forests, a humid habitat. Is it to keep the animal dry through enhanced evaporation from warm surfaces, or to aid in concealment? A second finding suggests that white face markings in ungulates, and possibly white rump patches, are instrumental in reducing heat load in open desert or grassland habitats because they reflect heat. Both parts of the body can be turned toward or away from the sun to regulate reflectance. Third, dark eyes are found in crepuscular and riparian species, suggesting that they counteract glare when the sun is horizontal or reflected off water (Ortolani 1999); indeed, Eskimos rub soot around their eyes to prevent snow blindness. Last, dark tails are found in lagomorphs living in cold climes, which may indicate differential melanocyte production in colder areas of the body. While robust, these findings have few conceptual underpinnings in common, except that they are all linked by being unrelated to concealment or communication.

Nonadaptive explanations

It would normally be improper to consider anything other than adaptive explanations for most biological traits, but there are hints of nonadaptive patterns of coloration in mam-

Box 4. The physiological basis of external coloration.

Agouti, a banded pattern appearing grey or brown, is probably the ancestral pelage color of mammals. Fur color derives primarily from melanin-based pigmentation deposited in shafts of hair, and thus differs from the hemoglobin that provides the red hue of primate sexual swellings and occasionally facial skin patches. Agouti is produced by alternating black eumelanin and reddish phaeomelanin banding on hairs. As reddish bands are reduced in number, pelage transforms from agouti to dark brown (black with little red). If depigmentation continues, black is lost, leading to light gray, silvery, cream-colored, and eventually colorless hair. If black bands are lost, however, pelage transforms from agouti to reddish-brown (red with little black). Then, if depigmentation continues, pelage turns red, orange, gold, straw, cream, and eventually colorless (Hershkovitz 1977).

Variation in pelage coloration is found in most populations. During development, both genetic and environmental influences, including ambient temperature, affect coat color. Coat color does not necessarily remain static throughout an animal's life; depigmentation may occur as a result of acute stress or age. Furthermore, in a minority of species, young have characteristic natal coats that differ from adult pelage. These include spotted coats in many artiodactyls (e.g., peccaries) and some felids (e.g., pumas), and black, cream, or ostentatious natal coats in some primates (e.g., gibbons).

Skin color is dependent on the melanic layer in the dermis. A blue hue is caused by light impinging on the melanic layer in the dermis and being reflected back through the epidermis; the way that the light scatters, and hence its hue, depends on the collagen and water content of the skin. Red skin results from a plexus of thin-walled blood vessels just below the epidermis. The degree of redness is linked to testosterone production in males and to increases in circulation during follicular development in females (Dixson 1998). Skin color is therefore more labile than fur color and is a priori more likely to signify short-term changes in health.

mals. In particular, forest-living guenons have an extraordinary diversity of facial and body coloration patterns (Kingdon 1988), and tamarins and marmosets show radically different hues on their foreheads, crowns, napes, mantles, and tails, even within species (Hershkovitz 1968). Along the headquarters of just one river, the Rio Jurua in Brazilian Amazonia, saddleback tamarins of both sexes show at least five color morphs ranging from blackish-brown to white, but chromatic types are radically different on opposite sides of the river, a known barrier to genetic dispersal as determined from mitochrondrial cytochrome *b* sequencing. Given that ecological factors and predation pressures are likely to be virtually identical on both river banks, it is difficult not to infer genetic drift as an explanation for different color morphs (Peres et al. 1996). More generally, there is a possibility that certain (unknown) selection pressures, which would constrain coloration over many parts of the body, are lifted for monkeys living in tropical rainforests (Hershkovitz 1968).

More children's questions

Classic hypotheses for selective advantages of coloration were among the first offered to vindicate Darwin's theory of natural selection (Blaisdell 1992), but only now are they receiving the systematic attention that they deserve. That said, many explanations are still *post hoc* and urgently require experimental testing. At present, most biologists believe that crypsis is the key evolutionary force driving the agouti-colored pelage observed in so many mammals. Nonetheless, it is still not clear why some species turn from agouti to white in winter while other sympatric species do not, nor can we yet explain the quite different striking coloration in species such as the giant panda or Burchell's zebra. Intraspecific communication is obviously important in explaining patches of color on the faces, ears, legs, and tails of mammals, but we are a long way from pinning down the content of these signals or understanding what observers they target (e.g., predators, prey, or potential mates). The virtual absence of sexual dichromatism in mammals, in spite of the prevalence of polygyny, remains a mystery; it stands in sharp contrast to the often striking differences between male and female coloration in birds, whose coloration is so important in intra- and intersexual displays. Superficially, this suggests a far smaller role of female choice in mammals than in birds. Aposematism, a clear example of interspecific signaling, explains coloration in mustelids, but why should mustelids need to be so noxious or pugnacious when other sympatric carnivores are not? Finally, although physical factors appear responsible for some types of coloration, we don't know why they are important in some environments but not in others; and 170 years after Gloger formulated his rule, we still don't understand why mammals obey it. Those "why" questions that children are so fond of have no easy answers. Better to ask them a trick question back: Which mammal is green?

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Evolutionary significance of ontogenetic colour change in animals

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Ontogenetic colour changes are non-reversible colour changes associated with normal progressive development of an individual of a species. This paper provides the first review of the evolutionary significance of this phenomenon in animals. Proximate mechanisms and environmental cues are briefly discussed and a conceptual framework for understanding the ultimate reasons for ontogenetic colour change is established. Changes in size, vulnerability, reproductive status, habitat and metabolism are often associated with ontogenetic colour change and can aid in understanding its adaptive significance. Neutral or non-adaptive ontogenetic colour changes due to phylogenetic inertia and developmental constraints are also considered. Existing studies of ontogenetic colour changes in marine invertebrates, terrestrial invertebrates, fish, amphibians, reptiles, birds and mammals are discussed within this framework. A need is identified for more experimental tests of hypotheses for the significance of ontogenetic colour change.

KEY WORDS:—Ontogenetic colour change – colouration – crypsis – mimicry – aposematism – predation – mating – thermoregulation – behavioural ecology – evolution.

CONTENTS

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INTRODUCTION

Studies of colouration patterns and their significance figure prominently in the history of evolutionary biology. At the end of the 19th century, zoologists used animal colour patterns to elucidate the principle of evolution by natural selection (Poulton, 1890; Beddard, 1892). Colt (1957) in the classic *Adaptive Coloration in Animals* offered a conceptual evolutionary framework for multitudinous animals colour patterns, and more recent workers have investigated pattern significance experimentally. Although the subject of ontogenetic colour change (OCC) has been neglected, other research areas are relevant to its study including crypsis (Robinson, 1970; Endler, 1978), mimicry (Wickler, 1968; Vane-Wright, 1980; Pasteur, 1982), aposematism (Edmunds, 1974; Harvey & Paxton, 1981), polymorphism (Ford, 1965; Allen, 1988; Endler, 1988), seasonal polyphenism (Shapiro, 1976, 1984), sexual dimorphism (Baker & Parker, 1979), and thermoregulation (Hamilton, 1973; Walsberg, Campbell & King, 1978; Willmer & Unwin, 1981).

Bückmann (1974, 1984, 1985) employed the term ontogenetic colour change in connection with his work on the great pussymoth caterpillar (*Cerura vinula*). He defined OCC as a morphological colour change that is a part of a species' normal development where timing and resulting colour are not influenced by the environment, but are determined by development. His definition was fashioned with respect to insects, but clearly the phenomenon is far more general. In this review I will use a broader definition that does not use a proximate mechanism of change (environmental versus developmental) to determine what is or is not OCC. In many cases where there is a change in colour with age, mechanisms for the change and their regulation by external conditions are not understood. In a few experimentally investigated cases, workers manipulated the environment to affect the OCC, but the change may be inflexible under normal conditions (Lee, 1966; Wenner, 1972). I consequently define OCC as a non-reversible colour change associated with normal progressive development of an individual of a species. External conditions may or may not affect the timing of the change and the resulting colour or pattern. I exclude reversible colour changes such as temporary breeding colours in some lizards, seasonal changes in snowshoe hares, and rapid changes in chameleons.

In the past, some animals' juvenile and adult phases were mistakenly classified as different species because taxonomists ignored OCC and worked with adult biased collections (Rietschel, 1975). Systematists rarely paid full attention to their subjects' entire life cycles. Yet, ontogenetic niche shift theory emphasizes the importance of studying all life stages in an attempt to understand species interactions. Ontogenetic shifts in diet and habitat can have large effects on species interactions and community structure and thus have ecological and evolutionary consequences (Stamps, 1983; Werner & Gilliam, 1984). Changes in colour associated with such behavioural changes can obviously influence an animals' success in its new environment.

OCC examples encompass everything from early development to sexual maturation to senescence. Colour changes can occur within the nymphal stages as in *Largus californicus* (Hemiptera) which are red as first instars and black in later instars (Booth, 1990). Colour changes from infant to juvenile occur in silvered leaf-monkeys (*Presbytis cristatus*); infants are orange and 6-month-old

juveniles are black (Napier & Napier, 1967). Colour changes from juvenile to adult occur in emperor fish (*Pomacanthus imperator*); juveniles are black and white and adults are multicoloured: blue, red and green (Fricke, 1980). From subadult to mature adult, male gorillas (*Gorilla gorilla*) develop silver-grey backs and males and females continue to turn grey with age (Schaller, 1963). OCC also occurs in higher plants in leaf, flower, and fruit colour change. Given such a wide range of circumstances, it is not surprising that there are many possible hypotheses for the significance of OCC.

In this review I will briefly introduce the proximate mechanisms for OCC and environmental cues that trigger OCC, before discussing ultimate reasons for OCC at greater length. Proximate mechanisms cause a colour change in individuals, while ultimate reasons are evolutionary explanations for why a species undergoes OCC. I will discuss ultimate reasons for OCC in the context of associated changes in an animal and its environment. Selected studies of the significance of OCC in animals (with examples of marine invertebrates, insects, arachnids, fish, amphibians, reptiles, birds and mammals) are included in the appendix and discussed in the text. As there are several areas in which I know of no OCC example, I have used examples of other types of colour change to help explain the concepts. These non-OCC examples are noted as such throughout; however, the section on proximate mechanisms is particularly lacking in OCC examples and the reader who wishes to skip this section is invited to move directly to the section on ultimate reasons.

COLOUR AND COLOUR CHANGE

Before describing mechanisms for OCC it is appropriate to briefly review the physics, visual perception, and biological production of colour. Colour has three attributes: hue, saturation and brightness (Hailman, 1977; Rossotti, 1983). Hue corresponds to wavelength of light: blue for short wavelengths, red for longer wavelengths. Saturation refers to spectral variance; monochromatic light is saturated, while white light, which includes all wavelengths, is unsaturated. Brightness is correlated with physical light intensity. Black, grey and white are neutral with respect to hue, are of zero saturation, and differ only in brightness (Rossotti, 1983). Attributes of a viewer's visual receptors as well as physical attributes of the reflected light and orientation of the coloured structure will determine the colour perceived. Animals that do not have colour vision may still detect differences in brightness (See Table 1). Some fish (Munz & McFarland, 1977), amphibians (Muntz, 1977) and birds (Meyer, 1977) undergo ontogenetic changes in their visual pigments which may affect their colour vision and be of interest when studying OCCs.

There are three main ways in which animals produce colour: through pigments, structural colours or bioluminescence. Pigments are substances that selectively absorb and reflect different wavelengths of light. The major classes of biological pigments are melanins, carotenoids, flavonoids, quinones, ommochromes, purines, pterins and flavins (Needham, 1974). Structural colours are those produced by interference, diffraction, or scattering of light by layers, surface structures or suspended particles. Angle of light incidence on the structures may affect the resulting colour, and some structures can change from transparent to brightly coloured simply by a change in viewing angle.

TABLE 1. Phylogenetic distribution of colour vision. Generalizations are based on various levels of evidence and a different number of species sampled

Taxa	Colour vision	Reference
Molluscs		
Gastropods	None	Smythe, 1975; Messenger, 1981
Bivalves	None	Smythe, 1975; Messenger, 1981
Cephalopods	None	Messenger, 1981
Arthropods		
Crustaceans	In most	Waterman, 1961; Smythe, 1975
Insects	In most	Mazokhin-Porshnyakov, 1969; Lythgoe, 1979; Menzel, 1979
Fish	In most	Walls, 1942; Ali & Klyne, 1985
Amphibians	In some	Walls, 1942; Ali & Klyne, 1985
Reptiles		
Snakes, crocodilians	None	Walls, 1942; Smythe, 1975
Turtles, lizards	In most	Walls, 1942; Smythe, 1975; Ali & Klyne, 1985
Birds		
Nocturnal	None	Walls, 1942; Lythgoe, 1979; Ali & Klyne, 1985
Diurnal	In most	Walls, 1942; Lythgoe, 1979; Ali & Klyne, 1985
Mammals		
Non-primates	None	Walls, 1942; Ali & Klyne, 1985
Primates		
Prosimiae	None	Walls, 1942; Ali & Klyne, 1985
Simiae	In most	Walls, 1942; Ali & Klyne, 1985

Bioluminescence is the production of light by living organisms. Bioluminescence may seem very different from pigment or structural colours, but the end result is the same: selected wavelengths of light coming from the organism's surface.

Colour change mechanisms are usually divided into physiological and morphological types (Bagnara & Hadley, 1973). Physiological colour change involves movement of existing pigment within chromatophores (pigment cells) or muscular movement of pigment cells and can occur over very short time periods. Morphological colour change, on the other hand, involves synthesis or degradation of pigments and therefore takes place over a relatively longer time period. Chameleon type reversible colour changes are physiological and most OCCs are morphological colour changes.

PROXIMATE MECHANISMS FOR ONTOGENETIC COLOUR CHANGES

Asking how a change in colour occurs during an individual's lifetime (the proximate mechanism) is different from asking why a species has come to have the trait of changing colour with age (the ultimate reason). Understanding the causal mechanisms, whether at the intrinsic molecular or extrinsic environmental level, may help us to understand the ultimate significance of the change. Although little work exists on mechanisms for OCC, other colour change studies (reversible or seasonal) suggest possible OCC causes.

Physiology affects colour changes

A change in pigment synthesis rate is perhaps the most direct mechanism for OCC. Eggs are often provisioned with carotenoids and other pigments which an embryo cannot produce, and integumental pigments are generally synthesized later in life (Needham, 1974). This transition from having only maternally derived pigments to synthesizing more complex pigments de novo is one way for OCC to occur. It may take time to acquire precursors for complex pigments, or other developmental processes may take precedence over pigment production. Dietary changes can lead to accumulation of different pigments in the integument. Changes in competitor density or predator density might affect an individual's food choices and thus pigment uptake.

Alternatively, if pigment synthesis ceases at a certain age, further growth and allometry may affect subsequent colour and colour pattern. Work on colour pattern development in lepidopteran wings, mammalian coats and molluscan shells has suggested some simple biochemical models for complicated colour pattern production through heterochronous pigment deposition (Murray, 1981; Nijhout, 1981; Nijhout & Wray, 1986; Ermentrout, Campbell & Oster, 1986; Meinhardt & Klingler, 1987).

Another physiological function that could influence colour change is excretion. Cochran (1975) discussed the long-standing idea that some pigments are packaging for waste products that can not be excreted from the body, but are instead sequestered in the integument out of metabolic pathways. Accumulation of such wastes during an animal's lifetime could result in OCC. However, knowing that a colour change is produced by accumulation of excretion products does not rule out the involvement of other functions (i.e. visually mediated ones).

OCC associated with maturation is probably controlled by hormones as temporary, breeding-season colour changes have been found to be under hormonal control. In insects, bursicon, ecdysone, juvenile hormone and the neuroendocrine complex affect colour change (Fuzeau-Braesch, 1985). In iguanid lizards, females develop secondary sexual colour patterns when gravid and progesterone and testosterone regulate colour change in some species (Cooper, 1984, 1986). Breeding-season colours in some birds (Witschi, 1961) and primates (Wickings, Marshall & Nieschlag, 1986) are also under hormonal control.

Bioluminescence in animals can be produced directly by the organism that uses it as in fireflies or by symbiotic bacteria as in lantern fish (Hastings, 1983). In some bioluminescent fish, ova are provisioned with bacteria (Buck, 1978), and ontogenetic changes in luminescence could be associated with bacterial colony growth rates.

Extrinsic factors affect colour changes

Abrasion and fading can account for colour changes. Changes in colour resulting from wearing down of pigmented structures occur in some bird feathers as melanic areas resist wear better than carotenoid rich or non-pigmented areas (Test, 1940; Burtt, 1979, 1986). In addition, prolonged exposure to sunlight can fade carotenoids in feathers (Test, 1940) and pterin pigments in lepidopteran scales (Taylor, 1973).

ENVIRONMENTAL CUES THAT TRIGGER ONTOGENETIC COLOUR CHANGES

Biotic environmental cues for OCC

An animal's biotic environment can vary with changes in habitat or habits that may be associated with OCC. Social circumstances can affect hormonal balance and even sex of an animal along with colour. Many labrid fish species have two colour morphs that are strongly associated with size: dark small individuals and brightly coloured large individuals (Roede, 1972; Warner, Robertson & Leigh, 1975; Hoffman, Schildhauer & Warner, 1985). In some species, small individuals are typically female and large individuals are typically males which changed sex from small females when they reached a size large enough to defend a territory. If a harem-holding male coral reef fish *Anthias squamipinnis* is removed, the largest female in the harem changes sex and colour (Shapiro, 1981).

Population density affects colour change in many insects including locusts (Fuzeau-Braesch, 1985). Key (1957) found that some phasmids (*Podacanthus wilkinsoni, Didymuria violescens* and *Ctenomorphodes tessulata*) raised in isolation were a uniform green and those raised at high densities were patterned with black, yellow and white. As high density rearing did not increase activity level, Key postulated that there was a direct nervous link between sensory stimulation and the endocrine system responsible for pigmentation.

Abiotic environmental cues for OCC

Possible triggers for OCC include daylength, insolation, temperature, humidity, carbon dioxide and background colour and texture. Although the following examples do not involve OCC, they may be instructive. Ultraviolet (UV) light (<500 nm wavelength) promotes melanization and light of >500 nm inhibits melanization in cabbage white butterfly pupae (*Pieris brassicae*) (Kayser & Angersbach, 1974). In contrast, melanization in adult flies (*Sarcophaga falculata*) was inhibited in those irradiated by UV light just after eclosion (Schlein, 1975). Flies held at 15°C or 31°C during eclosion were also less melanized than those at 26°C (Schlein, 1975). Desert beetles *Cryptoglossa verrucosa* change from light blue at low humidity to black at high humidity by changes in the waxy secretion on the cuticle surface (Hadley, 1979).

Homochromy, matching the background colour, can be either direct, where the animal perceives its background colour and changes colour to match it, or indirect, where environmental cues associated with a background colour are perceived and trigger a colour change (Fuzeau-Braesch, 1985). Indirect homochromy, in some insects, occurs when high humidity, normally associated with green vegetation, causes a cuticle colour change to green (Ibrahim, 1974). Migratory locusts (*Locusta migratoria*) kept in crowded conditions and exposed to 100% carbon dioxide for one minute per day change colour from a typical gregarious colour pattern (bright yellow and red) to either green or a background matching colour characteristic of the solitary type (Fuzeau-Braesch & Nicolas, 1981). Seasonal changes in colour, as in snowshoe hares, are triggered by changes in daylength (Hinton, 1976). Even substrate texture affects colour change. In the swallowtail butterfly *Papilio polytes* those larvae pupating on

rough surfaces become brown and those pupating on smooth surfaces become green, thus matching their background of brown bark or green leaves (Smith, 1978). Background colour, relative humidity, temperature and photoperiod also affected the resulting pupal colour, but the effect of texture was most significant in *P. polytes* which normally selects its pupation site in the dark (Smith, 1978).

ULTIMATE REASONS FOR ONTOGENETIC COLOUR CHANGES

Ultimate reasons for OCC deal with why a species, or higher taxon, has the trait, rather than how the colour changes happens in each individual. I am interested in selective factors that may determine the adaptive significance of OCC and in historical factors and constraints that may also influence colour changes in a species. In any study of the significance of colour there are many levels at which to ask questions. Biological pigments (biochromes) have chemical properties and may have biochemical, physiological, developmental, neurological and behavioural functions (Needham, 1974). Needham (1974) considered functions mediated by vision as epiphenomena that have evolved from primary, chemical biochrome functions. This is possible, but we must bear in mind that the evolutionary origin of a trait is more difficult to establish than are the agents responsible for its persistence (Huey & Bennett, 1986).

Biochrome functions may change during an animal's life, and thus the current utility of OCC may be understood by looking at the function of a colour in a given life stage and determining why other colours are more useful at other life stages. It is also conceivable that some OCCs are selectively neutral and are maintained simply by their association with strongly selected characters. In contrast to such selective arguments, we must also consider the possibility that changing colour has no current effect, or even a mildly deleterious effect, on an individual's fitness. Although an example of this has yet to be described, some would argue that this is due to our overwhelming bias towards adaptive explanations (Gould & Lewontin, 1979).

Colouration functions can be divided into four areas. The first two involve visual communication (intraspecific and interspecific), and the second two relate to physical properties of colour and pigments. Colour-mediated intraspecific communication (reviewed by Rowland (1979)) can affect aggregation, parental care, and species and mate recognition. Colour-mediated interspecific communication (reviewed by Baylis (1979)) can involve crypsis, mimicry and aposematism and is most often related to predation. Light-mediated functions of colour, where perception of colour by an observer is not involved, include absorption of solar radiation for thermoregulation and protection from damaging UV rays. Lastly, pigments' chemical and physical properties, as separate phenomena from the colours that they produce, can also be adaptive.

Colouration changes are often associated with changes in other characteristics of an animal or with changes in its biotic and physical environment. Environmental changes can be due to movement of the animal, movement of other organisms (predators, competitors, prey), and seasonal changes. Using changes in size, vulnerability, reproductive status, habitat and metabolism as a framework, I present examples of studies on the significance of OCC in the following sections. Some sections overlap slightly as, for example, changes in size and changes in habitat can also result in changes in vulnerability. These selected

examples are arranged systematically in the appendix. The criterion for inclusion in this list was that there existed a published account of the OCC with a discussion of its possible significance. I classified published interpretations as supported by either logical argument, observational data, or results from a manipulative experiment specifically designed to determine the colour changes' current utility. I have included a broad range of examples involving different types of animals and a variety of reasons for OCC. The list is not intended to be comprehensive as there are descriptions and photographs of animals that exhibit OCC in every field guide and natural history picture book; most accounts lack a critical analysis of hypotheses for the significance of the change and are therefore excluded.

OCC associated with change in size of the animal

Colour, size change and mimicry

As an animal gets larger, its ability to mimic certain models changes. Mathew (1934) coined the term transformational mimicry for cases where different models are mimicked sequentially during an animal's life. There are several examples of spiders and bugs that successively mimic different ant species as each instar gets bigger and changes colour (see arachnid and Heteroptera sections in the appendix). Evidence for Batesian mimicry is usually given in terms of direct observational data on numbers of models and mimics, their co-occurrence, and their similar behaviour. Two mantids mimic ants when young and leaves (*Evantissa pulchra*) or wasps (*Mantoida maya*) when older (Mathew, 1935; Jackson & Drummond, 1974).

Some animals are mimics during part of their lives and change to cryptic colouration as they outgrow the model. Juvenile African lizards (*Eremias lugubris*) mimic (in colour, size and gait) beetles that spray an acidic pungent fluid (Huey & Pianka, 1977). Adults are too large to mimic the beetle and instead are concealingly coloured. Similarly, the kingsnake (*Lampropeltis triangulum*) which is thought to mimic coral snakes as a red, yellow and black banded juvenile, changes to a wholly black adult. This OCC might have a thermoregulatory function, although a sympatric coral snake (*Micrurus alleni*) also turns black with age (Greene & McDiarmid, 1981). This may then be a case where the model undergoes OCC for thermoregulatory reasons and the mimic undergoes OCC to maintain the mimicry and perhaps to also gain a thermoregulatory advantage. Grass snakes (*Natrix natrix*) which are typically black have a yellow collar just behind the head which is more distinct in neonates than in adults. In experiments with coloured snakes models, wild birds attacked more solid black models than yellow collared models (Madsen, 1987). The author suggested that birds likely to prey on small snakes also ate insects and probably associated black and yellow snakes with stinging insects. According to this hypothesis it is expected that as snakes grow out of the vulnerable size range they will lose the yellow pattern. Eaton (1976) suggested that cheetah (*Acinonyx jubatus*) kittens, which are relatively defenceless, appear to mimic adult ratels (*Mellivora capensis*) which are aggressive to large predators and have a conspicuous white back and dark underparts. Change to a cryptic colour pattern occurs at 2.5 months when cheetah kittens become larger than ratels. The hypothesis that red salamanders (*Pseudotriton* spp.) are Batesian mimics of toxic red-spotted newt efts

(*Notophthalmus viridescens*) is supported by the fact that *Pseudotriton* species that are smaller than efts are red throughout their lives, but species which grow larger than efts change from red juveniles to cryptically coloured adults when their larger size would presumably prevent effective mimicry (Pough, 1974; Huheey & Brandon, 1974).

Colour, size change and crypsis

A colour pattern that is cryptic on a small individual may be conspicuous on a larger one (Endler, 1978). Hogarth (1978) described an example in which juveniles and adults achieved crypsis by different colour patterns. Shore crabs (*Carcinus maenus*) have contrasting pigment patches on their carapace when juveniles, but these patches are lost with age. He found that both patterned juveniles and unpatterned adults were cryptic from a human perspective.

Colour polymorphism studies may give some insight to an advantage of OCC. There is evidence for colour polymorphism maintenance by apostatic selection in which rare coloured morphs are not recognized by predators with search images for the more common coloured morphs (Croze, 1970; Atkinson & Warwick, 1983). Similarly, a species that exhibits OCC presents more than one colour morph to potential predators. Differences in size and colour could serve to confuse a predator with a narrow search image, if indeed such behavioural constraints are real (Guilford & Dawkins, 1987).

Colour, size change and thermoregulation

As an animal's size increases, surface area to volume ratio decreases. Small animals with a large surface area to volume ratio have more difficulties maintaining their body temperature, whether as a homeotherm or poikilotherm, than do larger animals. Colour can affect body temperature by affecting the amount of solar radiation that is absorbed (Kettlewell, 1973; Burtt, 1981). Melanin, a dark pigment, absorbs strongly in the infrared portion of the spectrum (Neville, 1975), however dark colouration does not always indicate a larger potential radiative heat gain. For birds, radiation penetrates white plumage more easily than black plumage, so that at high wind speeds ($3\ ms^{-1}$) white birds gain more radiative heat than black birds (Walsberg, Campbell & King, 1978). Walsberg *et al.'s* (1978) model also accurately predicts mammal radiative heat loads which are affected by coat colour and skin colour as well as optical properties of individual hairs (Walsberg, 1988).

Colour pattern, as well as colour, can affect heat gain through radiation absorption. In *Pieris* butterflies, wing melanization near the abdomen absorbs solar radiation, while areas lacking melanin further out on the wings reflect radiation down to the melanized areas when the butterfly holds its wings in a reflectance basking position (Kingsolver, 1985). This combination of wing melanization pattern and wing orientation raises body temperature to that needed for flight (Kingsolver, 1985).

In a variety of insects, Willmer & Unwin (1981) found that temperature excess (the difference between body temperature and ambient temperature) depended principally on size, but was increasingly influenced by reflectance (a measure partially related to colour) in larger insects. Therefore, within an insect's lifespan, smaller stages' colouration does not significantly affect body temperature and may have other functions, but larger stages' colouration will

affect body temperature and may limit the ability to respond to selection for different colours. Thermoregulatory needs may outweigh the need for crypsis at certain times in the life cycle. Southern green stink bugs (*Nezara viridula*) are black in the second instar and turn green in the third, fourth or fifth instar. Black fourth instars gain heat at a faster rate than green fourth instars which allows them to develop more repidly, but green instars are probably more cryptic (Johnson, 1984).

OCC associated with change in vulnerability

Colour, vulnerability and mimicry

Changes in vulnerability with age affect the value of mimicry to avoid predation. Predator mimicry associated with OCC may occur in characin fish (*Colossoma bidens*): juveniles mimic and school with juveniles of their predator, a piranha (*Serrasalmus notatus*), and may gain protection from predation as piranha are not cannibalistic (Zaret, 1977). Zaret argued that adult characins are probably large enough to avoid predation by piranha and therefore do not mimic them. Another form of mimicry is school-oriented mimicry where usually solitary fish species mimic schooling species to take advantage of the protection that the school offers from predation (Dafni & Diamant, 1984). Juveniles but not adults, of the venomous Red Sea blenny (*Meiacanthus nigrolineatus*) closely resemble young of cardinal fish species (Apogonidae) and are found among their schools. Adults are solitary and have a different and distinctive bright colouration to advertise their toxicity. Juveniles may be less venomous and more vulnerable to predation according to Dafni & Diamant (1984).

The above are cases in which mimicry serves as a protection from predation, however mimicry can also conceal a predator from its prey. Aggressive mimicry (*sensu* Wickler, 1968) was hypothesized in juvenile snapper (*Lutjanus bohar*) which mimic and school with planktivorous damselfish (*Chromis* spp.) in whose company they are not perceived as predators (Moyer, 1977). Perhaps adult snapper are better predators than the juveniles and do not need the camouflage of *Chromis*.

Colour, vulnerability and aposematism

Aposematic colouration warns potential predators of distastefulness, toxicity, danger or unprofitability of the prey. As with crypsis and mimicry, need or appropriateness of aposematism may change with age. If a toxic substance is accumulated gradually from food, then juveniles may not have enough to deter a predator and should not be aposematic. Similarly, aposematic colouration is not appropriate in juveniles which are too small to be dangerous to predators or too slow to escape from predators, but examples are lacking.

In many groups of animals, adults with bright colouration develop from relatively inconspicuous juveniles. For instance, beetle and wasp larva are not usually aposematic. The unprofitable prey hypothesis was proposed by Baker & Parker (1979) to explain bright colouration of some adult birds (usually males) that are difficult for predators to catch. According to this hypothesis, bright colours signal the predator that the prey is not worth chasing as it will most likely be able to escape. Juveniles and females are easier to catch and/or have less conspicuous behaviour patterns than males, so males are more likely to display

bright colours. This hypothesis was offered as an alternative to sexual selection for the evolution of brightly coloured plumage in male birds. Extending the unprofitable prey hypothesis from birds to other groups, Baker (1985) argued that Endler's (1980) observations on guppies (*Poecilia reticulata*), which are brightly coloured in streams with an inefficient predator and cryptic in streams with a better predator, supported the unprofitable prey hypothesis. Endler (1980) interpreted his results as evidence for a trade-of between sexual selection for brightly coloured males and predator selection for cryptically coloured males. In general, predators usually find juveniles easier to catch than adults and sexual selection does not act on prereproductives, therefore both hypotheses apply only to adults and both could account for OCC.

In at least some cases, aversion is more easily learned if distastefulness is associated with a bright colour than a drab colour (Gittleman, Harvey & Greenwood, 1980). If the main purpose of colouration is to warn potential predators, then one would expect conservation of bright colour patterns throughout life. This argument would apply both among an individual's life stages and among species in a Mullerian mimicry complex in which several species share a common warning colouration and all have some dangerous attributes. Ford (1971) thought that in a Mullerian mimicry complex general resemblance was sufficient to give all species mutual protection. Work on a hemipteran has shown, however, that in some cases general resemblance is inadequate to provide total protection across all life stages. Experiments with captive great tits (*Parus major*) as predators indicated that different stages of *Lygaeus equestris* (nymphs: red and black; adults: red, black and white) were not similar enough to give full mutual protection (Sillén-Tullberg, Wiklund & Järvi, 1982). After experience with the distastefulness of one stage, predators did not avoid the other stage. In such cases there are possibly stronger selective pressures, as yet unknown, for a change in colouration.

Aggregations of brightly coloured, distasteful animals increase the warning signal and also allow group-mates to benefit from a predator's learning experience with one of their number. Individual selection (Wiklund & Järvi, 1982; Sillén-Tullberg & Bryant, 1983) and kin selection (Harvey & Paxton, 1981; Harvey *et al.*, 1982) have been argued to account for the evolution of warning colouration. Guilford (1985), however, considered grouping of similar phenotypes the important factor, rather than the group members' relatedness, and discounted the involvement of kin selection. Aggregations may be advantageous for brightly coloured animals, but cryptically coloured animals are less easily detected if they are solitary.

There are many examples where changes in aggregating behaviour during an animal's life cycle are accompanied by changes in colour. Changes from bright to cryptic colours in a treehopper (*Platycotis vittata*) and an orthopteran (*Ephippiger ephippiger*) are accompanied by changes from gregarious to solitary behaviour (Wood, 1976; Hartley & Bugren, 1986). Bush locust nymphs (*Phymateus purpurascens*) are brightly coloured and gregarious, while adults are cryptically coloured and less gregarious (Rowell, 1967). Rowell found that visual stimuli were important in attracting individuals to each other, and hypothesized that dispersion with age was partly due to loss of bright nymphal colours. The palatability of the above species was not directly tested, but *Phymateus* spp. do have secondary deimatic displays and exude secretions from the thorax

(Edmonds, 1974). Changes in palatability are known to accompany changes in colour and gregariousness as in a treehopper (*Platycotis vittata*) in which brightly coloured, gregarious, young adults were rejected by *Anolis carolinensis* in laboratory studies, but cryptic, solitary, older adults were eaten (Wood, 1975). Aggregation can have functions other than predator protection, such as thermoregulation and feeding, and it is not clear in any of the above cases which evolved first, the colour change or the behaviour change. Sillén-Tullberg (1988) found phylogenetic evidence that the evolution of warning colouration preceded the evolution of gregariousness in four lepidopteran families.

Colour, vulnerability and crypsis

As animals grow they are exposed to different biological threats; need for crypsis can change along with vulnerability to predators or conspecific aggression. Some animals may only need the extra protection of camouflage when very young, as in deer (Cott, 1957), in others cryptic colouration may become useful after an initial guarded period, as in altricial birds (lacking feathers) that are in a secluded nest (Hinton, 1976). Juvenile birds are often more cryptic than adults and predator distraction by adults was hypothesized to account for colour differences between juvenile and adult plumage in some cases (Baker & Parker, 1979). An additional or alternative hypothesis involving sexual selection for bright breeding colours is discussed below in the section on OCC associated with reproductive status.

If there are differences in toxicity between different life stages then some may be cryptic while others are aposematically coloured. Bright red efts of the red-spotted newt (*Notophthalmus viridescens*) are more toxic than dark green adults, although adults are also avoided by predators after experience with them, and the significance of the colour change remains a mystery (Howard & Brodie, 1973). On the other hand, if certain life stages are more vulnerable to damage once attacked, crypsis may have advantages over aposematism even if levels of distastefulness are identical. Aposematically coloured larvae and adults of the monarch and European swallowtail butterflies (*Danaus plexippus* and *Papilio machaon*) survived attack by captive quails more often than their equally distasteful pupae which are cryptic in the wild (Wiklund & Sillén-Tullberg, 1985).

Colour, vulnerability and deflection marks

Certain colour patterns can direct predators' attacks to less vulnerable parts of an animal and give it a chance to escape. Hatchling skinks (*Eumeces fasciatus* and *E. laticeps*) have blue tails which change to black with age. Blue-tailed hatchling skinks were found to escape scarlet kingsnakes (*Lampropeltis triangulum elapsoides*) more often than tail-less or black-tailed (painted) hatchlings (Cooper & Vitt, 1985). The blue tail attracts attacks to the tail rather then the body and allows a lizard hatchling to autotomize its tail and escape. Adults may have less need for this type of protection from predators, but this idea has not been tested.

Colour, vulnerability and intraspecific aggression

Colour change from juvenile to adult may help to avoid or reduce intraspecific aggression. Specific juvenile colour patterns could allow adults to recognize prereproductive conspecifics and distinguish them from sexual or physical

competitors. OCC may also be useful to avoid confusion of juvenile conspecifics with prey species. Examples involving mating colouration will be discussed in the section on OCC and change in reproductive status. Avoidance of cannibalism and aggression are discussed here.

In addition to the above mentioned antipredator function, blue tails in hatchling skinks (*Eumeces fasciatus*) also inhibit attack by conspecific breeding males (Clark & Hall, 1970). A similar phenomenon may account for distinct natal and juvenile coat colours in many primates, especially colobines (Hrdy, 1976, 1977). These colour changes in primates are hypothesized to promote tolerance of infants by adults (Alley, 1980). In baboons (*Papio anubis*), a male holding an infant while involved in an antagonistic interaction with another male has an advantage. This phenomenon is called agonistic buffering (Strum, 1984).

Some juveniles are similar in size to adults of species that are preyed upon by their parents. Differences between juvenile colour and prey species colour may evolve given the advantages of preventing cannibalism of kin. Usefulness of such distinctive juvenile colouration may thus change as they grow larger than the prey species. Two examples where this was hypothesized to occur involve a predatory cichlid fish (*Cichla ocellaris*) (Zaret, 1977) and an anolid lizard (*Anolis cuvieri*) (Rand & Andrews, 1975; but see Gorman, 1977).

An alternative explanation for distinct juvenile colouration is that rather than helping to diminish intraspecific aggression, it may help to focus aggression towards inexperienced juveniles. When adults work together cooperatively, they may benefit from excluding juveniles from some activities, and this is easier if juveniles have different colour patterns. Under such circumstances there will be selective pressure for juveniles to change to adult colouration quickly. In African penguins (*Spheniscus demersus*) adults are aggressive towards juveniles with grey-brown heads and exclude them from cooperative feeding groups (Ryan, Wilson & Cooper, 1987). Juveniles that quickly moult to the adult black and white pattern can join adult foraging groups earlier. This is a confusing case where there seems to be an advantage to the adults but not to the juveniles, and it is therefore difficult to imagine how it would have evolved. It may be considered to be similar to the conflict of interest between adults and juveniles found during weaning.

In threespot damselfish (*Eupomacentrus planifrons*) Thresher (1978, 1979) found that bright juvenile colours initially elicited aggression from territorial adults, but the adults' attack readiness decreased after several interactions with a juvenile. The short-term effect was increased aggression by adults on juveniles, but the long-term effect was lowered aggression by adults on juveniles both mediated by the distinctive juvenile colouration. This behaviour may then allow juveniles to begin holding a territory within the colony of adults (Thresher, 1978).

Colour, vulnerability and parental care

OCC may facilitate mutual recognition between parents and their offspring with advantages for parental care. If adults have distinctive colour patterns, then young can identify then as capable of giving food, protection or other types of care. Red spots on adult gulls' beaks (*Larus* spp.) elicit an innate pecking response from young in the nest (Hailman, 1967). Likewise, distinctive juvenile

colour patterns may elicit care behaviour from adults. In some primates infant coat colour is hypothesized to stimulate protection and care by conspecific adults (Hrdy, 1976; Alley, 1980; see primate section in the appendix).

OCC associated with change in reproductive status

Prereproductives avoid risks associated with bright breeding colours

As breeding is not a concern of an immature animal, it may be best to postpone development of bright breeding colours if they elicit male aggression and increase predation risk. Likewise mating attempts with prereproductives are a waste of time for both adults and juveniles and may be avoided if sexually mature adults have a distinctive colouration. On the other hand, young adult males may be sexually mature but incapable of obtaining mates when in competition with older males. In this case maintaining juvenile colouration may be advantageous for sneaking copulations (Rohwer, Fretwell & Niles, 1980). If breeding colours are costly, they could be the result of genetic linkage of female preference and male trait and therefore subject to runaway sexual selection (Fisher, 1958). In this case, female preference would only act on reproductively active males and juvenile colours could be influenced by other selective pressures such as predation. Costly breeding colours could also be a sign of genetic quality that it is not possible to mimic (Kodric-Brown, 1985).

Recent research in this area has focused on delayed plumage maturation in male passerine birds. The female mimicry hypothesis is that subadult males "mimic females to overcome a handicap in competition with older males and thereby increase their probability of breeding in their first year" (Rohwer *et al.*, 1980: 406). When subadult males are able to enter adult male territories without incurring the owner's aggression, they may gain an advantage in holding a part of the area for their own breeding territory at some time in the future. Lyon & Montgomerie (1986) found little evidence in passerines to support this hypothesis and suggested that instead, dull second year male plumage is a subordinance status signal which limits mature male aggression. Mature males are not deceived into thinking that subadult males are females, but instead the delayed plumage maturation is a true signal of subordinance. Price (1984) found support for the status signalling hypothesis in Galapagos finches (*Geospiza fortis*) in which males become darker with age and females preferentially mated with the blackest males. Older, darker males should not be aggressive towards younger, paler males because females will not mate with younger males anyway. Foster (1987) found the juvenile plumage in young adult male swallow-tailed manakins (*Chiroxiphia caudata*) may enhance young male breeding success as well as survival. She concluded that the OCC in males swallow-tailed manakins was best explained by the juvenile mimicry hypothesis in which the plumage colour development is delayed relative to sexual maturity in order to deceive older males.

Lizards also use colouration for conspecific and sexual recognition. As conspecifics usually share more resources, including mates, than heterospecifics, species-specific colour patterns could help in focusing aggression towards significant competitors. An example involves two *Anolis* lizard species with different dewlap colours; red in *A. marcanoi* and yellow or white in *A. cybotes*. Intraspecific aggression between males was stronger than interspecific

aggression, and dewlap colour was the main cue used in species recognition (Losos, 1985). Juveniles do not have the distinguishing dewlap colours and therefore probably avoid intraspecific and interspecific aggression. An iguanid lava lizard from the Galapagos (*Tropidurus delanonis*) changes from cryptically coloured hatchlings to juveniles that mimic female colouration with red throat and chest. Adult male colouration releases aggression in territorial males and female colouration attracts males (Werner, 1978). Adult male fence lizards (*Sceloporus undulatus*) have bright blue belly and throat patches and adult females have predominantly white patches. Males used colour differences to recognize the gender of conspecifics; naturally blue or blue painted individuals were responded to as males (aggressively) and naturally white or white painted individuals, as females (Cooper & Burns, 1987). Juveniles of the species have white patches (Cooper, personal communication).

Fricke (1973) used the term intraspecific camouflage to refer to cases where juvenile fish have different colour patterns from adults to mask their species membership. Emperor fish (*Pomacanthus imperator*) change from non-territorial, black and white juveniles to territorial, brightly coloured adults. Experiments showed that adults preferentially attacked models with adult colours rather than juvenile colours, regardless of size of the model (Fricke, 1980). In the absence of adult aggression, juveniles were able to settle within adult territories. Whether adults consider juveniles as a different species or as prereproductive conspecifics is not known.

Although there are few documented cases of OCC in bioluminescent animals, the fact that light often functions in their mating behaviour suggests that juveniles do not use light or use it differently. A squid (*Sthenoteuthis* sp.) develops photophores only at maturity, which is circumstantial evidence that light is used in courtship, but no experimental evidence is available to test this hypothesis (Buck, 1978). Firefly beetle (Lampyridae) adults have species-specific light flash patterns that function in mate attraction (Buck, 1978). Larval fireflies are luminescent, but in most species their light organ is not the precursor for the adult light organ, and its function is unknown (Lloyd, 1971, 1979). Different firefly species produce different colours of light, but there are no reported cases where colour changes from larva to adult.

OCC associated with change in habitat

Colour, habitat changes, and mimicry, aposematism and crypsis

As some animals grow they move from one habitat to another and if their habitat background colours change then they too may have to change colour to remain cryptic. Background matching is the most commonly argued reason for OCC, and the appendix lists examples involving molluscs, crustaceans, insects, fish, amphibians, reptiles and mammals. Rarely, however, have such hypotheses been tested; most often they are only supported by a logical argument. Changes in habitat may also change the models available for mimicry and the opportunities for and advantages of aposematism as different toxins may be available for sequestering, and different predators may be present.

Psylla peregrina (Homoptera) undergoes OCC in association with a change in habitat and background colour (Sutton, 1983). Upon sexual maturation, adults change from green to red-brown and move from the outer foliage to the inner

branches of hawthorn trees. The colour changes presumably allows then to remain cryptic (Sutton, 1983). Another example is that of seal pups which are terrestrial at birth and become mostly aquatic after the first few weeks of life. Four out of five harbour seal subspecies (*Phoca vitulina*) give birth to pups on land at the end of May, and the fifth subspecies (*P. v. largha*) gives birth to its pups on ice from February to April (Ling & Button, 1975). Pups born on ice are white until the first moult at 3 weeks of age when they start to swim. Pups born on land moult their white fur *in utero* just before birth and are born with an adult type pelage. Ling & Button (1975) argue that natal pelage colours in all five subspecies are cryptic (white on ice, darker on land), and adult type pelage is useful for swimming. Density, length and type of hair changes along with the colour, with the natal coat being thicker and insulating when dry. Atlantic grey seal pups (*Halichoerus grypus*) are also white (Ling & Button, 1975), but they are rarely born on ice (an anonymous reviewer) so the explanation may not be generalizable. Colour change patterns in phocids are summarized in the appendix.

Seasonal changes can also lead to changes in background colours, however only certain cases of colour change associated with seasonal colour change can be considered OCC as initially defined. Longer lived animals such as snowshoe hares and ptarmigan undergo reversible colour changes with each season over a period of years, and such cases are excluded from my definition of OCC as non-reversible colour change associated with normal progressive development. However, animals with lifespans of one year or less can be said to undergo OCC in order to remain cryptic against the changing background colours of the seasons. Southeast-Asian stick insect (*Sipyloidea sipylus*) nymphs are green and resemble fresh green grass, and adults are brown and resemble dead grass. (Carlberg, 1981) although the timing of the change in relation to the season is not mentioned.

Colour, habitat changes and thermoregulation

In this section and the following sections involving water balance, photoprotection, abrasion protection and metabolism, I know of fewer examples of OCC than in the preceding sections which emphasized visual functions of colour. I have used examples of other types of colour changes to help explain the concepts.

If an animal changes habitat as it develops, its thermoregulatory needs will probably also change. As seen in the harbour seal, the white coat is not only cryptic on ice, but it is also more insulating when dry than the adult pelage. Once the seals start to swim they develop a subcutaneous layer of fat that replaces the insulation of the natal coat. It is also possible that the white colour allows more radiant heat to pass through to the skin as was found in white birds (Walsberg *et al.*, 1978). Colour can have multiple functions.

Circumstantial evidence for the thermoregulatory role of colour is found in distributional data for melanic morphs of arthropod species. Positive correlations between altitude and dark morph frequencies and latitude and dark morph frequencies occur in many insects (Watt, 1968; Halkka & Mikkola, 1977; Thompson, 1984; Stewart, 1986) and in *Daphnia* spp. (Hebert & McWalter, 1983). Mason (1976) found a negative correlation between amount of dark pigment in ambush bugs (*Phymata americana*) and mean April temperature over

several years. Dispersal to higher altitude or latitude within an individual's lifetime should favour development of darker colouration and this would result in OCC.

Colour, habitat changes and water balance

Habitats may also differ in relative humidity with the humid extreme represented by aquatic habitats. Colour differences can affect rate of dehydration, amount of wetting resistance and rapidity of surface drying. Although none of the following are examples of OCC, they help to show how a change in water balance could be usefully accompanied by a change in colour. In diurnal boreal chorus frogs (*Pseudacris triseriata*), green individuals desiccate faster in sunlight than brown ones (Hoppe, 1979). Green frogs also absorb more solar radiation than brown frogs in this species and this could explain differences in hydroregulation (Hoppe, 1979). Dark mutants of *Drosophila melanogaster* lose less water and light mutants lose more water than wild type flies (Kalmus, 1941). Wax filaments that, at low humidity, produce a light blue colour in the desert beetle (*Cryptoglossa verrucosa*) also reduce water loss (Hadley, 1979). Kalmus (1941) noted that parts of insect cuticle where wetting should be avoided were frequently dark. Quinones darken insect exoskeleton and reduce its permeability to water (Needham, 1974). Dark animals are often found in humid environments and water evaporates more quickly from dark than light surfaces, keeping the animal drier (Burtt, 1981).

Colour, habitat changes and photoprotection

As with thermoregulation and hydroregulation, habitat shifts can affect the need for protection from damaging UV radiation. Water is an effective barrier to UV radiation, and UV radiation exposure varies with altitude and latitude (Hamilton, 1973). Consequently, dispersal from aquatic to terrestrial habitats or movements in altitude or latitude could select for changes in the concentration of UV radiation absorbing and reflecting pigments. The following examples illustrate photoprotective pigment functions, but are not examples of OCC. Melanin protects the nervous system from UV radiation damage by acting as a sink from free radicals that could damage cells (Kayser, 1985). Kayser & Angersbach (1974) found an increase in production of UV radiation absorbing pigments, melanin and bile pigment, in *Pieris brassicae* pupae following irradiation with UV light. As mentioned above, human skin pigmentation, which is generally darker in areas with more intense sunlight, reduces photolysis of light-sensitive nutrients (Branda & Eaton, 1978). Carotenoids also protect against photosensitized oxidation by quenching oxygen and inhibiting free radical reactions (Krinsky, 1979). Rothschild, Mummery & Farrell (1986) found that in some aposematic Lepidoptera, as carotenoids accumulate so does toxic aristolochic acid, and they hypothesized that carotenoids play a photoprotective role in protecting lepidoptera from their sequestered, protective toxins. When a toxic lepidoptera was eaten by a predator, the acidity of the predator's gut would eliminate the protective effects of the carotenoids.

Colour, habitat changes and abrasion protection

Dark insect cuticle is more resistant to mechanical stress, and parts of the cuticle subjected to stress, such as muscle attachment points, are often dark

(Kalmus, 1941). Melanin in bird feathers also resists abrasion (Burtt, 1979, 1982, 1986). If changes in risk of abrasion occur with age, then there is potential for OCC associated with selection for abrasion resistant pigments to vary although I know of no example. Comparing plumage colours in oceanic birds and Arabian desert birds, no desert birds had greater than 40% of the body surface white, although some species of oceanic birds were 100% white (Burtt, 1981). Because desert air sometimes contains large abrasive particles and oceanic air contains scattered, small particles Burtt suggested that there would be strong selection for dark, abrasion resistant plumage in desert birds and a relaxation of this pressure for oceanic birds. The effects of abrasion on feather size may be significant in loss of flight power and manoeuvrability (Barrowclough & Sibley, 1980).

OCC associated with metabolism

Colour changes and nutrient synthesis and degradation

Changes in the need for certain nutrients with age could favour OCC when pigmentation affects synthesis or degradation of those nutrients. Ultraviolet light is damaging to mammal skin, but can also be useful in calciferol (vitamin D) synthesis. In humans, dark pigmentation common at the equator inhibits excessive calciferol synthesis, which is toxic in large amounts, and lighter pigmentation at higher latitudes allows enough calciferol synthesis to prevent rickets (Loomis, 1967). As calciferol needs are highest early in life, the author suggests that this is why human skin colour is usually lighter in infants and darkens with age. Human skin pigmentation also prevents photolysis of circulating folate, and other light-sensitive nutrients which are critical for reproducing females and for children (Branda & Eaton, 1978), although these workers do not consider age effects. Pigments that provide protection from damaging radiation are discussed further in the section below on colour, habitat change and photoprotection.

Colour changes and excretion

As discussed in the section above on proximate mechanisms for colour change, some pigments serve excretory functions (Cochran, 1975). Larval protein breakdown during lepidopteran metamorphosis results in release of toxic tryptophan which is then used in ommochrome synthesis (Bückmann, Willig & Linzen, 1966). Ommochrome pigments serve to sequester tryptophan and simultaneously cause an OCC. Pteridines, which are rich in nitrogen, metabolically inert and highly insoluble, can also serve an excretory function (Kayser, 1985). Harmsen (1966) suggested that pteridine deposition in lepidopteran cuticular scales was an efficient mechanism of dry excretion of nitrogenous waste produced during imaginal development. A gradual accumulation of pteridines throughout metamorphosis would support the hypothesis that their primary function is in excretion, and synthesis of pteridines late in metamorphosis would support the hypothesis that their primary function is in colouration (Harmsen, 1966). These hypotheses have apparently not been tested.

Colour changes and pathology

Although I know of no OCC examples in which the following functions occur, routine infection or damage by a certain age could cause OCC. Quinones,

precursors to melanin, are oxidizing agents which destroy enzymes, and Hackman (1974) suggested that they may play a role in primitive, non-specific defence systems. Insects often deposit a melanin layer around endoparasites and in response to mechanical damage (Pye, 1974; Hoffmann, 1984).

Constraints and OCC

> "Speaking generally, animals change colour during development simply because at different times during development they are subjected to different selective pressure" (Hinton, 1976: 391).

In the above discussions I have focused on functions of colours and pigments as if there were usually some adaptive significance to the particular colour pattern displayed by an animal. Despite the prevailing selectionist view, expressed above by Hinton, one must remember that there are alternative hypotheses: other colours could be equally useful, a colouration pattern may have no adaptive significance whatsoever, and colouration may even be maladaptive. The colour of an animal can also be influenced by phylogenetic inertia or by genetic and developmental linkage with other traits. Even if selection influences the evolution of a colour trait, there may be conflicting pressures (i.e. sexual selection for bright colours and selection for cryptic colours to avoid predation) resulting in a compromise in the final colour.

One possible example of a maladaptation involves age-related colour changes due to sunlight fading of pterin pigments in *Anartia fatima* wing scales. These butterflies are brown with red spots and bands of yellow, cream, or white (Emmel, 1972). Taylor (1973) presented evidence that the difference between the yellow morphs and the white morphs was due to fading with age. Ironically, the males preferentially approach the white females for mating, even though the yellow females are in the best reproductive condition (Emmel, 1972; Taylor, 1973). Emmel (1973), however, subsequently criticized Taylor's methods and interpretation and gives evidence for a genetic polymorphism which generates yellow and white morphs thus calling into question the existence of an OCC.

Another case of OCC where the colour change is possibly maladaptive is in the red-spotted newt (*Notophthalmus viridescens*): the change from very toxic bright red efts to less toxic dark green adults remains unexplained (Howard & Brodie, 1973). Predators learn to avoid both efts and adults, but the colour change would seem only to confuse a predator and require that it relearn the association of distastefulness with the new colour. If the two stages have different predators, then the colour change may be appropriate to the predators' visual capabilities.

Current utility vs. *adaptation*

There is a distinction between traits that were built by natural selection for their current role (adaptations) and traits that merely have current utility (exaptations) (Gould & Vrba, 1982). If, for example, a change in pigments is originally favoured by natural selection for their biochemical properties during different life stages (adaptation), the resulting colour change may later function in intraspecific communication (exaptation or current utility). It is much easier to show evidence of current utility for a trait (Huey & Bennett, 1986) so I have emphasized examples where manipulative experiments gave evidence for current utility of an OCC. Extrapolation of the result from one species to another is

risky, however, and even extrapolation from one population to another may be problematic if current or past selective regimes differ.

Utility and cost of pigments

OCC may, in some instances, be constrained by associated metabolic costs. In defence of the idea that colour or pigments are often of significant use to an animal and are costly to maintain, is the fact that in clades in which some species have become cave dwellers, burrowers, or deep sea inhabitants those species lose pigments through evolutionary time (Hinton, 1976; Culver, 1982; Edmunds, 1987). Edmunds (1987) interprets pigment loss in these cases as an adaptation conserving energy when colour has no selective value. According to the neutral mutation hypothesis pigments are not selected for or against in dark environments, so neutral mutations affecting pigment synthesis pathways tend to accumulate (Culver, 1982). Culver (1982) found both supporting and conflicting evidence for both selection and neutral mutation hypotheses of colouration. Both hypotheses assume that light-mediated interactions are important in explaining the significance of colouration, but the neutral hypothesis does not assume that pigments are metabolically costly.

The observation that synthesis pathways for many pigments in animals are long and complex and cannot produce other products also provides indirect evidence for metabolic cost of pigments (Needham, 1974). More specifically, nitrogen-rich pteridine pigments are costly to produce in species that are nitrogen-limited in growth. Morphs of *Colias* butterflies that are white, due to decreased pteridine synthesis, are at a metabolic advantage over red, orange or yellow morphs as they eclose earlier with greater fat body reserves (Watt, 1973; Graham, Watt & Gall, 1980). The fact that animals preferentially store and chemically modify carotenoids from plants is additional evidence that pigments are not just passively accumulated (Rothschild, Mummery & Farrell, 1986).

Developmental and phylogenetic constraints

Developmental and phylogenetic constraints must also be considered as an alternative to selective explanations for the existence of traits. Unfortunately, they are often poorly defined as agents of evolution and it is difficult to separate out their effects from the effects of selection. The word constraint has become commonly used to imply restriction, but in its original use it also implied directing force (Gould, 1989). Constraints not only limit the direction that selection can take a trait, but they can also channel the change along certain pathways. Of the three ways of looking at evolution of traits (functionalism, historicism and formalism), the first two involve constraints (Gould, 1989). Functionalism attributes form to immediate utility. This is the Neo-Darwinian world view where selection determines the course of evolution. Historicism considers the importance of the form of ancestors in determining the forms possible for currently existing organisms. Selection is constrained (limited and directed) by what came before. Formalism uses abstract laws of form and defines properties of an organism as physical consequences of their immediate structure. Selection is not exempt from the laws of chemistry and physics, and the commitment to materials by ancestors determines what selection currently has to work with. Gould (1989) notes that Darwin avoided recognizing history as of

equal importance as selection by claiming that historical constraints to current selection were merely the result of past selection.

While it is generally agreed that developmental constraints can influence evolution, their relative importance compared to selection, drift and other evolutionary factors remains unknown (Maynard Smith *et al.*, 1985). Correlations among traits are expected from the mechanics of development, and covariances can be used as a measure of the strength of developmental constraints. These correlations, however, could also be acted upon by selection with a consequence being that genetic variance-covariance matrices can and do change (Maynard Smith *et al.*, 1985). While pigment development may be affected by the development of other traits, selection may act on those patterns of interaction.

Phylogenetic constraints (historicism) must be considered when searching for potential significance of a trait (Gould & Lewontin, 1979). Using methods such as those of Felsenstein (1985) and Chevrud, Dow & Leutenegger (1985), comparative studies of the correlations of morphology or colour traits with ecological and life-history traits in closely related taxa factor out phylogenetic effects, leaving any independent associations for further investigation. The technique used involves an autocorrelation model which divides the variance of a trait into phylogenetic variance, specific variance, and covariance between phylogenetic and specific values. These approaches are conservative in that they do not address how much of the phylogenetic trend is due to past and/or present selection.

DISCUSSION

OCC thus occurs under extremely diverse circumstances. In this review of published work, it is difficult to make broad generalizations about the phylogenetic distribution of OCC as we lack a random sample of all organisms that do and do not undergo OCC. It is also difficult to identify the most important or common functions of OCC due to the paucity of experimental analyses of known cases.

Adequacy of data

The examples of OCC in the appendix are not a random sample of all animals; entire phyla, such as coelenterates, annelids and echinoderms are without representation. Several factors create this bias. The vast majority of organisms are microscopic but biologists' attentions are biased towards vertebrates in disproportion to their abundance. Animals with shorter life spans that live in more accessible habitats are sometimes more likely to be studied. Some animals, such as insects, are traditionally collected only as adults, so even species identification of immatures can be difficult. By choosing to list only examples with a published account of the OCC along with a hypothesis for its significance, I have ignored many cases where OCC is known to occur but has not been studied. There are many striking examples of OCC, such as Malaysian tapirs (*Tapirus indicus*), which have yet to be formally investigated. Similarly, although many recently metamorphosed amphibians have colour patterns different from adults (Duellman & Trueb, 1986), there are few studies on

OCC in amphibians. I also suspect that many studies are not published as they failed to produce positive results; this further adds to the sample bias.

Adequacy of analyses

Of the published studies on OCC, the hypotheses considered for its significance are not a random sample of all possible functions. Perhaps because humans are visually oriented, there are more studies of visually mediated functions of colour than studies of physical or chemical functions. Logical arguments for adaptive significance of OCC based on human subjective visual perception pervade the literature. As human visual perception does not include the UV part of the spectrum (unlike various arthropods, amphibians, reptiles, and birds (Silberglied, 1979)) this method of analysis is particularly biased. Many groups, especially in the mammals, are entirely without colour vision, so analyses should focus on changes in brightness rather than changes in hue.

Of the many studies that I have chosen to include in this review, some were only marginally related to OCC with their main focus being on some other aspect of the animal's colouration or general biology. However, one study stands out as a particularly good test of a hypothesis for the significance of OCC. Wiklund & Sillén-Tullberg (1985) worked with butterflies (*Danaus plexippus* and *Papilio machaon*) that changed from aposematic larvae to cryptic pupae to aposematic adults. They designed predation experiments with captive quail to test the hypothesis that these colour changes were useful in avoiding or surviving predation attempts. All three stages had equal amounts of cardiac glycosides and were therefore equally distasteful. The pupae did not have any defensive behaviour, while adults fluttered their wings or flew away and larvae everted their bright strongly smelling osmetrium. Pupal cuticle is also not flexible and tough like that of the larvae and adults. All three stages were presented in a dish so the pupae were not cryptic as they would be in a natural setting. Larvae and pupae were attacked more often than adults and once attacked the probability of being killed was higher for pupae than for adults or larvae. Given the pupa's lack of defensive behaviour and its vulnerability to damage once attacked, Wiklund & Sillén-Tullberg (1985) argue that the best strategy would be to avoid detection while in this stage. The adults and larvae seemed to benefit from the association of their aposematic colouration and defensive behaviours with their distastefulness although their colours were not manipulated to specifically test this.

Many studies are on the function of colours at different life stages and do not specifically address the function of the change in colour. Fricke (1980) performed experiments with the territorial coral reef fish *Pomacanthus imperator*. He made models of the black and white juvenile and of the multi-coloured adult in both adult and juvenile sizes for each colour type. Territorial adults preferentially attacked adult-coloured models regardless of size. This behaviour allows juveniles to settle within adult territories and may explain the advantage to the juvenile of having its colouration. However, the function, if any, of the adult colouration is not mentioned and the disadvantage to the adults in allowing juveniles into their territories is not known.

Intraspecific communication in adult fence lizards (*Sceloporus undulatus*) was experimentally examined by Cooper & Burns (1987). The males change from

juveniles with white belly and throat patches to adults with blue patches while the females do not change colour with age (Cooper, personal communication). In this study they used unpainted males and females, males and females painted the colour of the opposite sex, and males and females painted their normal colour (to control for the effect of painting) to assess males' responses to individuals of different colours. Males responded aggressively to naturally blue or blue-painted indivduals, and did not respond aggressively to naturally white or white-painted individuals regardless of their sex. While this work clearly shows the function of the colour differences between males and females, no work has been done on the reaction of males to juveniles, and the significance of the OCC is not directly discussed.

Given the limited resources available to researchers, it is not surprising that only one or a few possible hypotheses are tested in each case. Getting positive results for the utility of a colour change does not, however, rule out other non-conflicting functions. In some cases the colour change may be a compromise between several functions and just testing for one function may give misleading negative results. I also suspect that our reliance on the adaptationist programme is responsible for the paucity of examples of neutral or maladaptive colour and colour change.

Generalizations

In spite of all of the above qualifications, I can make some generalizations about the evolutionary significance of OCC (Table 2). Background matching to avoid predation is the most commonly suggested function of OCC in invertebrates, but this hypothesis is most often supported only by logical argument based on human subjective perception. Other examples involving invertebrates suggest that OCC functions in antipredation mimicry and aposematism. From this sample it would seem that predation is the main selective force affecting colour changes in invertebrates.

Association of a change in colour with sexual maturity and use of the colour change in mate recognition is commonly found in fish, reptiles, and birds. Several other cases involving intraspecific aggression are also found in vertebrates. This implies that intraspecific communication is more important in affecting OCC in vertebrates than in invertebrates.

Mammals, not known for bright colours, can also show dramatic changes in coat colour in some orders such as primates. Mammals that change colour often do so between infancy and the juvenile stage, when they become less vulnerable, but before they actually reach maturity. Several primates change colour after infancy and some also change colour again at maturity. The dependent nature of primate infants and the relatively long period spent in a juvenile state could explain why an infant to juvenile OCC may be useful in these animals. I am investigating the patterns of single and double colour changes in the gibbons (*Hylobates* spp.) in more detail (Booth, unpublished).

Future work

Analysis of an OCC presupposes availability of information on colour, ecology and habits at all stages of an animal's life. Individuals must also be

TABLE 2. Summary of ontogenetic colour change studies listed in appendix. Numbers in table refer to species listed in the appendix. Each example can be a single species, a genus with several species or some other taxonomic group. Letter subsections are used if there is more than one hypothesis for the same colour change

	Marine invertebrates	Terrestrial invertebrates	Fish	Amphibians	Reptiles	Birds	Mammals
1. Change in size							
a. Mimicry		8,9,14,15,20,23		44	48,53,54		63
b. Crypsis	5						
c. Thermoregulation		22					
2. Change in vulnerability							
a. Mimicry			34,38,39				
b. Aposematism		11,12,13,17,18,21				55	
c. Crypsis		27,28		43a			70
d. Deflection marks					49a		
e. Intraspecific aggression			30,31,33,37,40		46a,49b	62	
f. Parental care						60	65–68,71,72
3. Change in reproductive status							
a. Avoid risks	3		29,32,41		47,51,52	56–59,61	
4. Change in habitat							
a.1. Mimicry		16					
a.2. Aposematism							
a.3. Crypsis	1,2,4,6,7	19,24,26b	35,36	42,45	46b,50		64
b. Thermoregulation							
c. Water balance							
d. Photoprotection							
e. Abrasion protection							
5. Metabolism							
a. Nutrients							69
b. Excretion		10,26a					
c. Pathology							
6. Constraints		25		43b			

followed through time to preclude confusing differential mortality of genetic colour morphs with OCC. Some cases will be easier to study due to constancy of other traits while the colour is changing. For example, hemimetabolous insect nymphs and adults may differ in colour, but they are similar in form, whereas OCC in holometabolous insects is accompanied by drastic changes in form. Changes from aquatic to terrestrial habits and morphologies by metamorphosing amphibians and some insects are other examples of confounding changes that occur along with OCC. Other difficult cases include sequentially hermaphroditic fish that change sex and colour when social circumstances dictate. Factoring out the effect of form change or sex change from the effect of colour change may be challenging. On the other hand, another change associated with OCC can sometimes help to elucidate or distinguish between possible hypotheses for its significance. In species that are sexually dimorphic at maturity, but not at birth, there is necessarily an OCC in at least one of the sexes. Hypotheses on the significance of these colour changes with maturity will logically focus on the mating status of individuals changing colour. An interesting endeavour would be to look at presence or absence of OCC in parthenogenetic species closely related to species that use OCC in sexual reproduction.

Future studies of the significance of OCC must give more serious consideration to phylogenetic and developmental constraints and must include well-designed experiments to test hypotheses of function. Physical and chemical functions of colour and pigments in addition to the more popular visually mediated functions should be explored, and neutral and maladaptive examples searched out. Until more rigorous experimental investigation of such diverse cases is undertaken, no grand synthesis of OCC will be possible. The study of ontogenetic colour change can combine the best of a tradition of natural history that goes back to Darwin with the most recent ideas in molecular genetics, development, phenotypic plasticity, selection and phylogenetic constraints. This review will have achieved its goal if it stimulates such studies and leads to the development of a stronger theoretical understanding of this widespread phenomenon.

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APPENDIX

List of studies of ontogenetic colour change categorized as involving a logical argument (LA), observational data (OD) or a manipulation experiment (MX)

Species (numbers 1–72 used in Table 2)	Ontogenetic colour change and associated changes	Hypothesized significance	Category: reference
Molluscs			
1. In general	Pelagic larva: transparent littoral adult: pigmented	Background matching	LA: Cott, 1957
2. *Aplysia parvula* and *A. punctata*	Small: pink, feed on pink-colured algae larger: green, migrate to green-coloured algae	Background matching	LA: Faulkner & Ghiselin, 1983; LA: Cott, 1957
3. *Sthenoteuthis* sp.	Juvenile: no photophores adult: bioluminescent	Bioluminescence used in mating	LA: Buck, 1978
Crustaceans			
4. Some	Pelagic larva: transparent littoral adult: pigmented	Background matching	LA: Cott, 1957
5. *Carcinus maenas*	Juvenile: polymorphic, white patterns adult: not polymorphic, dull colour	Pattern only cryptic when small	OD: Hogarth, 1978
6. *Hippa pacifica*	Megalopae: translucent, white upon reaching beach Juvenile and adult: match sand after moults	Background matching	OD: Wenner, 1972
7. *Idotea montereyensis*	Juvenile: red, feed on inshore red algae adult: green or brown, migrate out to eelgrass	Background matching	OD: Lee, 1966 & 1972
Spiders			
8. *Castianeira rica*	Instars 2,3: black 4,5: yellow/orange male: red-orange female: red-brown	Transformational mimicry of ants	OD: Reiskind, 1970
9. *Myrmarachne* spp.	Instars: mimic small ant species adult: mimic larger ant species	Transformational mimicry of ants	OD: Edmunds, 1978
M. foenisex	Instars: red/brown adult: orange	Transformational mimicry of ants	OD: Edmunds, 1978

	M. elongata	Instars: red/brown adult: black	Transformational mimicry of ants	OD: Edmunds, 1978
	M. legon	Instars: dark brown adult: black	Transformational mimicry of ants	OD: Edmunds, 1978
	M. plataleoides	Instars: dark brown adult: red	Transformational mimicry of ants	OD: Mathew, 1934
Insects				
10.	In Lepidoptera and some Hemiptera	Accumulation of pteridine pigments	Dry storage excretion	MX: Harmsen, 1966
	Orthopterans			
11.	*Astroma riojanum*	Instar 1: grey instars 2–4: yellow to black instars 5,6: brown male: tan or brown female: dark brown	Differences in palatability associated with colour and predator avoidance behaviour	MX: Schultz, 1981
12.	*Ephippiger ephippiger*	Instar 1: grey instars 2,3: green later instars: green in isolation, red/brown in pairs adult: darker	Solitary: cryptic pairs: aposematic	OD: Hartley & Bugren, 1986
13.	*Phymateus purpurascens*	Instars 1–3: black instars 4,5: pink, black, red, and green instar 6: green and red adult: dark green	Gregarious nymphs use visual cues, loss of bright colour leads to dispersion	OD: Rowell, 1967
	Mantids			
14.	*Evantissa pulchra*	Nymphs: black adult: green	Transformational mimicry of ants then leaves	OD: Mathew, 1935
15.	*Mantoida maya*	Early instars: black later instars: black and yellow	Early mimic ant later mimic wasp	OD: Jackson & Drummond, 1974
	Phasmatids			
16.	*Sipyloidea sipylus*	Nymphs: green adult: brown, pink wings, red spots on thorax	Grass mimicry	OD: Carlberg, 1981
	Homopterans			
17.	*Anthianthe foliacea*	Early instars: black, gregarious final instars: red and black, solitary adult: green, solitary	Black and red and black: aposematic green: cryptic	LA: Hinton, 1976

APPENDIX—*continued*

Species (numbers 1–72 used in Table 2)	Ontogenetic colour change and associated changes	Hypothesized significance	Category: reference
18. *Platycotis vittata*	Nymphs: red, black, and white, gregarious adult: white, blue, and orange, gregarious *older*: dark green, solitary	Group: aposematic solitary: cryptic	LA: Wood, 1976
19. *Psylla peregrina*	Immature adult: green mature adult: brown in September	Background matching	OD: Sutton, 1983
Heteropterans			
20. *Hyalymenus limbativentris* and *H. tarsatus*	Instars 1–3: black instar 4: yellow and black instar 5: yellow, brown, or black adult: black, not an ant mimic	Transformational mimicry of ants	MX: Oliveira, 1985
21. *Lygaeus equestris*	Nymphs: red and black adult: red, black, and white spot both aposematic	Birds ate fewer nymphs after tasting adults, should be more similar to gain full protection	MX: Sillén-Tullberg, Wiklund, & Järvi, 1982
22. *Nezara viridula*	Instar 1: red instar 2: black instars 3–5: some black, some green adult: green	Thermoregulation advantage for black nymphs, cryptic adults	MX: Johnson, 1985
23. *Riptortus pedestris*	Instar 1: dark brown instar 2: yellow/brown instar 3: red/yellow, dark red, or black instar 4: most black instar 5: dark brown adult: dull brown, not an ant mimic	Transformational mimicry of several ant species	OD: Mathew, 1935
Lepidopterans			
24. Several species	Larvae, pupae, and adults different colours	Background matching; mimicry of needles, twigs, and bird feces by larvae, pupae and adults cryptic	LA: Cott, 1957
25. *Anartia fatima*	Young adult: brown with yellow, cream, or white bands older adult: brown with faded white bands	Debate about polymorphism, males prefer white females, maladaptive?	MX: Emmel, 1971; critique: Taylor, 1973; rebuttal: Emmel, 1973

26a	*Cerura vinula*	Instars 1,2: black instars 3,4: black and green instar 5: brown and green spinning larva: red	Ommochrome formation to remove toxic tryptophan	MX: Bückmann, Willig, & Linzen, 1966
26b.		Pupa: brown	Background matching	LA: Bückmann, 1984
27.	*Danaus plexippus*	Larva: aposematic pupa: cryptic	Aposematic forms survive attack, cryptic pupa fragile	MX: Wiklund & Sillén-Tullberg, 1985
28.	*Papilio machaon*	Adult: aposematic		
Fish				
	Reef fishes			
29.	10 families	Juvenile: more cryptic	Species camouflage	OD: Thresher, 1984
30.	8 families	Juvenile: less cryptic	Intra-juvenile advertisement	
31.	6 families	Juvenile and adult: similar conspicuousness, different colour	Poster colour, adult habituation	
32.	*Chaetodon* spp.	Juvenile: different colour from adult, or with eyespots adult: different colour from juvenile, without eyespots	Mask juvenile species identity, juvenile live within adult territories	OD: Fricke, 1973
33.	*Cichla ocellaris*	<2 months: horizontal bar, no gold iris, no tail ocellus, with parents >2 months: vertical bars, gold iris, tail ocellus, solitary	Cannibalism inhibition while under parental care	MX: Zaret, 1977
34.	*Colossoma bidens*	Juvenile: silver with red breast, school with juvenile piranha adult: silver grey	Predator mimicry, juvenile mimic juvenile piranha	LA: Zaret, 1977
35.	*Cypsilurus furcatus*	Juvenile: tan and orange-brown, live in seaweed adult: blue-grey and silver, pelagic	Background matching	LA: Cott, 1957
36.	*Eupomacentrus* spp.	Juvenile: bright colours adult: dull colours	Juvenile cryptic on certain substrata	OD: Itzkowitz, 1977
37.	*Eupomacentrus planifrons*	Juvenile: yellow with black and blue spots adult: golden tan with large black area	Bright colour of juvenile draws attacks by adults until adults get habituated	OD: Thresher, 1978 & 1979

APPENDIX—*continued*

Species (numbers 1–72 used in Table 2)	Ontogenetic colour change and associated changes	Hypothesized significance	Category: reference
38. *Lutjanus bohar*	Juvenile: mimic planktivorous *Chromis* spp. adult: does not mimic	Aggressive mimicry	OD: Moyer, 1977
39. *Meiacanthus nigrolineatus*	Juvenile: 3 lateral dark stripes, swim with schools of cardinal fish adult: 1 dark stripe, darker background, solitary	School-orientation mimicry, juveniles mimic cardinal fish	MX: Dafni & Diamont, 1984
40. *Pomacanthus arcuatus*	Juvenile: bright colours adult: dull colours	Only juveniles are aggressive, bright colours elicit aggression	LA: Lorenz, 1962
41. *Pomacanthus imperator*	Juvenile: black and white pattern adult: brightly coloured horizontal stripes, territorial	Limit aggression against juvenile, ease into territorial system	MX: Fricke, 1980
Amphibians			
Salamanders			
42. *Aneides flavipunctatus*	Juvenile: similar throughout range, many white spots, grey-green body adult: variable by site, fewer white spots, more melanin	Adults background matching	OD: Larson, 1980
43. *Notophthalmus viridescens*	Juvenile: red, toxic adult: dark green, less toxic	Both avoided by predators, maladaptive?	LA: Howard & Brodie, 1973
44. *Pseudotriton m. montanus* and *P. m. diasticus*, *P. r. ruber*	Juvenile: red adult: brown	Juveniles mimic toxic red efts, adults larger than efts are cryptic	LA: Pough, 1974; LA: Huheey & Brandon, 1974
Frogs			
45. *Rana septentrionalis*	Juvenile: dark spots adult: larger dark spots	Cryptic on different backgrounds	LA: Kramek & Stewart, 1980
Reptiles			
Lizards			
46a. *Anolis cuvieri*	Juvenile: tan with brown adult: green or brown	Cannibalism inhibition	MX: Rand & Andrews, 1975;
46b.		Crypsis: juvenile on ground, adult in trees	LA: Gorman, 1977

47.	*Anolis marcanoi*	Juvenile: tan adult: red throat	Species recognition, male intraspecific aggression	MX: Losos, 1985
	A. cybotes	Juvenile: tan adult: yellow or white throat		
48.	*Eremias lugubris*	Juvenile: black and white, conspicuous adult: pale red-tan, blend with sand	Juveniles mimic noxious beetle	OD: Huey & Pianka, 1977
49a.	*Eumeces fasciatus* and *E. laticeps*	Hatchling: black body, blue tail adult: turn brown as mature	Distracts predator attention away from body, may inhibit attack by female adults	LA: Jopson, 1938; MX: Cooper & Vitt, 1985
49b.	*E. fasciatus*		Inhibits attack by breeding males	MX: Clark & Hall, 1970
50.	*Iguana iguana*	Juvenile: bright green, match foliage, bright black eyes, eye spots on eyelids adult: dull green, brown, or grey, match branches, no eyelid spots	Background matching, eyes and eyespots aid juveniles in aggregation	LA: Green *et al.*, 1978; LA: Burghardt, 1977
51.	*Sceloporus undulatus*	Juvenile: white patches adult female: white belly and throat patches adult male: blue belly and throat patches	Sexual recognition by males	MX: Cooper & Burns, 1987
52.	*Tropidurus delanonis*	Hatchling: white ventrum, black, grey, brown dorsum juvenile: like female young male: change from female-like colour to cryptic colour to male colour adult female: red throat and chest, brown dorsum adult male: black, blue, and yellow throat and chest, dorsum yellow-rust with black dots	Hatchlings cryptic, juveniles tolerated by females, female colour attracts males, male colour releases aggression in other males	MX: Werner, 1978
	Snakes			
53.	*Lampropeltis triangulum*	Juvenile: red, yellow, and black bands adult: solid black	Mimics toxic coral snake which also turns black with age, thermoregulation?	LA: Greene & McDiarmid, 1981
54.	*Natrix natrix*	Juvenile: grey or black body, yellow collar with black borders adult: contrast between collar and body colour fades	Predation by small birds less on models with yellow collar, mimicry of aposematic insects	MX: Madsen, 1987

APPENDIX—*continued*

Species (numbers 1–72 used in Table 2)		Ontogenetic colour change and associated changes	Hypothesized significance	Category: reference
Birds				
55.	In general	Juvenile: cryptic adult: bright	Unprofitable prey hypothesis	OD: Baker & Parker, 1979
56.	Passerines	Juvenile: cryptic subadult males: dull colour like females adult female: breeding colours dull adult male: breeding colours bright	Female mimicry Breeding threshold model Subordinate status signal by young males	OD: Rohwer, Fretwell, & Niles, 1980 OD: Studd & Robertson, 1985 OD: Lyon & Montgomerie, 1986; Montgomerie & Lyon, 1986
57.	*Chiroxiphia linearis*	Juvenile: green, over 39 months and 5 molts turns blue, black, and red male adult: blue and black with red crown	Enhance young male survivial	OD: Foster, 1987
	C. caudata	Juvenile: green, over 27 months and 3 molts turns blue, black, and orange-red male adult: blue and black with orange-red crown	Enhance young male survival and breeding success	OD: Foster, 1987
58.	*Geospiza fortis*	Juvenile: brown adult females: brown adult males: progressively blacker with age	Females prefer black males, may be cost to adult plumage	OD: Price, 1984
59.	*Icterus galbula*	1st year males: intermediate between females and mature males mature males: black and orange	Female-mimicry, less aggression by mature males	MX: Flood, 1984
60.	*Larus atricilla*	Chicks: single colour beak adult: red spot on beak	Directs food begging pecks of chicks	MX: Hailman, 1967
61.	*Septophaga ruticilla*	1st year males: dull mature males: orange and black	Predation less on dull males	OD: Proctor-Gray & Holmes, 1981; critique: Rohwer, 1983
62.	*Spheniscus demersus*	Juvenile: grey-brown heads, white under adult: black and white pattern	Adults aggressive towards juveniles, exclude from cooperative feeding groups	OD: Ryan, Wilson, & Cooper, 1987

Mammals				
	Carnivores			
63.	*Acinonyx jubatus*	<2.5 months: silver mantle, black underparts >2.5 months: tan with black spots	Juveniles mimic ratel *Mellivora capensis*	LA: Eaton, 1976
	Pinnipeds			
64.	Most Northern phocids	Born: white 1st moult: silver-grey	White cryptic on ice, most ice breeders born white, first coat for insulation, second coat for swimming	LA: Ling & Button, 1975
	Most Southern phocids	Born: grey or brown 1st moult: silver-grey		
	Primates			
65.	In general	Distinctive infantile colouration	Elicits attention, protection, tolerance, and caregiving	OD: Alley, 1980 LA: Wickler, 1972
66.	Several Colobinae	Natal coat contrasts with adult coat	Natal coat attracts conspecifics for care	OD: Hrdy, 1976, 1977
67.	*Cercopithecus aethiops*	Infant: brown or black adult: grey with white beard and orange brow	Natal coat stimulates protection by adults	OD: Booth, 1962; OD: Gartlan, 1969; OD: Struhsaker, 1971
68.	*Colobus guereza* and *C. polykomos*	Infant: white adult: black and white	Colour change signals infant independence	OD: Horwich & Manski, 1975
69.	*Homo sapiens*	Juvenile: pale adult: darker	Calciferol synthesis	LA: Loomis, 1967
70.	*Hylobates hoolock*	Infant: grey/white juvenile: black adult male: black adult female: yellow/brown	Infant camouflaged against mother	LA: McCann, 1933
71.	*Papio anubis*	Infant: black with pink face juvenile and adult: brown	Males use infants in agonistic buffering	OD: Strum, 1984
72.	*Presbytis entellus*	Infant: dark brown adult: white	Adult females interested in brown infants	OD: Jay, 1962
	P. johnii	Infant: red-brown hair, pink skin adult: black hair and skin	Natal coat releases material behaviour	OD: Poirier, 1968

Genetics of Pigmentation in Dogs and Cats

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Abstract

Color variation in companion animals has long been of interest to the breeding and scientific communities. Simple traits, like black versus brown or yellow versus black, have helped to explain principles of transmission genetics and continue to serve as models for studying gene action and interaction. We present a molecular genetic review of pigmentary variation in dogs and cats using a nomenclature and logical framework established by early leaders in the field. For most loci in which molecular variants have been identified (nine in dogs and seven in cats), homologous mutations exist in laboratory mice and/or humans. Exceptions include the *K* locus in dogs and the *Tabby* locus in cats, which give rise to alternating stripes or marks of different color, and which illustrate the continued potential of coat color genetics to provide insight into areas that transcend pigment cell biology.

INTRODUCTION

History of Mammalian Coat Color Genetics

Because of its utility as a model organism, the laboratory mouse is often used as both a starting and an ending point to study Mendelian variation in cats, dogs, and other domestic animals. Molecular identification of cat and dog color mutants often begins with candidate genes nominated on the basis of their effects in mice; conversely, specific hypotheses about gene action in domestic animals are often evaluated in transgenic or knockout mice. Even in 1917, Sewall Wright (1, p. 373) wrote that "most experimental work has been carried on with four rodents—the mouse, rat, rabbit, and guinea-pig, and the factors have been determined with very much more certainty than in the larger animals," but that "[d]ogs and cats are even richer in color varieties than the rodents, but the factors are none too well understood."

Wright's summary and analyses of coat color genetics in dogs (2) and cats (3) drew on work in dogs from Barrows & Phillips (4) and C.C. Little (5) and in cats from P.W. Whiting (6, 7). Classical references on dog color genetics include books by C.C. Little (8) and Øjvind Winge (9); Roy Robinson (10) carried out analogous work on the cat, and Tony Searle (11) authored an insightful and prescient comparative analysis. These works are classical in the sense that, for the most part, they predate molecular biologic analyses but are still used as reference works by many breeders and fanciers.

We first briefly review the history of mammalian coat color genetics as well as dog and cat domestication. We then summarize current information about dog and cat coat color, organized by categories of phenotypes (and associated biologic mechanisms). Homologous systems exist in dogs and cats for genes that affect pigment cell development and survival, generalized dilution, and pigment-type switching, and there are several examples in which mutations in orthologous genes give rise to similar phenotypes, e.g., the chocolate phenotype. For phenotypes that involve alternating stripes or marks of color, however, there is no obvious homology between dogs and cats, and we consider two different categories separately: irregular alternating stripes, as seen in brindled dogs or tortoiseshell cats, and periodic alternating marks, as seen in tabby or spotted cats.

Throughout the review, and as in our recent work summarizing dog coat color and texture (12), we use the term "locus" to refer to a phenotypic trait that segregates in a Mendelian fashion; "gene" to refer to a fragment of coding DNA associated with and/or responsible for a specific trait; and "allele" to refer to the different variants that are observed, sometimes at a phenotypic level (where the gene has not yet been identified, e.g., *Ticking* in dogs or *Silver* in cats) and often at a molecular level (where the gene has been identified, e.g., *Spotting* in dogs or *Blotched* in cats). Although there are many similarities between dog and cat color genetics, development of the fields has proceeded, for the most part, independently; consequently, the terminology can be confusing, e.g., *White spotting* in cats involves a different gene than *Spotting* in dogs, and *Ticking* in dogs means something very different from *Ticked* in cats.

At present, an established and widely accepted nomenclature for gene names and symbols in domestic dogs and cats does not exist; some genes are named historically according to the scientists who first described them, some genes are named according to guidelines for humans or model organisms, and some terms (genes, alleles, and phenotypes) are used commonly by breeders and fanciers. In the laboratory mouse, most genes known as previously existing coat color mutations are renamed after molecular identification, e.g., *albino* became *Tyrosinase*, *Extension* became *Melanocortin 1 receptor*, and *White spotting* became *Kit*. For this review, we use a modified version of the historical nomenclature, in part to make the review more accessible to breeders, and in part because some loci do not yet have molecular identities. In general, and in accord with most

systems for genetic nomenclature, we use italicized text to refer to loci or alleles and roman text to refer to phenotypes. In addition, we add a superscript plus symbol ($^+$) to alleles to indicate the ancestral or wild-type version, which can be especially relevant for domestic animals when considering molecular mechanisms that underlie selected traits. Finally, in accord with the International Society for Animal Genetics recommendation that nomenclature for domestic animals be consistent with that in humans, we use all capital letters for dog and cat gene symbols when their molecular identity has been established. We have also authored a recent book chapter on dog coat color and texture (12); in some cases, relevant portions of that work are included here. Summaries of color loci, genes, and alleles in dogs and cats are provided in **Tables 1** and **2**, respectively.

Population Histories for Dogs and Cats

Domesticates of dogs and cats were derived from relatively monomorphic species, the gray wolf (*Canis lupus*) and the African wild cat (*Felis silvestris lybica*), respectively. Like other domestic species, their origins trace to the Fertile Crescent region of East Asia; however, dogs were among the first species to undergo domestication (between 15,000 and 40,000 years ago), whereas cats were much later (between 4,000 and 11,000 years ago). Their early population histories also are very different. Dogs exhibit a pattern of molecular diversity that suggests an early bottleneck (13), likely owing to strong directional selection for specialized tasks (14). By contrast, domestic cats show no reduction of genetic diversity compared with their wild ancestors (15, 16), which suggests weak selection over an extended period of time: As Driscoll et al. (17) have suggested, dogs were selected, but cats were tolerated.

More recent population histories of dogs and cats—breed establishment and maintenance—are also different. With the spread of agrarian societies, both species followed similar trajectories, rapidly dispersing and expanding to reach population sizes advantageous for diversifying selection. In dogs, these populations eventually served as the foundation for modern breeds, as many as a thousand of which have been described worldwide (18). Most dog breeds were established in Eurasia within the past few hundred years and subsequently maintained as closed breeding lines under strong selection for desired traits, which resulted in a radiation of morphological and behavioral diversity (19). For example, the Newfoundland breed acquired a thick, water-resistant double coat and webbed feet adapted for aiding fishermen in the icy waters off the Newfoundland coast (18). Similarly, Dachshunds (whose breed name means badger dog in German) developed olfactory machinery honed for hunting, along with short legs and an elongated body to aid in pursuing badgers in underground burrows (19). Because of the strong population bottlenecks associated with dog-breed formation, the association of neighboring SNPs tends to be strong; long haplotype blocks [0.5–1 megabase (Mb)] are apparent within breeds, and large allele frequency differences (i.e., genetic structure) are apparent across breeds (13, 14). Today, most dogs in Western societies are associated with a breed or represent recent outcrosses.

By contrast, 98% of the estimated 700 million domestic cats worldwide are not affiliated with a breed (16). Breed establishment in cats was motivated more by preservation of appealing form, most prominently coat color and pattern traits, rather than utilitarian trait development. A few breeds, such as the Siamese and the Egyptian Mau, date their origins to the eighteenth century or earlier, but most of the currently recognized breeds were established in the past hundred years (20). Moreover, unlike dog breeds, cat-breed barriers are often relaxed, with outcrosses permissible between some breeds. As a consequence, the genetic diversity within cat breeds is higher than that observed in dog breeds, and the genetic structure of cat breeds is substantially lower than that of dogs.

Thus, selective pressures on dogs and cats probably differed over the course of domestication, with dogs, but not cats, under strong artificial selection for traits that are, or were, of practical

Table 1 Mendelian coat color loci in dogs

Locus/Category	Gene	Allele[a]	Phenotype	References[b]
Spotting				
S (Spotting)	*MITF*			*26*, *27*, *28*
		S^+	Solid coat (no spotting, wild type)	
		s^i	Irish spotting pattern	
		s^p	Piebald spotting pattern	
		s^w	Extreme white spotting	
T (Ticking)	?			
		T	Ticking in white areas	
		t^+	No ticking (probably wild type)	
M (Merle)	*PMEL*			*41*, *49*
		M	Merle pattern	
		m^+	Nonmerle (wild type)	
Dilution				
B (Brown)	*TYRP1*			*63*
		B^+	No dilution of eumelanin (black, wild type)	
		b^s, b^d, b^c	Diluted eumelanin (liver, brown, chocolate)	
C (Albino)	?			
		C^+	No dilution of pheomelanin (yellow, sable, fawn, wild type)	
		c^{ch}	Dilution of pheomelanin (white, cream)	
D (Dilute)	*MLPH*			*75*, *76*
		D^+	No dilution of eumelanin (black, wild type)	
		d	Dilution of eumelanin (silver, blue)	
G (Graying)	?			
		G	Graying of eumelanin with age	
		g^+	No graying	
H (Harlequin)	*PSMB7*			*55*
		H	Harlequin pattern (in a merle background)	
		h^+	Merle pattern (in a merle background)	

(*Continued*)

Table 1 (*Continued*)

Locus/Category	Gene	Allele[a]	Phenotype	References[b]
Tw (Tweed)	?			
		Tw^T	Large, smooth patches (in a merle background)	
		tw^+	Small, jagged patched (in a merle background, probably wild type)	
Pigment-type switching				
A (Agouti)	*ASIP*			*94, 95, 96*
		A^y	Yellow, sable, fawn	
		a^w	Agouti-banded hair, light-colored ventrum	
		a^t	Black-and-tan	
		a	Recessive black	
K (Kurokami)	*CBD103*			*82, 83*
		K^B	Black	
		k^{br}	Brindle (black and yellow stripes)	
		k^+ (or k^y)	wild type (allows expression of Agouti phenotypes)	
E (Extension)	*MC1R*			*99, 102, 103, 105*
		E^m	Melanistic mask	
		E^+	Extension, wild type	
		e	Recessive yellow	

[a]Alleles for each locus are listed in order of dominance. In situations where questions remain about the molecular nature of specific alleles (*e.g.*, grizzle, saddle-tan), the relevant references but not the allele names are included.
[b]Primary references for molecular characterization of the indicated loci.

utility. However, because both were kept as companion animals, there likely were similar selective pressures for cosmetic traits. These considerations help to explain why cats exhibit a similar, even greater degree of diversity than dogs with regard to color and color patterns, whereas dogs exhibit a greater degree of diversity than cats with respect to behavior and morphology.

As in other organisms, color variants are low-hanging fruit for cat and dog geneticists. Early leaders in the field have previously determined many allelic and epistatic relationships, and, for most loci, orthologs have been previously identified and characterized in the mouse. Thus, with few exceptions (four loci in dogs and one in cats), molecular identification of dog and cat pigmentation genes has been accomplished using candidate approaches rather than genome-wide mapping. On the one hand, this provides strong evidence that pigmentary mechanisms and pathways are strongly conserved across mammals; on the other, it highlights the potential of genome-wide approaches to identify cat and dog color traits that have no obvious homology with traits in other model organisms and therefore promise potential new insight and novelty.

Table 2 Mendelian coat color loci in cats

Locus/Category	Gene	Allele[a]	Phenotype	References[b]
Spotting				
W (White)	*KIT* (?)			*36*
		W^T	White coat	
		w^b	High degree of spotting	
		w^l	Low degree of spotting	
		w^+	Solid (wild type)	
Dilution				
B (Brown)	*TYRP1*			*60, 61*
		B^+	No dilution of eumelanin (black, wild type)	
		b	chocolate	
		b^1	cinnamon	
C (Albino)	*TYR*			*60, 65, 69*
		C^+	No dilution of melanin (yellow, sable, fawn)	
		c^b	Burmese (temperature-sensitive dilution pattern, pointed coat)	
		c^s	Siamese (temperature-sensitive dilution pattern, extreme pointed coat)	
		c	Albino	
D (Dilute)	*MLPH*			*77*
		D^+	No dilution of eumelanin (black)	
		d	Dilution of eumelanin (silver, blue)	
I (Inhibitor)	?			*81*
		I	Progressive dilution of pigmentation along hair shaft (silver, smoke)	
		i^+	No dilution (wild type)	
Pigment-type switching				
A (Agouti)	*ASIP*			*100*
		A^+	Agouti-banded hair (wild type)	
		a	Recessive black	

(*Continued*)

Table 2 (*Continued*)

Locus/Category	Gene	Allele[a]	Phenotype	References[b]
E (Extension)	*MC1R*			*104*
		E	Extension (wild type)	
		e	Amber, age-dependent fading of tabby pattern	
O (Orange)	?			*89, 90*
		O	Orange coat	
		o^+	Nonorange, color determined by hypostatic loci	
Pattern				
Ti (Ticked)	?	Ti^A	Absence of tabby pattern	*109, 111*
		Ti^+	Expression of tabby pattern	
Ta (Tabby)	*TAQPEP*	Ta^M or Ta^+	Mackerel tabby pattern (vertical stripes, wild type)	*109, 112*
		Ta^b	Blotched (or classic) tabby pattern (whorls)	

[a]Alleles for each locus are listed in order of dominance.
[b]Primary references for molecular characterization of the indicated loci. For two loci (*I* and *O*) we include references for linkage, although the causative molecular lesions and genes are not yet known.

PIGMENT CELL DEVELOPMENT AND SURVIVAL: *SPOTTING, TICKING, ROAN, AND MERLE*

White spotting is exceedingly common in dogs and cats. As discussed below, the genetics of white spotting is more complex than other color traits but, nevertheless, is influenced predominantly by a single major locus. In contrast, 25 white-spotting loci have been identified in mice, and ten white-spotting genes have been cloned (21).

An understanding of melanocyte development provides a context for appreciating the pathophysiology of white spotting. During embryogenesis, melanocyte precursor cells, melanoblasts, derive from the neural crest and migrate to the epidermis and hair follicles in the skin (as well as to parts of the eye and inner ear). Once there, they proliferate and differentiate into pigment-producing melanocytes. A second type of pigment cell—that found in the retinal pigment epithelium (22)—expresses many of the same melanogenic enzymes and proteins as the melanocyte but arises directly from neurectoderm rather than from the neural crest, and it has a shape and physiology very different from those of the melanocyte. White spotting on the coat usually indicates an absence of melanocytes caused by a failure of melanoblast migration, proliferation, or survival during development (23).

"The curious association of deafness with blue-white color" was described in cats by Wright (3) and by Darwin and in dogs by Pearson, Nettleship & Usher (24). In mice, white spotting is often associated with other abnormalities, including intestinal problems, craniofacial malformations, deafness, or blood and germ cell defects. These pleiotropic aspects of white spotting reflect gene action that affects either a shared cell lineage (in the case of white spotting, deafness, and intestinal and craniofacial malformations, which trace back to a common neural crest precursor cell) or

a common set of signaling pathways used by different cell lineages (in the case of blood and germ cell defects) (21). The different range of associated pleiotropy in mice and companion animals likely reflects contrasting motivations for selection; mutations in dogs and cats will have been selected and maintained by breeders only if they have little or no effect on overall health and fitness. By contrast, laboratory mice are a well-established and rich resource for collecting and studying mutations that affect a variety of disease processes.

Spotting ($S^+ > s^i > s^p > s^w$) in Dogs

In his book on dog coat color genetics, Little (8) described four alleles for the dog *Spotting* locus—*Solid* (S^+), *Irish spotting* (s^i), *Piebald spotting* (s^p), and *Extreme white spotting* (s^w)—which differ based on the degree of pigmented body surface. In some breeds, s^w/s^w animals are completely or almost completely white, e.g., the Boxer, Bull Terrier, or Greyhound, whereas in others, s^w/s^w animals may have residual pigmentation that overlaps with the piebald spotting phenotype (e.g., the Italian Greyhound or Great Pyrenees). Phenotypic heterogeneity of spotting within breeds that are fixed for a specific *Spotting* allele points to both modifier loci and stochastic effects.

Irish spotting refers to the presence of white markings on the face, legs, and ventrum, which often extend to form a white collar around the neck. Dogs with an Irish spotting phenotype may be homozygous for s^i or compound heterozygotes for the presumptive wild-type allele S^+ and a more severe *Spotting* allele (i.e., S^+/s^p and S^+/s^w). This difference in the genetic basis has a practical implication: The desired Irish pattern is fixed in certain breeds, such as the Basenji (s^i/s^i), but is maintained by balancing selection (and therefore not fixed) in others, such as the Boxer (S^+/s^w) (25, 26). Italian Greyhound siblings exhibiting an Irish pattern and extreme white spotting with residual pigmentation are depicted in **Figures 1*a*** and **1*b***, respectively.

Microphthalmia-associated transcription factor (*MITF*) was identified as the gene responsible for white spotting in dogs by genome-wide association (27) and heralded the application of similar approaches to a variety of nonpigmentary traits. Dog population history—punctuated by an initial bottleneck that occurred at least 15,000 years ago (at domestication) and a second series of bottlenecks that occurred in the past few hundred years (at breed formation)—enables an efficient and powerful strategy for association-based gene mapping. Comparing 10 white (s^w/s^w) with 9 solid (S^+/S^+) Boxers, Karlsson et al. (27) localized the gene to an ~1-Mb interval. Analysis of the same interval in Bull Terriers allowed fine mapping, which localized the putative molecular lesion(s) to a small area close to the transcriptional initiation site of *MITF*. In addition, using a candidate approach, Rothschild et al. (28) showed that *MITF* cosegregated with white spotting in the Newfoundland, the Schipperke, and the Beagle.

Mitf is a basic helix-loop-helix transcription factor involved in the development of several cell types, including mast cells, osteoclasts, the retinal pigment epithelium, and melanocytes. In melanocytes and the retinal pigment epithelium, Mitf activates the expression of many melanogenic enzymes and proteins (29, 30). In laboratory mice, alleles that disrupt the Mitf protein usually affect both melanocytes and the retinal pigment epithelium; the latter cell type is important for proper eye development, which is why many *Mitf* alleles in the mouse cause microphthalmia in addition to white spotting. Because *Mitf* is expressed in many different cell types, it makes use of several alternative promoters and transcriptional initiation sites (31, 32), including a so-called melanocyte-specific promoter, *Mitf-M*.

In the work referred to above (27), DNA sequencing revealed dozens of distinct molecular alterations in noncoding regions of s^w but not S^+ chromosomes, including a short interspersed nuclear element (SINE) element insertion ~3 kb upstream of the *MITF-M* promoter as well as a polymorphic homopolymer tract ~100 bp upstream of the *MITF-M* promoter that is longer in

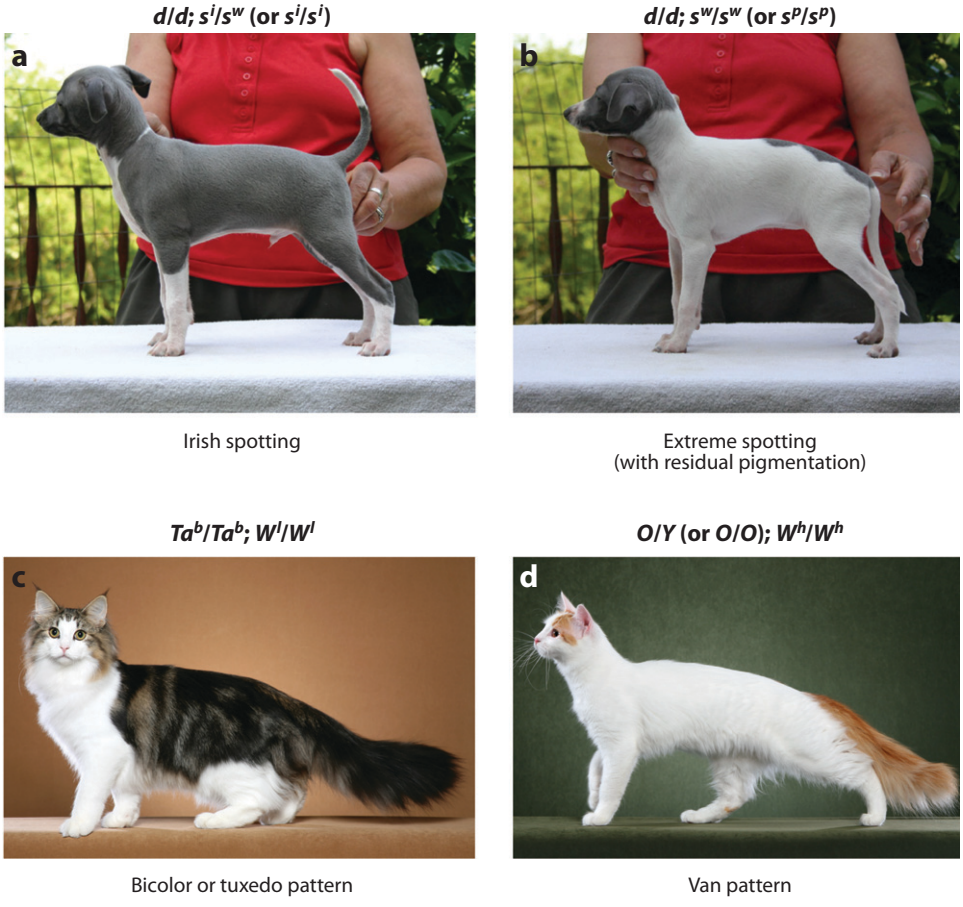

Figure 1

Different genes in dogs and cats cause similar types of spotting. (*a*,*b*) In these Italian Greyhound puppies, one sibling has Irish spotting, and the other has extreme spotting with residual pigmentation. The animal in (*b*) might also be described as an extreme version of piebald spotting (s^p/s^p). The spots represent areas in which melanocyte survival has been impaired owing to reduced activity of *Mitf*. Both puppies must also be heterozygous or homozygous for the K^B allele, because pigmented areas are blue (diluted eumelanin) rather than pale fawn (diluted pheomelanin). (*c*,*d*) In cats, similar differences in white spotting appear to be caused by the *Kit* gene (*W*), although specific molecular lesions have not yet been identified. In cats, *W* alleles associated with more white areas are dominant to those associated with less white areas. Thus, these animals may not necessarily be homozygous at *W* (e.g., the genotype W^h/W^h could be W^h/W^l or W^h/w^+). In (*c*), the pigmented area exhibits a blotched tabby pattern; therefore, the genotype must be *A*/*A* (or *A*/*a*); Ta^b/Ta^b. In (*d*), the pigmented area is orange; therefore, the genotype must be *O*/*Y* or *O*/*O*. Parts (*a*) and (*b*) are adapted from (12).

s^w- than in *S*-bearing chromosomes. The situation with other *Spotting* alleles was confusing: Breeds fixed for piebald spotting (s^p/s^p) carry the s^w-associated SINE element insertion, whereas breeds fixed for Irish spotting (s^i/s^i) do not. However, all three spotting alleles (s^i, s^p, and s^w) carry a homopolymer tract that is longer than the version found in solid (*S*/*S*) dogs. At present, the data suggest that (*a*) a series of regulatory mutations occurred sequentially on a single *MITF* haplotype to generate increasingly severe spotting phenotypes, and (*b*) the underlying mechanism involves reduced expression of *MITF* in developing melanoblasts and/or melanocytes, which places those cells close to a threshold below which they cannot survive.

Dogs with extensive white spotting exhibit an increased risk of deafness because melanocytes in the skin are closely related to those in the stria vascularis of the inner ear; the latter are required for normal function of the cochlea (33). Thus, diminished expression of *MITF* impairs the survival of pigment cells in both the skin and the inner ear. The association between white spotting and deafness extends across most if not all breeds; in general, the risk of deafness is related to the extent of spotting and ranges from ~2% to ~20% in dogs with extensive white spotting (34).

White Spotting ($W^T > W^h > W^l > w^+$) in Cats

In many ways, white spotting in the domestic cat is similar to that in dogs; there is a broad phenotypic range, animals with extensive depigmentation exhibit an increased risk of deafness, and spotting preferentially affects the ventral trunk but spares the head and tail. Historical breeding studies (7, 35) described four phenotypic classes—total depigmentation, high degree of spotting, low degree of spotting, and a fully pigmented coat—that were interpreted in terms of a single locus with four alleles, e.g., W^T, W^h, W^l, and w^+ (the wild-type allele with no spotting). More recently, dominant inheritance of a completely depigmented coat was considered potentially epistatic rather than allelic to partial white spotting (10), which has led many breeders and fanciers to adopt the symbols *W*, for *dominant white*, and *S*, for *white spotting*. Distinguishing epistatic from allelic relationships based solely on breeding studies can be challenging; furthermore, blue eyes are often seen in completely depigmented cats but are observed rarely in spotted cats. Breeders and fanciers also use the terms bicolor (also known as tuxedo) and Van pattern: The former (**Figure 1*c***) refers to restricted spotting that occurs mainly in the ventrum; the latter is a pattern of extensive depigmentation that spares the head and tail, and it is named after a breed of cat from the Lake Van region of Turkey that frequently displays this phenotype. Here we use the *W*- series nomenclature to describe white spotting in cats, in part because available mapping data point to a different gene from that responsible for *Spotting* in dogs (see below).

In contrast to dogs, white spotting (including complete depigmentation with blue eyes) in cats is dominant to the absence of spotting. Available genetic mapping data also point to a different genetic locus than spotting in dogs. Cooper et al. (36) demonstrated linkage between white spotting and a microsatellite marker near the *KIT-PDGFRA* region on cat chromosome B1. *Kit* encodes a receptor tyrosine kinase that facilitates melanoblast and melanocyte survival and/or migration during development. *Kit* is an especially frequent target for dominant white-spotting mutations in the mouse, with more than 100 different alleles, many of which also impair the survival of blood cells and germ cells. *KIT* is also a good candidate to explain the ocular phenotype in blue-eyed, white cats, which is associated with reduced pigmentation in the iris, the choroid, and the tapetum lucidum (a cell layer behind the retina) but spares the retinal pigment epithelium itself and is generally not associated with defects in visual function (37).

If mutations in *KIT* do cause white spotting in cats, one potential explanation for the absence of defects in blood and germ cells (unlike *Kit* protein–coding mutations in mice) is that the underlying molecular abnormalities in cats are regulatory and alter expression of *KIT* only in melanocytes, analogous to the likely situation for *MITF* and white spotting in dogs. If so, the difference in dominance hierarchies between dog and cat white spotting could reflect different phenotype thresholds (i.e., pigment cells are more sensitive to a reduction in *KIT* expression than in *MITF* expression). Indeed, regulatory alterations that affect *KIT* expression also underlie coat color phenotypes in cattle (38), horses (39), and pigs (40). Resolving the molecular nature of white spotting in cats, and determining whether dominant white is allelic or epistatic to white spotting, should be possible with additional molecular genetic analysis.

Ticking ($T > t^+$) and *Roan* ($R > r^+$) in Dogs

Ticking and roan are dominant modifiers of white spotting in dogs. Ticking refers to small spots or "ticks" of pigmented hair that emerge in regions that would otherwise be white, whereas roan refers to a uniform mixture of white and pigmented hairs in white regions. Ticking and roan may appear anywhere but are most frequently observed on the muzzle and forelimbs, and they are found in hounds, pointers, spaniels, setters, and Dalmatians. Although ticking and roan are distinct phenotypes, their potential allelic relationships are not clear.

Ticking and roan do not manifest until three to four weeks of age (8) and may represent a second wave of melanocyte precursor differentiation, proliferation, or colonization of hair follicles. The underlying genetics of ticking is especially interesting because corresponding traits have not been recognized in cats or in mice. However, the terminology is confusing because, in other animals, ticking is also used to describe bands or ticks of pheomelanic pigment on individual hairs, better known as the Agouti phenotype (see below). Little suggested that the distinctive spotting pattern of Dalmatians represents homozygosity for *T* and s^w (8). However, several breeds, including Cocker Spaniels, segregate the ticking phenotype (19), which provides the opportunity to map the ticking locus using genome-wide association. Because white spotting is itself a derivative trait, ticking and roan likely represent derivative traits selected on a white spotting background, with the recessive alleles representing the ancestral or wild-type variant.

Merle ($M > m^+$) in Dogs

Merle, which is referred to as dapple in some breeds, is a pattern of irregularly shaped areas of diluted pigmentation (**Figure 2**). The mutation is semidominant: Animals homozygous for the presumptive ancestral or wild-type allele, m^+, are normally pigmented; M/m^+ animals have mild to moderate dilution in some areas of the coat; and *M/M* (so-called double merle) animals are mainly

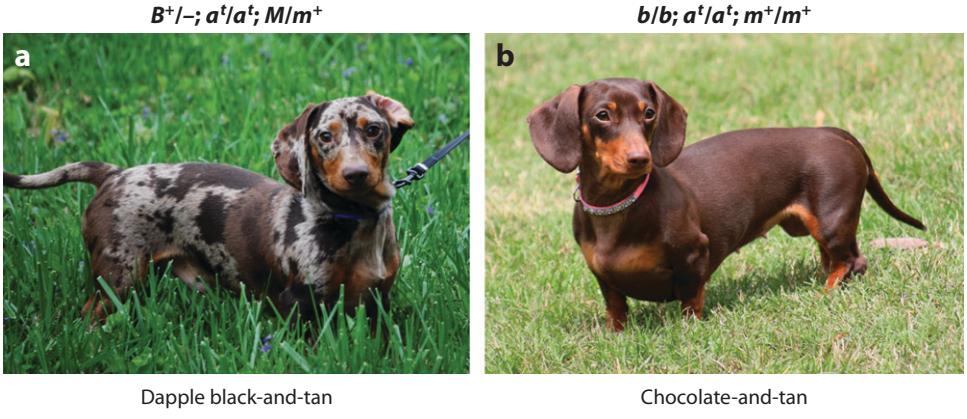

Figure 2

Both Dachshunds have a tan point pattern owing to a genotype of a^t/a^t (or a^t/a). The a^t allele restricts *ASIP* expression (and production of tan pheomelanin) to an area above the eyes, on the sides of the muzzle, on the chest, and on the legs and feet. In (*a*), the merle pattern (referred to as dapple in the Dachshund) is caused by a mutation in *PMEL*, which dilutes black eumelanin to brown and grey patches. In (*b*), recessive loss-of-function alleles of *TYRP1* dilute black eumelanin to brown (or chocolate). Because both *PMEL* and *TYRP1* function in eumelanogenesis but not in pheomelanogenesis, the mutations do not dilute tan pheomelanin areas. Adapted from (12).

white. Characteristically, small patches of normal color appear within areas of diluted pigmentation in both *M*/*M* and *M*/m^+ dogs. In addition, *M*/*M* animals occasionally exhibit deafness and ocular problems (microphthalmia, abnormal irises, and/or blindness); consequently, most guidelines recommend against interbreeding *M*/m^+ dogs, and the phenotype is not fixed in any breed. Melanin in dogs, like in other mammals, is of two basic types: eumelanin (which usually appears brown or black) and pheomelanin (which usually appears red, yellow, or cream-colored). Cell physiology and genetic control of pigment-type synthesis are described further below, but it should be noted that eumelanic areas are primarily affected in merle dogs, as is apparent in a dapple (or merle) Dachshund compared with a nondapple chocolate-and-tan Dachshund (**Figure 2**).

Using genome-wide association mapping of a Shetland Sheepdog cohort and subsequent screening of candidates, Clark et al. (41) discovered that a molecular alteration in *Silver* (*Silv*), first identified in the laboratory mouse and now referred to as *Pmel*, underlies the merle phenotype. Linkage studies from Hédan et al. (42) suggested the same locus was responsible for the merle phenotype in multiple breeds. *Pmel* encodes a melanocyte-specific transmembrane glycoprotein whose intramelanosomal domain is cleaved and localizes to the matrix of eumelanosomes, where it forms fibrillar amyloid structures that serve as substrates for precipitation and deposition of melanin (43, 44). Mutations of *Pmel* cause pigmentation phenotypes in other species, including silvering in mice (45) and horses (46) and a series of plumage phenotypes in chickens (47). Pmel is localized primarily to eumelanosomes, which helps to explain why diluted areas in merle dogs tend to spare pheomelanic areas.

One of the most striking aspects of the *M* allele is that it is genetically unstable. Matings of mutant *M*/*M* to wild-type m^+/m^+ dogs produce true-breeding nonmerle offspring at a rate of 3–4% (48), a hallmark of so-called germ line reversion, or a reverse mutation of *M* to *m* (discussed further below). This observation suggests that the molecular alteration responsible for the *M* mutation is itself unstable and can revert both in germ cells (as above) and in somatic cells, giving rise to the normal patches of color within areas of diluted pigmentation. In all dogs carrying *M*, a small insertion in the *PMEL* gene was observed for which an internal A_n tract exhibited a shortened length in putative *M* to *m* revertants (49). The insertion itself is a SINE mobile genetic element and is associated with production of abnormal cDNAs predicted to give rise to aberrant protein products. Clark et al. (41) suggest that reversion is not caused by excision of the SINE (which happens rarely, if at all) and instead is caused by shortening of the A_n tract owing to errors in DNA replication during cell division. According to this suggestion, there are three groups of alleles: the ancestral allele that lacks the SINE insertion (referred to as m^+); the derivative allele with the SINE insertion that disrupts *PMEL* function (referred to as *M*), for which the A_n tract is 91–101 nucleotides long; and a revertant allele (referred to here as m^*), which carries the SINE insertion with a shorter A_n tract of 54–65 nucleotides (49). An important implication of this idea is that both the *M* and the m^* alleles would exhibit instability, the former for tract shortening to a normal phenotype and the latter for tract expansion to an abnormal phenotype, in which case the phenomenon of *M* reversion might more accurately be referred to as pseudoreversion. Indeed, the idea of an unstable m^* allele likely underlies what has been described as a cryptic or phantom merle, in which a dog with little or no pigmentary abnormalities produces typical merle offspring.

The previous discussion also provides a hypothesis for considering merle-associated phenotypes from a cellular and developmental perspective (**Figure 3**). Microphthalmia and deafness are a hallmark for death of retinal pigment cells and melanocytes in the inner ear, respectively; thus, the *M* mutation likely increases the risk of *Spotting*-associated deafness, and vice versa. By contrast, *white spotting* is unlikely to modify the risk of *M*-associated microphthalmia, because even though

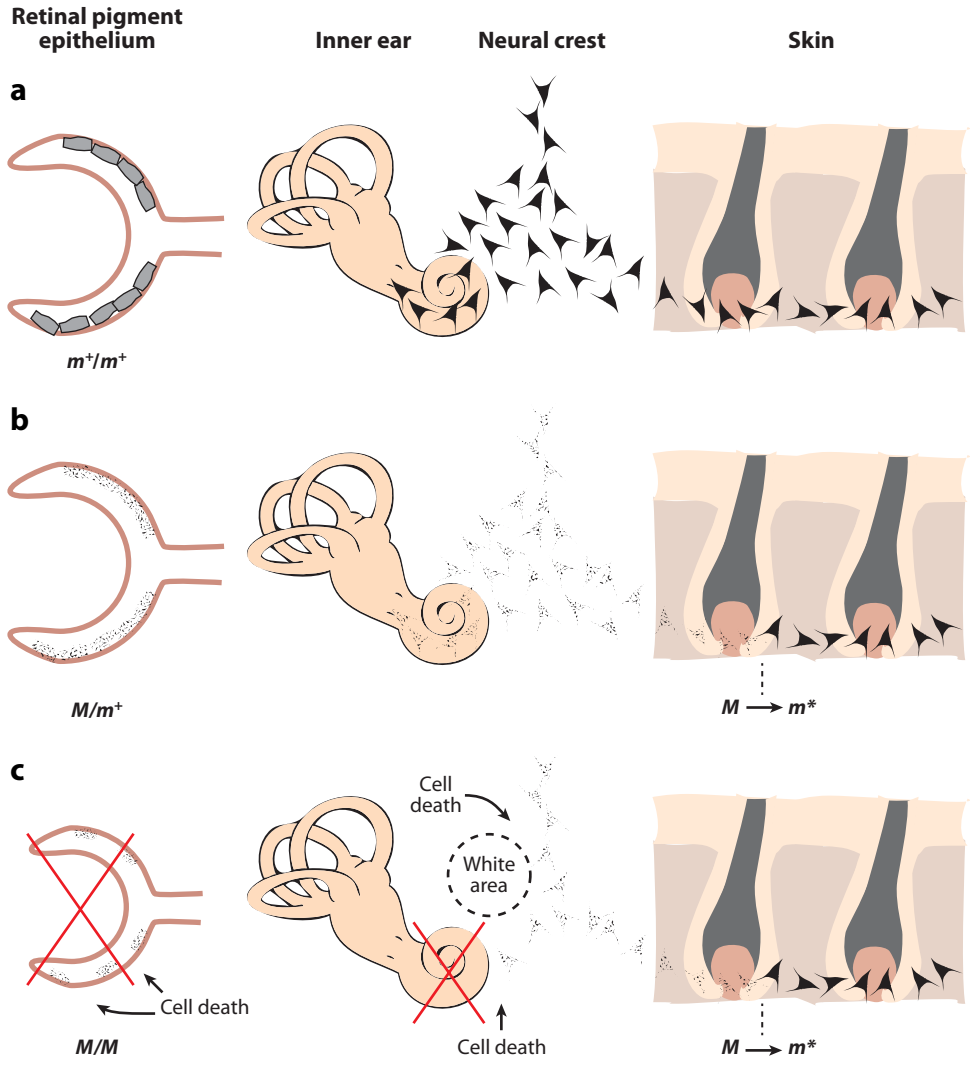

Figure 3

Proposed cellular and developmental basis for *Merle*-associated phenotypes. (*a*) The *Merle* gene product, PMEL, is a component of both the neurectoderm-derived retinal pigment epithelium and neural crest–derived melanocytes in the inner ear and the hair follicle, as shown in m^+/m^+ nonmutant animals. (*b*) In dogs heterozygous for *Merle* (M/m^+), a defective PMEL protein compromises eumelanosome formation, which leads to a generalized pigmentary dilution that affects the appearance of both the retinal pigment epithelium and the skin. (*c*) In dogs homozygous for *Merle* (*M*/*M*), increased levels of defective PMEL protein cause pigment cell death, which itself leads to abnormal retinal development, deafness, and large white areas on the coat. (*b*,*c*) The molecular nature of the *M* mutation is unstable, which facilitates frequent conversion to what we refer to as a pseudorevertant m^* allele. Pseudoreversion is shown here at late stages of melanocyte development but may also occur much earlier, giving rise to large patches of normal color within a diluted area.

MITF is expressed in both melanocytes and retinal pigment epithelial cells, the *MITF* molecular lesion responsible for *white spotting* seems to affect expression only in melanocytes. In mice, homozygosity for a targeted null allele of *Pmel* gives rise to a very subtle coat color phenotype (50)

that is much milder than the original mouse allele, $Pmel^{Silv}$, caused by a single nucleotide insertion that truncates the last 25 C-terminal residues of Pmel (51).

Taken together, these considerations suggest that pigmentary dilution in merle dogs is caused by a structurally abnormal PMEL protein that interferes with eumelanosome formation. Death of skin melanocytes probably accounts for white areas of the coat in *M/M* animals but is unlikely to explain the pigment dilution in merle dogs, because death of follicular melanocytes during hair growth yields a characteristic hair phenotype with loss of pigment at the base of the hairs (52). Finally, the location and size of normal color patches that occur within diluted areas of merle dogs probably reflect the time during melanocyte development when reversion occurs, with large and small patches signifying early and late events, respectively. In both cases, the shape of normal color patches should reflect the developmental history of a melanocyte clone and is consistent with recent studies in laboratory mice (53).

Harlequin (*H*) and *Tweed* (Tw^T) are both dominant modifiers of the merle pattern that have no apparent effect on coat color in nonmerle backgrounds. Dilute regions of merle pattern are, instead, white in a harlequin background (*H/h*; M/m^+) and contrast sharply with the region of full pigmentation. *H/H* genotypes cause lethality during embryogenesis (54), and Clark et al. (55) showed that the phenotype is associated with a coding variant in the *20S proteasome 2 subunit* (*PSMB7*). In tweed dogs, dilute regions of the merle pattern are larger on average, with varying shades of pigment intensity and smooth boundaries (56); there is, at present, no information on the molecular basis of *Tweed*.

GENERALIZED PIGMENT DILUTION: *BROWN, CHINCHILLA, DILUTION, PROGRESSIVE GRAYING, INHIBITOR* (*SILVER*)

As indicated above, melanocytes produce two types of pigments, black eumelanin and red pheomelanin, in specialized organelles called melanosomes. Mature melanosomes are then transferred to surrounding keratinocyte cells that populate the hair and skin. Pigment dilution reflects decreased pigment production or transfer and results from mutations in genes that function in melanosome biogenesis, pigment synthesis, or melanosome transport. Eumelanin and pheomelanin differ according to amino acid content (pheomelanin is cysteine-rich, eumelanin is not), solubility (eumelanin is more highly polymerized and therefore more insoluble than pheomelanin), and structure (eumelanin exists in highly structured ovoid granules, whereas pheomelanin exists in granules that are less structured and more spherical). As with *Pmel*, many components of the pathways for eumelanin and pheomelanin synthesis are different (**Figure 4**); thus, dilution phenotypes often affect either eumelanin or pheomelanin specifically (11, 57).

At least five loci are known to modify the intensity of coat color in the domestic dog and cat: *Brown* (*B*), *Chinchilla* (*C*), *Dilute* (*D*), *Progressive graying* (*G*), and *Inhibitor of melanin* (*I*, also known as *Silver*). In this section, we discuss the phenotypes associated with these loci as well as their molecular characterization.

Brown ($B^+ > b$) in Dogs and ($B^+ > b > b^1$) in Cats

In several mammals, recessive alleles of *Tyrosinase-related protein 1* (*Tyrp1*) cause the dilution of black to brown pigment but do not alter the intensity of red or yellow pigment (58–61). *Tyrp1* encodes an intramelanosomal enzyme that catalyzes the oxidation of intermediates in eumelanin synthesis (62).

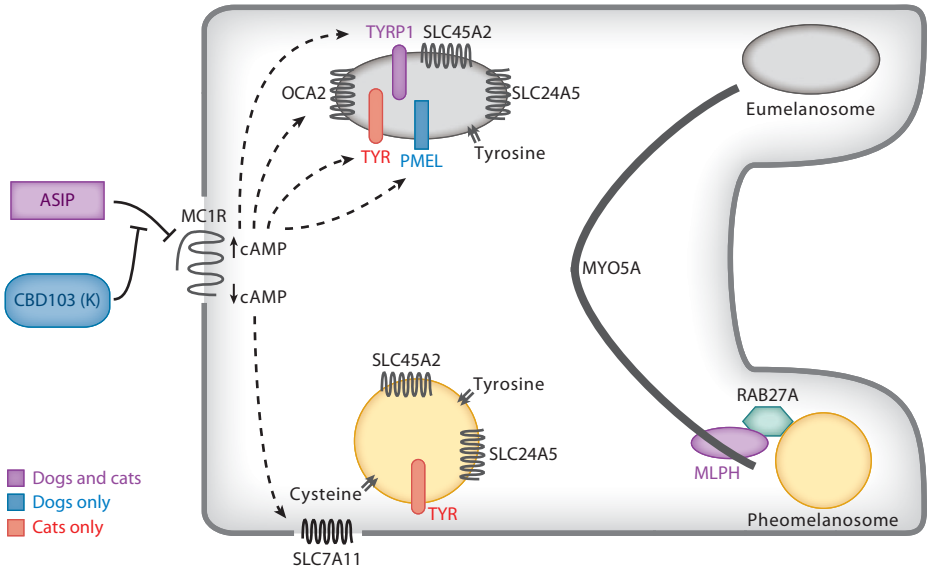

Figure 4

The role of dog and cat coat color genes in melanocyte cell biology. Mutations of each gene product shown here give rise to pigmentary phenotypes. MC1R and its second messenger cAMP control switching between synthesis of eumelanin and pheomelanin. High levels of basal MC1R signaling cause increased expression of TYR, TYRP1, OCA2, and PMEL, which leads to increased eumelanin synthesis. Low levels of cAMP cause increased expression of the cysteine transporter, SLC7A11, which leads to increased pheomelanin synthesis. CBD103 (encoded by the *K* locus) prevents ASIP from inhibiting MC1R, thereby promoting eumelanin synthesis. The illustration is drawn to emphasize the differences between eumelanin and pheomelanin synthesis; in reality, biogenesis of the different organelles is more complex and involves a common precursor organelle and several distinct protein-trafficking steps. As melanosomes mature, they are transported to dendritic tips via a process that depends on the unconventional myosin MYO5A, a GTP-binding protein (RAB27A), and an adapter protein (MLPH). Adapted from (12).

Schmutz et al. (63) identified three *TYRP1* coding mutations in dogs, each on a different haplotype, responsible for an indistinguishable brown coat color in different allelic combinations. A survey of 28 breeds found that the *TYRP1* alleles were widespread, with all three alleles present in several breeds (63). The identification of three molecularly distinct but functionally equivalent alleles in dogs is somewhat surprising because selection for a specific phenotype is expected to sweep a single allele to high frequency. A potential explanation is that selection for brown coat color occurred in multiple isolated populations, each of which contributed to the formation of modern breeds.

In contrast, the domestic cat has two derived *TYRP1* alleles, *chocolate* (*b*) and *light brown* or *cinnamon* (b^1), responsible for distinct coat color dilutions (60, 61). A light, reddish-brown color (cinnamon) characteristic of the Abyssinian breed is associated with an early *TYRP1* nonsense mutation. A darker brown color (chocolate) common to Burmese, Siamese, and Havana Brown breeds is associated with two predicted coding alterations, a conservative amino acid substitution and an in-frame, 18–amino acid insertion caused by abnormal splicing. The allelic hierarchy, $B^+ > b > b^1$, corresponds to dilution intensity, with darker alleles dominant to lighter alleles. The nature of the mutations and the allelic relationships suggest that *chocolate* and *light brown* are partial and complete loss-of-function alleles, respectively. The effects of *TYRP1* variation on dog and cat coat color are apparent in **Figure 5**.

Figure 5

Effect of *TYRP1* and *MC1R* mutations. (*a*) In dogs, a uniform appearance of eumelanin can be caused by either the K^B mutation or the *a* mutation; however, the latter is rare. Here, all three retrievers carry the dominant black allele of *CBD103* (K^B). Recessive loss-of-function *TYRP1* alleles (*B* locus) cause the dilution of black-to-brown pigment in the chocolate (sometimes referred to as liver) Labrador Retriever, and a recessive loss-of-function *MC1R* allele (*e*) causes a yellow coat in the Golden Retriever. Adapted from (12). (*b*) In cats, uniform appearance of eumelanin is caused by homozygosity for the *a* mutation. The *b* allele of *TYRP1* causes a mild dilution of eumelanin in the chocolate cat; the b^l allele causes more dilution of eumelanin in the cinnamon cat. The genotype of the cinnamon cat (*b*, *right panel*) is given as *a/a;* b^1/b^1, but could alternatively be $Ti^A/-$; *A*/–; b^1/b^1. In the latter scenario, the dominant Ti^A allele at the *Ticked* locus would suppress the tabby pattern, and individual hairs would have subapical pheomelanic (yellow) bands.

Tyrosinase ($C^+ > c^s$, $c^b > c$) in Cats

Historical studies in laboratory mice identified the most common cause of oculocutaneous albinism as an allele of the C locus, later identified as *Tyrosinase* (*Tyr*). Tyrosinase is a transmembrane melanosomal protein whose intramelanosomal domain catalyzes the initial and rate-limiting step of both eumelanin and pheomelanin synthesis. Identification of *TYR* variation in dogs and cats is

based on comparison with similar phenotypes in laboratory mice, where allelic variation of *Tyr* gives rise to several characteristic phenotypes. These include *Himalayan* (c^b), *Albino* (c), and *Chinchilla* (c^{cb}), which are associated with acromelanism (restriction of pigment to body extremities), complete albinism, or preferential dilution of pheomelanin, respectively. Similar allelic series have also been described for several other species (11).

The distinctive nature of the acromelanic phenotype is easily recognizable, because pigment is typically restricted to body regions where heat loss is greatest (the muzzle, ears, feet, and tail). In a historical demonstration of temperature sensitivity in cats, Iljin & Iljin (64) observed that Siamese cats that were maintained at low temperatures developed increased coat pigmentation over a two-month period. Conversely, when a region of hair was shaved and bandaged to increase body-surface temperature, a large proportion of the regrowing hair was completely devoid of pigment. Two *TYR* alleles, *Siamese* (c^s) and *Burmese* (c^b), are responsible for distinct color varieties in the domestic cat (**Figure 6**). The Siamese cat (c^s/c^s) has a pronounced dilution very similar to that recognized in the Himalayan mouse, with dark extremities and an almost white body. The Burmese cat (c^b/c^b), in contrast, has a moderate gradient of pigment dilution and a substantially darker body than the Siamese. Both alleles are recessive to C^+ but are additive in combination; compound heterozygotes (c^s/c^b) display an intermediate mink phenotype, common in the Tonkinese breed. c^s and c^b are independent missense mutations that occur at conserved residues in known *TYR* functional domains (60, 65). The acromelanic phenotype is also easily recognizable in other mammals including the rabbit, the guinea pig, and humans; perhaps surprisingly, there are no reports of a similar phenotype in dogs.

A *Tyr*-null mutation causes oculocutaneous albinism, recognized in many species as a completely white coat, red eyes, and, often, visual defects owing to a requirement for melanin in retinal pigment epithelial cells to support normal projection of retinal axons. In fact, much of the work on the relationship between retinal pigment epithelial cells and retinal axon pathfinding was carried out in Siamese cats, which have monocular rather than binocular vision because of the retinal axon abnormalities (66). Complete albinism is rare in dogs but occurs infrequently in some breeds, such as the Pekingese, and is assumed, though not proven, to result from a *TYR* loss-of-function allele (c) (67). A more common cause of white coat color in dogs is extreme white spotting (s^w/s^w) on a pale background (either a^y/a^y or *e/e*, see below), which is especially common in spotted breeds such as the Borzoi. These dogs typically have dark eyes with small amounts of residual pigmentation on the coat and skin and produce spotted pups when mated to a solid-colored dog. Some

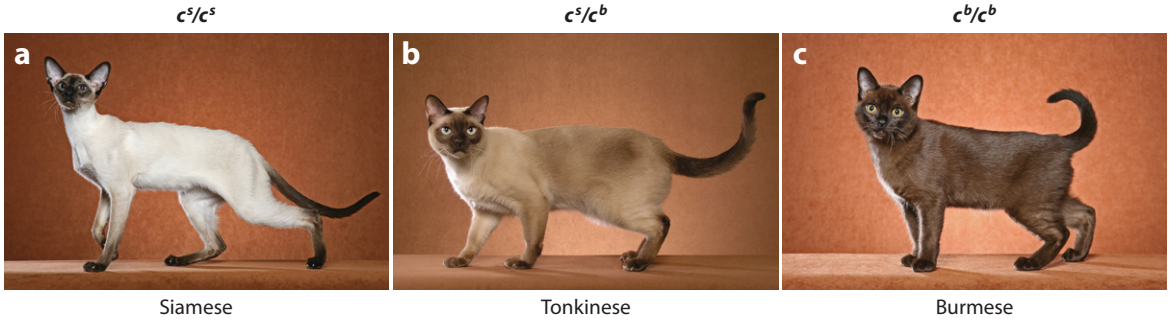

Figure 6

Effect of different *TYR* alleles in cats. The c^s allele impairs tyrosinase activity more than the c^b allele. Thus, different *TYR* allelic combinations are responsible for a range of pigment dilution in breeds such as (*a*) the Siamese, (*b*) the Tonkinese, and (*c*) the Burmese. In each case, impairment is temperature sensitive; pigmentation is relatively spared in areas of the body where heat loss is greatest.

dark-eyed white dogs, however, are found in predominantly solid- and dark-colored breeds, such as the German Shepherd, and provide evidence for a third locus (in addition to s^w and *c*) that segregates as a monogenic trait with a recessive inheritance pattern (68). In cats, a likely null mutation in *TYR* has been identified in a Siamese pedigree that included both acromelanic and completely white cats (69). However, most white cats are not true albinos but instead carry dominant white (W^T) or a c^s or c^b allele on a pheomelanic background (**Figure 7**).

Tyr mRNA is normally downregulated during pheomelanin synthesis. Thus, hypomorphic alleles of *Tyr* may provide enough activity for eumelanin but not for pheomelanin synthesis (70), causing preferential dilution of pheomelanin. This phenomenon underlies the characteristic chinchilla phenotype—a banding pattern of eumelanin alternating with cream-colored pheomelanin—apparent in many species. Little (8) speculated that a *Tyr* allele, equivalent to c^{ch} in other animals, was responsible for variation in pheomelanin intensity in dogs, for example, the dark-red coat of an Irish Setter compared with that of a yellow Labrador Retriever, both of which are caused by the same *Mc1r* allele (see below). However, Schmutz & Berryere (71) observed that *TYR* markers did not segregate with pheomelanin dilution in either Golden Retriever or Labrador Retriever pedigrees. In cats, similar modifiers of pheomelanin intensity, referred to in the cat fancy as rufousing factors, might be explained by hypomorphic *TYR* alleles. An additional candidate that has not yet been investigated in either dogs or cats is *Slc7a11*, which encodes a cysteine transporter that underlies pheomelanin dilution (the *subtle gray* mutation) in mice (72).

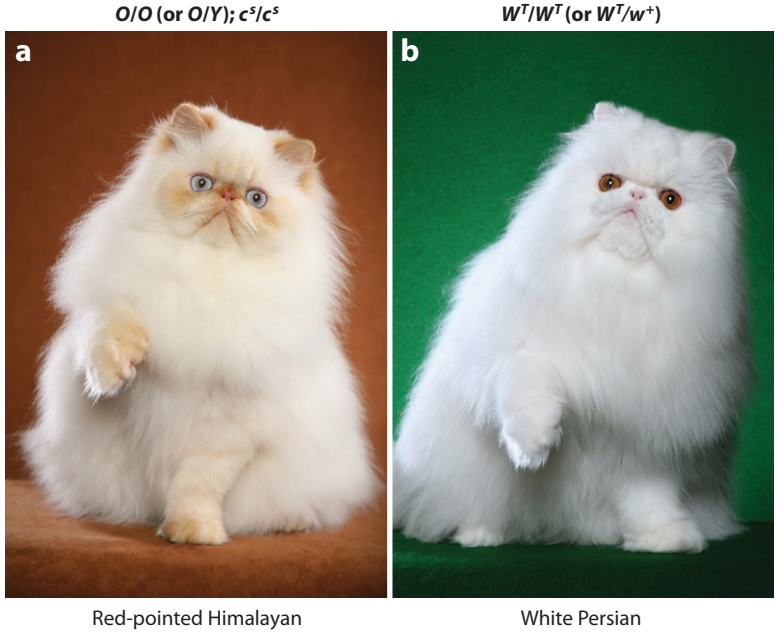

Figure 7

White coat color in cats caused by different mechanisms. (*a*) The acromelanic phenotype associated with the presence of a pale orange color in the pigmented areas indicates that melanocytes are present in the skin, that melanogenesis is inhibited in most areas of the body because of the c^s mutation, and that the cat is homozygous or hemizygous for the *O* mutation. (*b*) Complete absence of pigment in this white Persian is probably caused by loss of melanocytes owing to heterozygosity or homozygosity for the dominant white (W^T) mutation.

Dilution ($D^+ > d$) in Dogs and Cats

Recessive alleles at the *Dilute* (*D*) locus are responsible for eumelanin and pheomelanin dilution to a similar bluish-silver color in both the cat and the dog. There is a characteristic histology, including perinuclear melanosome clumping in melanocytes and an abnormal melanosome distribution in the hair shaft (11, 73). Studies in mice show that the underlying mechanism involves a disruption in the melanosome transport machinery. This machinery is made up of melanophilin (Mlph), a member of the exophilin subfamily of Rab effector proteins, which forms a ternary complex with a Ras-related GTPase, Rab27a, as well as a myosin motor protein, Myo5a, which is involved in the transport of melanosomes along the actin cytoskeleton (74). In mice, mutations in the genes encoding each of these components produce a similar bluish-gray coat color, but *Rab27a* and *Myo5a* mutations also affect hematopoiesis and nervous-system function, respectively.

In humans, mutations in *MYO5A*, *RAB27A*, and *MLPH* are responsible for distinct subtypes of a rare recessive disorder known as Griscelli syndrome (GS). All three subtypes present with a characteristic hypopigmentation, but in most cases, *MYO5A* (*GS1*) and *RAB27A* (*GS2*) forms are accompanied by severe neurological and immune deficiencies, respectively, and are usually life threatening. In contrast, GS3, caused by *MLPH* mutations, is nonsyndromic, affecting only pigmentation. The observations in humans and mice imply a pigmentation-specific role for Mlph, which makes it an ideal target for artificial selection in domesticates.

Molecular genetic studies have confirmed that *MLPH* is responsible for the dilute phenotype in the dog and cat. Leeb and colleagues (75) reported both linkage and association between three different *MLPH* haplotypes and coat color dilution in multiple dog breeds. Some dilute animals carried *MLPH* variants predicted to alter protein-coding sequence, but the same variants were observed in non-dilute animals, which led the authors to suggest an underlying regulatory mutation or mutations. More recently, the same group discovered that all dilute dogs carried the same haplotype in the 5′ untranslated and flanking regions of *MLPH*, together with a 5′ UTR variant that likely affects splicing, which led to greatly reduced levels of normal skin *MLPH* mRNA in skin (76). In cats, Ishida et al. (77) demonstrated association between an *MLPH* frameshift mutation and color dilution in multiple breeds of cats. Thus, in both species, a single mutational event appears to have been selected and widely distributed.

Progressive Graying ($G > g^+$) in Dogs

Progressive graying (*G*) is a dominantly inherited, progressive dilution of eumelanin from black to gray (8) observed in some dog breeds, most notably Poodles, but not in the domestic cat. Graying in dogs shares some similarities to graying with age in horses (78), which is also dominantly inherited and is caused by an unusual regulatory alteration that increases expression of two nearby genes (*Syntaxin-17* and *Nr4a3*). However, gray horses also exhibit vitiligo and increased susceptibility to melanoma (79). These additional skin findings are not observed in dogs that carry *G*, and the molecular basis of dog graying has not yet been determined.

Inhibitor of Melanin ($I > i^+$) in Cats

In the domestic cat, variation at *Inhibitor of Melanin* (*I*) is responsible for a dominantly inherited pigment dilution that occurs in a tip-to-base gradient along the hair shaft, with hair tips being more fully pigmented. Unlike most other mutations of melanogenic components that cause dilution by affecting the intensity of pigment granules, dilution in cats carrying the *I* mutation is caused by a decreased number of pigment granules (80). The uneven follicular

distribution of pigment granules could result from the gradual disruption of melanin transfer or biosynthesis, or from premature cessation of melanogenesis owing to melanocyte regression and/or apoptosis. No similar forms of pigment dilution are reported in other species, including the dog. *I*, which has also been referred to as *SILVER*, was recently mapped to a ~3.5-Mb interval on cat chromosome D2 with 17 genes, none of which had a previous connection to coat color (81).

The extent of dilution may depend on hair length and pigment type. In a silver tabby (a cat that carries *I* and in which a tabby pattern is visible, see below), pigmentation is restricted to hair tips in light components of the tabby pattern (where individual hairs are normally Agouti banded) but is not noticeably diluted in dark components (where individual hairs are not banded), which results in a sharp contrast that highlights the tabby pattern (**Figure 8**). This observation suggests that the effect of *I* may intensify in pheomelanic compared with eumelanic areas. However, the *I* allele also dilutes solid black (*a*/*a*, see below) coat color in long-hair breeds, such as the Persian and the Angora. In this background, pigmentation extends further down the hair shaft but is diluted or absent at the base, which results in a faded appearance known as smoke. The background-dependent color variation resulting from *I* has spawned a phenotype-based nomenclature among cat fanciers; for example, interaction of *I* with *Tabby*, *Orange*, and/or *ASIP* alleles can yield cats described as cameo, chinchilla, shaded, silver, smoke, or tipped.

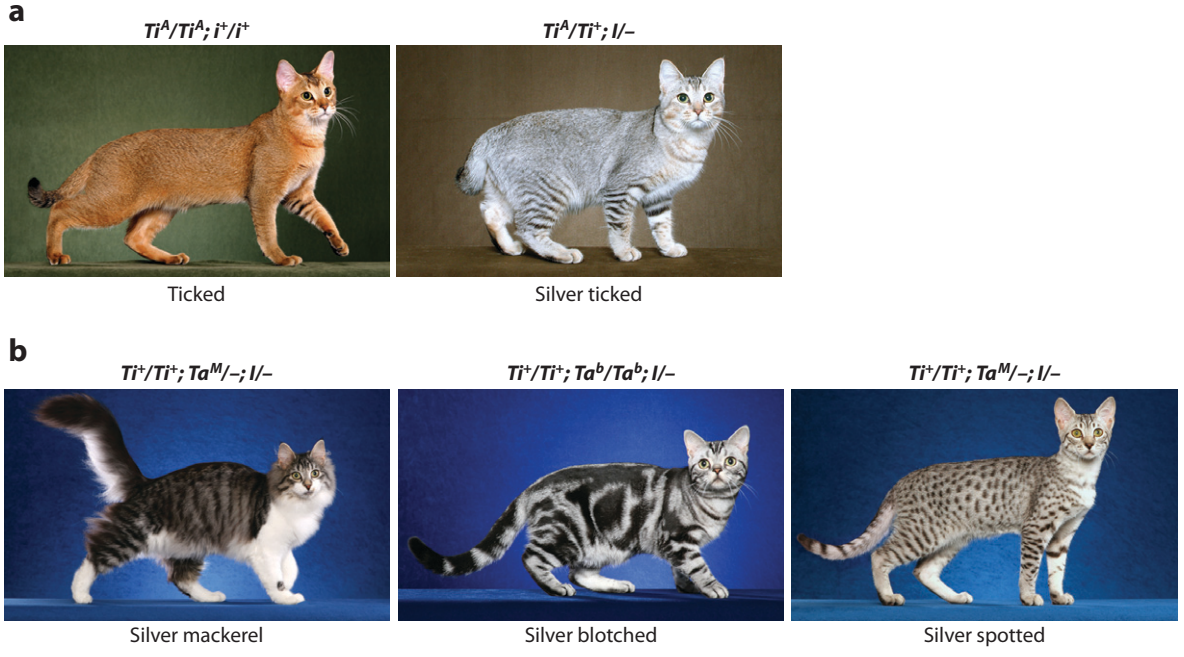

Figure 8

Variation of tabby markings in cats. (*a*) Ticked cats lack tabby markings on most of their body; residual markings on the legs or head are more apparent in heterozygotes (Ti^A/Ti^+) than in homozygotes (Ti^A/Ti^A). In the silver ticked cat (*right*), only hair tips are fully pigmented owing to the action of the dominant *I* allele. (*b*) Ti^+/Ti^+ cats display tabby markings. All cats shown here are also silver. The *I* allele does not noticeably dilute pigment within tabby markings, which creates a sharp contrast between the markings and background areas. Homozygosity for Ta^b in the middle cat causes the blotched pattern. The genetic basis of the spotted pattern is complex and not yet known, but spotted patterns are not observed in Ta^b/Ta^b cats. (The *Tabby* genotype for the mackerel and spotted patterns shown in the left- and right-hand panels, respectively, could be Ta^M/Ta^M or Ta^M/Ta^b.)

PIGMENT-TYPE SWITCHING: *K*, *ORANGE*, *ASIP*, AND *EXTENSION*

The synthesis of either eumelanin or pheomelanin is regulated in a time- and location-specific manner by an intercellular signaling pathway within the hair follicle (73). The main components of this pathway are encoded by the pigment-type switching genes *Melanocortin 1 receptor* (*Mc1r*) and *Agouti signaling protein* (*Asip*). Mc1r is a G protein–coupled receptor expressed by melanocytes, and its signaling activity promotes eumelanin synthesis. The signaling activity of Mc1r is primarily regulated by Asip, a secreted ligand that functions as an inverse agonist, which thereby antagonizes Mc1r signaling. Increased activity of Mc1r promotes eumelanin synthesis, and decreased activity of Mc1r promotes pheomelanin synthesis (**Figure 4**). In many mammals, including dogs and cats, transient expression of *Asip* during hair growth gives rise to a band of pheomelanin on a eumelanic background of individual hairs, a phenotype often referred to as Agouti (after a South American rodent) or Agouti banding.

Mutations in *Mc1r* or *Asip* commonly result in phenotypes that alter the timing and/or distribution of eumelanin and pheomelanin. *Mc1r* gain-of-function or *Asip* loss-of-function mutations cause exclusive production of eumelanin. Conversely, *Mc1r* loss-of-function or *Asip* gain-of-function mutations cause exclusive production of pheomelanin. As a consequence, *Asip* has a characteristic allelic hierarchy, with dominant yellow and recessive black alleles. *Mc1r* has a reverse hierarchy, with dominant black and recessive yellow alleles (11), and, as expected for a ligand-receptor relationship, *Mc1r* alleles are epistatic to *Asip* alleles. In most domestic species, *ASIP* and *MC1R* alleles are responsible for pigment-type variation. However, in the dog and cat, pigment-type switching is additionally determined by two alternative loci, *K* and *Orange*, respectively, both of which represent new entry points to study pigment-type switching and related pathways.

K ($K^B > k^{br} > k^y$ or k^+) in Dogs

Enabled by genome-wide molecular markers, pedigree and linkage analysis revealed that dominant black and another unusual dog coat color phenotype, brindle, are not alleles of either *ASIP* or *MC1R* but instead map to a novel pigment-type switching locus (*K*) with three alleles: *Dominant black* (K^B), *Brindle* (k^{br}), and the ancestral allele (k^y), listed here in order of dominance (82). A combination of pedigree and population-based mapping approaches identified the K^B mutation as a 3-bp deletion in *β-defensin 103* (*CBD103*) that predicts an in-frame glycine deletion (83). Several lines of evidence suggest that CBD103 is an MC1R ligand that promotes eumelanin synthesis by inhibiting ASIP antagonism of MC1R (83).

β-defensins comprise a diverse and rapidly evolving family of secreted peptides (84, 85) with a role as endogenous antibiotic agents that participate in both acquired and innate immune responses (86, 87). The link between *CBD103* and the melanocortin system indicates that β-defensins do more than just defend and points to a potentially intriguing connection between pigmentation and immunity. Nevertheless, a similar pigmentary function for β-defensins in other species has yet to be uncovered.

Brindle (k^{br}), which describes the irregular pattern of black stripes on a fawn or yellow background, is common in many breeds. Little initially assigned k^{br} to the *Extension* locus, but mapping data revealed it as an allele of the *K* locus (82). The brindle phenotype is only apparent on pheomelanic areas of the coat, and its expression requires a functional MC1R (*K* alleles are hypostatic to *E* alleles) (82). The extent of brindle striping varies considerably. Some dogs are yellow with only a few black stripes, whereas others are so heavily striped that they appear black.

Orange ($O > o^+$) in Cats

Another exceptional pigment-type switching locus, X chromosome–linked *Orange* (*O*), is responsible for the common orange coat color in the domestic cat. *Orange* is a classic example of sex-linked variation that was also commented upon by Darwin, Whiting (7), and Wright (3) (who termed it a "curious association. . .of femaleness with tortoise-shelled color") nearly 50 years before Mary Lyon's paper on X-inactivation (88). Hemizygous males and homozygous females with the variant *Orange* allele, *O*, are orange owing to exclusive production of pheomelanin. Heterozygous females (*O*/*o*) have a mosaic coat pattern, known as tortoiseshell (or calico when in a white-spotting background), in which patches of orange and nonorange hairs are interspersed. As alluded to above, the pattern is a consequence of random X chromosome inactivation early in development, epigenetic cellular inheritance of the inactivation state, and incomplete mixing of cell clones later in development. Pigment cells will produce either orange or nonorange pigment (with the nonorange color determined by the genetic background at other pigmentation loci), depending on which X chromosome is inactivated. The *O* locus maps to a region of the X chromosome devoid of known pigmentation genes (89, 90).

In a white-spotting background, the variegated pattern is referred to as calico and is characterized by larger and more distinct color patches. Patch size is correlated with the amount of white spotting, which suggests a relationship between pigment cell death caused by *W* alleles and the extent of cell mixing after X inactivation (**Figure 9**). Thus, in tortoiseshell cats with normal numbers of developing pigment cells, mixing of different clones results in a tight mosaic patchwork

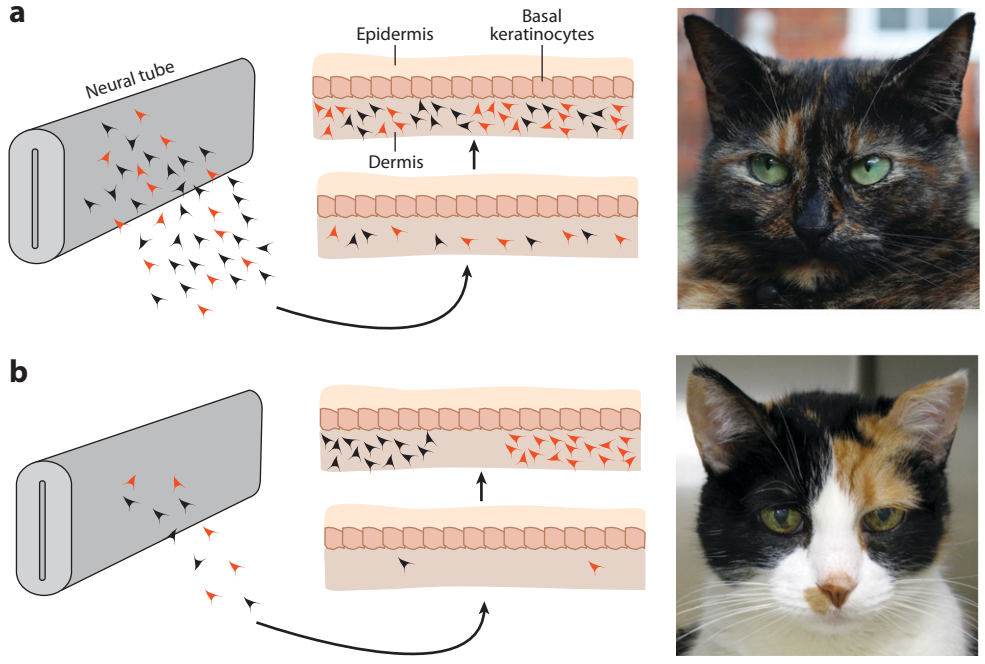

Figure 9

Both (*a*) the tortoiseshell and (*b*) the calico phenotypes are caused by heterozygosity for *Orange*, O/o^+. Calico cats also carry a *white spotting* mutation (such as W^J); impaired survival of melanocytes after X inactivation allows clones of melanocytes (distinguished by whether or not they have inactivated the *O*-bearing or the o^+-bearing chromosome) to expand into larger body areas of the developing skin than would be possible in the absence of a *white spotting* mutation.

of coat color. In contrast, calico cats have a reduced number of developing pigment cells, which allows for broader clonal expansion, less mixing of different clones, and, consequently larger and more distinct color patches. The interaction between patch size and white spotting provides strong evidence that the product of *Orange* acts in a melanocyte cell autonomous way to influence pigment-type switching (11). Consistent with these observations, *O*/*O A*/*A* and *O*/*O a*/*a* cats have an identical appearance, which indicates that *O* is epistatic to *ASIP* (90).

Agouti ($a^y > a^w$, $a^+ > a^t > a$ in Dogs and $A^+ > a$ in Cats)

In contrast to *K* and *Orange*, variation in *Asip* commonly affects coat color and pattern in many different species of mammals. In mice, expression of *Asip* is localized to the dermal papilla, a permanent structure of the hair follicle positioned immediately underneath hair follicle melanocytes, and is regulated in a temporal and spatial manner by two alternative promoters (91). One promoter is active in ventral body regions and is responsible for so-called countershading patterns, where the ventral areas are yellow or cream colored (92, 93). The second promoter is active transiently during the hair cycle and is responsible for the yellow subapical band (i.e., the Agouti phenotype) on individual hairs.

In dogs, the effects of *ASIP* and the overall gene structure are similar to that in other mammals, and a coding-sequence variant predicted to inactivate *ASIP* causes recessive inheritance of black coat color in a few breeds, including the German Shepherd and Australian Shepherd (94). Assuming that *ASIP* regulation in dogs is homologous to that in mice, the a^w allele can be most easily explained by the existence of ventral-specific and hair cycle-specific isoforms that give rise to Agouti-banded hairs and a pale ventrum; this is the phenotype seen in the wolf, and a^w therefore represents the ancestral or wild-type version.

The remaining alleles (a^y, a^t, and possibly a^s as described further below) likely reflect derivative alleles that affect regulation of *ASIP*. Dogs carrying an a^y allele are predominantly yellow (commonly referred to as fawn, tan, or sable), though hairs often have black tips that give a sandy appearance. The a^y allele is associated with two *ASIP* coding variants in 22 breeds, which suggests a single common origin (95). However, the coding variants may have little or no effect on function and instead may be in linkage disequilibrium with a regulatory mutation that expands the timing of hair cycle expression to extend throughout most of hair growth.

The a^t allele in dogs gives rise to a characteristic phenotype with a black or brown dorsum and yellow (or tan) markings on the head, ventrum, and legs, known as black-and-tan, brown-and-tan (in animals with a *TYRP1* mutation), or sometimes just tan points (**Figure 2**). The a^t allele is associated with a SINE insertion in dogs from 35 breeds (96), but the underlying molecular mechanisms and causal relationships are not yet clear. Spatial distribution of yellow or tan regions in black-and-tan dogs varies considerably among breeds. The Doberman Pinscher and the Dachshund display minimal tan points, whereas in the Airedale Terrier, yellow regions extend dorsally to cover a considerable portion of the coat, limiting dark regions to a saddle shape on the back and sides. The latter phenotype is described as saddle or saddle-tan and is often attributed to variation at *ASIP* (by virtue of a fifth allele, a^s), although some have suggested that saddle is caused by nonallelic modifiers of the a^t pattern (8, 97, 98). A closely related phenotype, referred to as grizzle in the Saluki and domino in the Afghan Hound, is defined by expansion of pheomelanic areas in an otherwise tan points animal to encompass most of the inner and distal extremities and muzzle, leaving eumelanic regions along the top and sides of the body and outside of the limbs. From a phenotypic perspective, grizzle or domino lies between tan points and saddle but has been associated with an *MC1R* rather than an *ASIP* variant as described below (99).

In the cat, we consider the ancestral allele to be *Agouti* (*A*) rather than *white-bellied Agouti* (a^w) because African wild cats do not exhibit a pale ventrum. (A pale or yellow-colored ventrum is, however, very prominent on many other felids, including most of the *Panthera*.) There is considerable variation in hair banding among domestic cats, which may be attributable to heterogeneity in *ASIP* expression during the hair cycle. Nonetheless, only a single derived *ASIP* allele (*a*) has been described: a frameshift mutation predicted to inactivate the protein, which is responsible for recessive inheritance of a black coat (100).

Extension ($E^m > E^+ > e$ in Dogs, $E^+ > e$, *amber* in Cats)

The *Extension* (*E*) locus has two alleles in the domestic cat ($E^+ >$ e) and three alleles in dogs ($E^m > E^+ > e$). Little assigned a fourth allele, E^{br}, to the dog series, which he postulated was responsible for the brindle phenotype, but which is now recognized as an allele of the *K* locus (K^{br}) (82, 101). In both dogs (102) and cats (103), the wild-type allele, *E,* encodes a functional MC1R that allows for signaling by melanocortin ligands.

Yellow coat color in most dog breeds results from the dominant *ASIP* allele, a^y, as described above. In the Labrador Retriever, the Golden Retriever, and the Irish Setter, however, uniformly yellow or red coat color owes to recessive inheritance of the *e* allele, identified as a nonsense mutation (R306ter) that truncates the final 11 amino acids of the receptor (102, 104). The R306ter variant is found on two different haplotypes in the dog, which indicates an independent origin for each and implicates the site as a hotspot for mutations (102).

The dog E^m allele causes localized distribution of eumelanin on the muzzle resembling a darkened mask in a pheomelanic (a^y) background. The phenotype was perfectly associated with an *MC1R* coding variant (M264V) in a survey of 12 breeds (105). However, an M264V substitution is not predicted to be especially detrimental, and a valine at this position is found in other vertebrate species; thus, the M264V variant could be in linkage disequilibrium with a regulatory mutation that increases *MC1R* expression levels. In either case, E^m likely affects the MC1R signaling levels rather than the regional distribution of receptors, which implies that specific areas of the body have different thresholds for pigment-type switching that can be revealed by perturbations in MC1R signaling efficiency (105). The same may be true for the grizzle or domino phenotype described above, which appears in animals that carry the a^t-associated SINE insertion together with a novel MC1R variant, G78V, for which the symbol E^G has been proposed (99). Because the grizzle phenotype is associated with expansion of pheomelanic areas, the underlying mechanism likely involves reduced MC1R activity. This is difficult to reconcile with the suggestion that the putative E^G allele is dominant to E^+ (99), and more work will be necessary to unravel the molecular bases and *ASIP-MC1R* interactions that underlie the tan points, saddle, and grizzle phenotypes.

In cats, the *O* allele at the X-linked *Orange* locus is responsible for pheomelanic coat color in most individuals, but an autosomal recessive form, known as amber, segregates in a Northern European breed, the Norwegian Forest Cat. Peterschmitt et al. (103) identified a *MC1R* missense substitution (D84N) that was predicted to inactivate the protein and was perfectly associated with amber coat color. The *O* mutation and the *e* mutation both promote the production of pheomelanin instead of eumelanin, but their effects can be distinguished in the context of tabby markings (described further below). In $O/O\ E^+/E^+$ cats, the light component of the tabby pattern is orange, and the dark component is a more intense orange; in $o^+/o^+\ e/e$ cats, the light component of the tabby pattern is also orange, but the dark component is brown or black and gradually fades with age (103).

COAT COLOR PATTERNS: *TICKED*, *TABBY*, AND *SPOTTED*

Color patterns fall into two categories based on developmental origin and appearance. In one category are variegated or random patterns in which the placements of the different colored areas are random—white spotting, brindle stripes, and merle and tortoiseshell patches. These patterns arise from developmental events that are stochastically initiated (e.g., X inactivation in tortoiseshell females or melanoblast death in white spotting) and then stably maintained via cell lineage. Patterns initiated by stochastic events are common in domesticates but are relatively infrequent in natural populations.

In contrast, color patterns with predictable, nonrandom characteristics are common in natural populations and must arise from developmental processes that are programmed to be spatially constrained. As described below, we find it helpful to consider such patterns as involving two phases: One operates during development and establishes the regional boundaries of the eventual pattern, and a second implements and maintains the color differences during hair growth and cycling.

Nonrandom color patterns can be further divided into those that exhibit periodicity and those that do not. The tan points pattern in dogs (**Figure 2**) exemplifies the latter: The distribution of yellow-colored hair in a Dachshund or a Doberman Pinscher—head markings, distal extremities, muzzle, and axillary areas—represents a set of body regions in which dermal papilla cells are developmentally programmed to express *ASIP*. In this case, studies in laboratory mice suggest that expression from the ventral-specific *Asip* promoter provides an obvious link between pattern establishment and pattern implementation: The ventral-specific *Asip* isoform is expressed in a specific population of dermal fibroblasts during fetal development and then is later refined and maintained in dermal papilla cells of ventral hair follicles (106, 107). From this perspective, the tan points pattern highlights a set of body regions that respond to the same developmental instructions. Furthermore, variation in the spatial distribution of tan markings across dog breeds likely reflects genetic variation in how those developmental instructions are established or interpreted.

Periodic stripes, spots, and rosettes represent another type of nonrandom pattern, exemplified by tabby markings in cats. Unlike the situation with tan points and *ASIP*, however, there is no obvious link between pattern establishment and pattern implementation; the boundaries between stripes and interstripes do not correspond to developmental compartments recognized by virtue of differences in gene expression or cell lineage, and there is no visible manifestation of pattern boundaries during development. For these reasons, we often refer to the developmental phase of periodic patterns as establishment of a prepattern. Periodic color patterns are of long-standing interest to theoretical biologists because of the work of Alan Turing (108), a mathematician and computer scientist who applied the reaction-diffusion theory of self-organizing patterns to biological processes.

Tabby patterns are a composite of two features: (*a*) a light background color in which individual hairs have Agouti bands and (*b*) dark markings in which individual hairs are not banded. The phenotypes are often referred to categorically as four common and heritable types: mackerel, blotched, spotted, and ticked (**Figure 8**). Mackerel, blotched, and spotted describe variation in the size and shapes of the markings. In mackerel cats, the dark component forms periodic vertical stripes; in blotched cats, the dark component is organized into whorls; and in spotted cats, the dark component forms cheetah-like spots characteristic of two breeds, the Ocicat and the Egyptian Mau. The ticked phenotype is characteristic of the Abyssinian and Singapura breeds, in which dark tabby markings are eliminated from most of the body, leaving a coat composed of Agouti-banded (or ticked) hairs.

Nearly 100 years ago, Phineas Whiting recognized that three of the four common tabby patterns, which he described as banding factors, have a simple genetic basis. Whiting (7) postulated a single locus with three alleles: *Ticked* (T^a), *Mackerel* (T^m), and *Blotched* (T^b). More recently,

application of a genome-wide panel of molecular markers to pedigrees segregating the different combinations of coat color patterns revealed the involvement of two loci instead of one (109, 110). In this updated view of cat pattern genetics, the *Tabby* locus determines the size and shape of the markings, with the *Mackerel* (Ta^M) allele dominant to the *Blotched* (Ta^b) allele. A second locus, now known as *Ticked*, determines the presence or absence of a visible pattern, with the *Ticked* allele (Ti^A) dominant to the presumed ancestral allele (Ti^+) (109–111) (**Figure 8**).

Tabby (Ta^M or $Ta^+ > Ta^b$) in Cats

Allelic variation at the *Tabby* locus alters the shape but not the color of markings, which suggests that the *Tabby* gene participates in pattern establishment but not in pattern implementation. Genome-wide linkage mapping followed by genetic association recently led to identification of *Transmembrane aminopeptidase Q* (*TAQPEP*) as the *Tabby* gene (112). *TAQPEP* encodes a type II, membrane-spanning protein whose metalloprotease-containing ectodomain can be shed into the extracellular space, perhaps contributing to a potential reaction-diffusion process.

Ta^M represents the ancestral, wild-type version, consistent with the mackerel pattern apparent in African wild cats. Two nonsense mutations and a missense mutation at an evolutionarily conserved site (S59ter, W841ter, and D228N) account for the Ta^b alleles; homozygosity or compound heterozygosity for any combination of Ta^b alleles results in indistinguishable blotched patterns (112). Even though *Blotched* is recessive, blotched cats are extremely common in Northern Europe and Scandinavia (113, 114). Blotched, often referred to as classic tabby, was thought by Linnaeus to be a distinguishing feature of domestic compared with wild cats and has been speculated to provide a fitness advantage (114, 115). However, with one notable exception (see below), clear-cut *TAQPEP* loss-of-function mutations have not been identified in other felids (or other mammals). Instead, existence of three different *Ta* alleles more likely simply reflects independent artificial selection for an appearance that cat fanciers found interesting, perhaps analogous to the situation that pertains for the *TYRP1* locus in dogs.

A frameshift mutation in *TAQPEP* is also responsible for a naturally occurring pattern variant of the cheetah, *Acinonyx jubatus*, known as the king cheetah, in which spots coalesce into larger, less organized markings and multiple longitudinal black stripes appear on the dorsum (112). The king cheetah initially was thought to be a distinct species (116), but its pattern was later reclassified and ultimately recognized as a recessively inherited trait when it was introduced into captive breeding programs (117). The observation that loss-of-function *TAQPEP* alleles have similar effects on different pattern types—stripes in cats and spots in cheetahs are broadened and coalesced and exhibit reduced periodicity—suggests that TAQPEP is an important component of a general patterning mechanism.

Ticked ($Ti^A > Ti^+$) in Cats

Cats that carry the Ti^A allele do not exhibit tabby markings regardless of their *Tabby* genotype; thus, *Ticked* is epistatic to *Tabby*. A residual tabby pattern is observed occasionally on the legs, face, and tail, usually in Ti^A/Ti^+ heterozygotes (**Figure 8**). In domestic cats, the ticked phenotype is uncommon in the Western hemisphere but is reportedly found at higher frequencies in some parts of East Asia (11, 118).

Genome-wide linkage analysis in a pedigree segregating blotched tabby and ticked phenotypes allowed Lyons et al. (109) to map the *Ticked* locus to a 17-cM region on chromosome B1. (*Tabby* and *Ticked* were thought to represent a single locus at that time, which highlights the challenges of distinguishing epistatic from allelic relationships.) Eizirik et al. (110) later confirmed the location

of *Ticked*; comparative homology did not identify known pigmentation genes in other mammals as potential candidates.

One of the more interesting aspects of *Ticked* is its evolutionary origins. The African wild cat (indeed, all *Felis* sp.) exhibit tabby markings; thus, Ti^A is a derived allele. The ticked phenotype is apparent, however, in more distantly related felids, including the lion (*Panthera leo*), the caracal (*Caracal caracal*), the African golden cat (*Caracal aurata*), the jaguarondi (*Puma yagouaroundi*), and the puma (*Puma concolor*). Phylogenetic considerations (119) suggest that the ticked phenotype has arisen independently at least four times during felid evolution (*Felis*, *Puma*, *Caracal*, and *Panthera*). In at least two of these cases (the lion and the puma), tabby markings are apparent in young animals; thus, there may be several different loci in which mutations can suppress tabby markings.

Spotted

Not to be confused with white spotting, the spotted phenotype in the domestic cat involves the arrangement of dark tabby markings into a nonrandom pattern of cheetah-like spots on a lighter Agouti background. Observational studies (109) indicate that *Spotting* interacts with but is not allelic to *Tabby*: Spotting patterns can be observed only when the *Tabby* genotype is Ta^M/Ta^M or Ta^M/Ta^b and when the *Ticked* genotype is Ti^+/Ti^+. Furthermore, there exists a range of spotting patterns, which has given rise to phenotypic designations such as large spot, small spot, or broken mackerel. Thus, *Spotted* likely involves multiple loci.

CONCLUDING REMARKS

As indicated in **Tables 1** and **2**, molecular variants exist for 9 of 12 coat color loci in dogs and for 7 of 10 coat color loci in cats. Of the 16 loci with likely molecular identities, only 3 do not have obvious homologs in other mammals, but these examples (*Harlequin* and *K* in dogs, *Tabby* in cats) provide new insights into areas that transcend pigment cell biology. The ability of β-defensins to modulate melanocortin receptor activity is relevant to general aspects of cell and animal physiology, and identification of an extracellular metalloprotease as a component of periodic patterns provides an entry point for questions of long-standing interest to theoretical and developmental biologists.

Thus, just as observations on tortoiseshell cats provided important evidence in characterizing the process by which cells undergo X inactivation in females, certain aspects of dog and cat color variation have continued potential to inform additional basic questions in biology. Besides the likely Mendelian traits (*Tweed*, *Graying,* and *Ticking* in dogs; *Inhibitor*, *Orange*, and *Ticked* in cats), several complex traits remain unsolved mysteries, including variation in white spotting, variation in red-pigment dilution, and *Spotting* in cats. Together with genome sequences and genomic technology, continued partnership between basic scientists and companion animal breeders has the potential to solve these mysteries.

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Preface

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Animal coloration: production, perception, function and application

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Research on animal coloration is a vibrant area of biology currently involving evolutionary biologists, behavioural ecologists, psychologists, optical physicists, visual ecologists, geneticists and anthropologists. The proliferation of recent work requires that we take stock of the field, aiming to identify major themes, questions and future directions. This was the goal of the year-long Wissenschaftskolleg zu Berlin 'focus group' (2015–2016), in which many of the authors in this issue participated either as fellows or visitors. The result is this 19-chapter theme issue, in which we pinpoint the breakthroughs and challenges in animal coloration research, focusing on production, perception, function and evolution. We also explore animal coloration research as it applies to humans. This theme issue is by no means exhaustive but our goal has been to summarize and synthesize the state of animal coloration research in 2017 and to chart courses for the future.

The basic principles underlying animal coloration were formulated in a flurry of research during the second half of the 1800s and early part of the last century. These include the functional significance of coloration, involving protective coloration [1], disruptive coloration and countershading [2], sexually selected coloration [3], mimicry [4,5] and aposematism [6]. Use of colour phenotypes as genetic markers to study developmental processes and natural selection in the wild was critical to the early development of genetics and evolutionary theory [7]. In 1940, Cott's important volume [8] was a major milestone in our understanding of the functional significance of colour patterns, while Kettlewell [9] and Ford [10] spearheaded understanding of polymorphisms. In the 1960s and 1970s, we recognized that non-humans see the world differently from us, particularly in relation to ultraviolet perception [11]. Owing to the advent of spectrophotometry and digital imaging—combined with elegant laboratory and field studies, and large-scale comparative analyses—the field has since mushroomed. Now, the diversity and rapid pace of modern animal coloration research make it a particularly exciting interdisciplinary field.

This issue provides an entry point to recent developments in the main areas of animal coloration research: colour production, perception, function and evolution, and application. We present the articles in this order. Each topic covered in this special issue touches on the interdisciplinary nature of animal coloration research [12]. We also emphasize that this field not only draws on many disciplines but also contributes fundamental knowledge to those disciplines, and generates solutions for societal problems too [13]. We hope that this theme issue will enable readers to make better sense of this broad, growing and dynamic area of biology.

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THE ROYAL SOCIETY PUBLISHING

Guest editor profiles

Tim Caro is a Professor of Wildlife Biology at the University of California at Davis. He conducts research on the adaptive significance of mammalian coloration in the field and from a comparative perspective. His recent work has investigated the function of zebra stripes and the coloration of giant pandas. He organized a year-long focus group working on animal coloration at the Wissenschaftskolleg zu Berlin from which this series of papers emerged.

Mary Caswell Stoddard (Cassie) is an Assistant Professor in the Department of Ecology and Evolutionary Biology at Princeton University. She investigates animal sensory ecology and physiology, with a focus on avian vision and coloration. Using a multidisciplinary approach, including techniques from computer vision and optics, she has explored the evolution of structural and pigment-based plumage colours, cuckoo egg mimicry, shorebird clutch camouflage, avian colour perception and pattern recognition.

Devi Stuart-Fox is an Associate Professor at the University of Melbourne. Her research focuses on the function and evolution of animal coloration, particularly colour change and colour polymorphism. She has worked on a wide variety of species in different parts of the world including chameleons in South Africa, gliding lizards in Malaysia and numerous species across Australia. Her research uses a variety of approaches including evolutionary genetics, pigment cell biology, behaviour and macroevolutionary analyses.

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