



ANNUAL REVIEWS **Further**

Click [here](#) for quick links to Annual Reviews content online, including:

- Other articles in this volume
- Top cited articles
- Top downloaded articles
- Our comprehensive search

Evolution of Coloration Patterns

Meredith E. Protas¹ and Nipam H. Patel^{2,3}

¹Department of Integrative Biology, ²Department of Molecular and Cell Biology, and ³Howard Hughes Medical Institute, University of California, Berkeley, California 94720-3140; email: nipam@uclink.berkeley.edu

Annu. Rev. Cell Dev. Biol. 2008. 24:425–46

First published online as a Review in Advance on July 1, 2008

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

This article's doi:
10.1146/annurev.cellbio.24.110707.175302

Copyright © 2008 by Annual Reviews.
All rights reserved

1081-0706/08/1110-0425\$20.00

Key Words

pigmentation, morphological change, genetic mapping, mimicry

Abstract

There is an amazing amount of diversity in coloration patterns in nature. The ease of observing this diversity and the recent application of genetic and molecular techniques to model and nonmodel animals are allowing us to investigate the genetic basis and evolution of coloration in an ever-increasing variety of animals. It is now possible to ask questions about how many genes are responsible for any given pattern, what types of genetic changes have occurred to generate the diversity, and if the same underlying genetic changes occur repeatedly when coloration phenotypes arise through convergent evolution or parallel evolution.

Contents

BACKGROUND.....	426
FUNCTIONS OF COLORATION...	426
EARLY GENETIC STUDIES OF COLORATION AND STUDIES OF COLORATION IN MODEL SYSTEMS.....	431
NATURAL COLOR VARIATION IN NONMODEL SPECIES.....	432
CANDIDATE GENE APPROACH: GENETIC ASSOCIATIONS IN WILD POPULATIONS.....	433
CANDIDATE GENE APPROACH: COMPARATIVE EXPRESSION ANALYSIS.....	434
COMPLEMENTATION CROSSES WITH MUTANTS IN A MODEL SPECIES.....	434
MAPPING APPROACH.....	435
THE GENETIC BASIS OF ENVIRONMENTAL EFFECTS ON COLORATION.....	438
MANY GENES OR FEW GENES?..	438
REGULATORY VERSUS CODING CHANGES?.....	439
SAME GENES OR DIFFERENT GENES?.....	439
STANDING VERSUS NOVEL VARIATION?.....	441
CONCLUSION.....	442

BACKGROUND

The remarkable diversity of coloration patterns seen in animals is one of the most striking features in the natural world. Examples include *Melanitis* butterflies that resemble dried vegetation, flounder and flatfish that are pigmented on only one-half of their body and alter their appearance to match their surroundings, the bold black and white stripes of zebras, brilliantly colored tropical fish and birds, and cavefish that lack pigment altogether (**Figure 1**). Countless examples exist of unique colors and patterns for all types of animals. This varia-

tion, and the ease of observing it, has made the study of coloration patterns a popular and tractable subject for scientific inquiry. In the past few decades, genetic, molecular, biochemical, and cellular approaches in both model and nonmodel species have allowed us to understand many details of the basis for color patterns during development. In addition, it is possible to surmise, and sometimes experimentally validate, the selective pressures behind animal coloration, allowing one to approach the question of how these patterns evolved.

FUNCTIONS OF COLORATION

When one is studying coloration from an evolutionary perspective, the first question that comes to mind is, What is the function of a particular pattern? In cases such as the dead-leaf butterfly, functional significance is fairly obvious: Appearing leaf-like may cause predators to misidentify potential prey (**Figure 2**). The functional and evolutionary significance of other examples, such as a zebra's stripes, is not as obvious. Many functions of coloration have been proposed: concealment, thermoregulation, warning of toxicity, mimicry, sexual selection, and linkage to beneficial characteristics such as immunity and salinity tolerance (reviewed in Roulin 2004). Finally, it is always possible that a certain coloration pattern evolved by chance through processes such as genetic drift.

Concealment is a very common function of coloration. Many animals, for example, sea dragons, when viewed in their natural habitat, blend in almost perfectly with their surroundings (**Figure 3a**). This is all the more striking when multiple populations of a single species living in different habitats have distinct coloration forms that match each environment (**Figure 4**). This phenomenon has been observed in many species; rock pocket mice, beach mice, deer mice, and fence lizards are just some examples within the vertebrates (Hoekstra 2006, Hoekstra et al. 2006, Mundy 2005). Rock pocket mice of the southwestern United States and Mexico provide a particularly clear example. Most live predominantly on

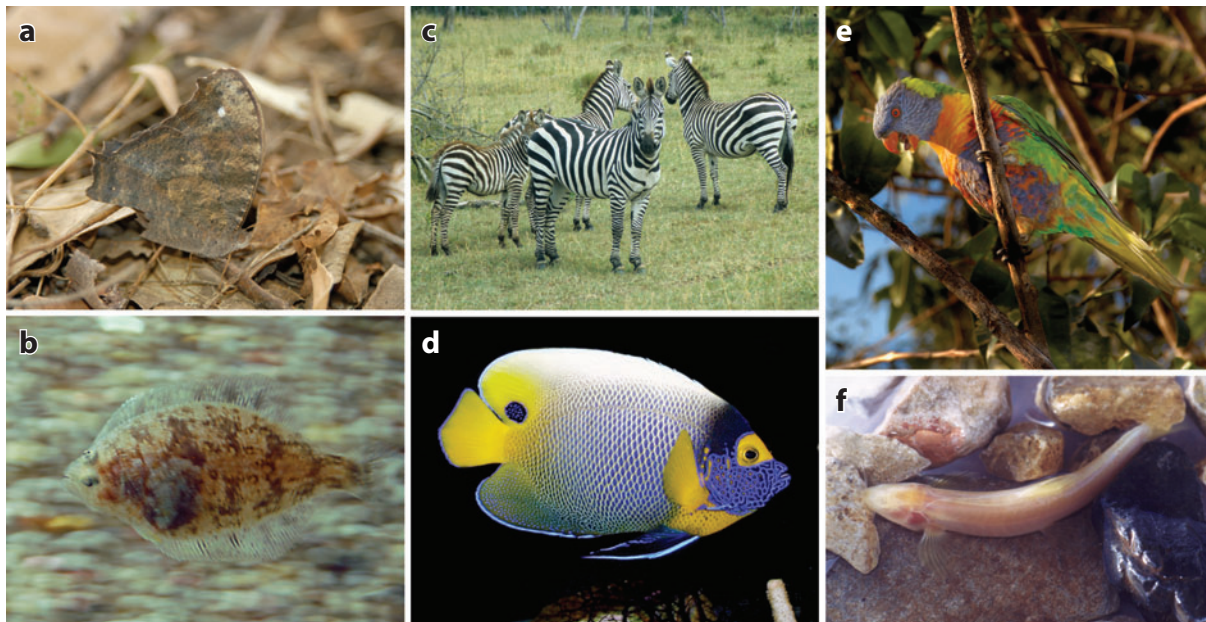


Figure 1

Some samples of the diversity of animal coloration patterns. (*a*) *Melanitis* butterfly, whose underside resembles the dried vegetation on which it sits. (*b*) Flatfish that can change coloration on the upper side of its body to blend into the background. (*c*) A group of zebras with their striking black and white stripes. (*d*) Blueface angelfish. (*e*) Rainbow lorikeet. (*f*) The unpigmented blind cave loach *Nemacheilus troglotactaractus*, from Thailand (image courtesy of R. Borowsky).

lightly colored rocks, and these mice have light-colored fur. However, there are also some populations of rock pocket mice that colonized dark lava flows (some of which are just a few thousand years old), and these individuals have dark fur (Hoekstra & Nachman 2003). It is thought that the melanic form provides better camouflage in the lava flow environment, lessening the chance of predation.

Coloration is often a constant feature of an animal throughout its life. However, there are cases when the animal's coloration changes at different times and in different environments. This ability to change coloration is often advantageous for animals that move around on different-colored substrates. One classic example is that of the cuttlefish (**Figure 3b**). Cuttlefish skin changes in both color and texture as the animal moves. It is thought that this change of color and pattern is advantageous for camouflage as well as for signaling to other individuals (Barbato et al. 2007).

Another mechanism by which coloration patterns disguise an animal is by disruptive coloration, a phenomenon in which a color pattern breaks up the animal's form so that it is difficult to identify the real outline of the animal. A common way of disguising the boundaries of an animal is to hide the eyes by an eye mask pattern and thus distort one of the most identifiable features of a prey item. Also potentially disruptive are black and white lines intersecting the outline of the animal, e.g., tapirs and pandas (Caro 2005).

Whereas many forms of coloration camouflage an animal, there are many examples of conspicuous coloration used as a warning to potential predators. Black, red, orange, and yellow often indicate that the species is distasteful. The yellow and black ant, *Crematogaster inflata*, produces chemicals, including 5-*n*-alkyl resorcinols, that make them unpalatable to some predators, and the predators learn to avoid these ants (Ito et al. 2004). Often, multiple



Figure 2

The within-population morphological diversity of dead-leaf butterflies of the species *Kallima inachus*. The butterflies were all collected within a small geographic region. Although all the butterflies resemble dead leaves on their undersides, the variation on the basic leaf pattern is quite remarkable. This variation may help ensure that predators cannot cue on a specific pattern element to distinguish the butterflies from the general leaf litter.

unpalatable species not only will use the same bright colors but will closely mimic other species in pattern as well. This phenomenon, in which multiple species share the same warning coloration pattern, is called Müllerian mimicry and is particularly well studied in butterflies and moths (**Figures 5** and **6**) (reviewed in Parchem et al. 2007). Batesian mimicry, in contrast, refers to instances in which a palatable species mimics the coloration pattern of an unpalatable one and thereby evades predation (**Figure 5**). Ants of the genus *Camponotus* may utilize Batesian mimicry; *Camponotus* individuals overlap in territory with the previously mentioned unpalatable *C. inflata* and have a very similar coloration. Chick-feeding experiments determined that *Camponotus* individuals were palatable whereas *C. inflata* individuals were not. Chicks that had previously eaten *C. inflata*, however,

rarely attempted to eat *Camponotus* (Ito et al. 2004).

Some animals also employ coloration to startle predators. For example, some moths, when resting, rely primarily on cryptic coloration but, when startled, raise their forewings to expose a brightly colored area on their hindwings (**Figure 3c,d**). A variation of the startle response is to have a striking coloration pattern (false heads, eye spots, or vivid coloration of tails) in a noncritical part of the animal to draw the predators' attention away from the most vulnerable area of the body.

Coloration also seems to play an important role in sexual selection (**Figure 3e**). Studies in birds and other animals with color polymorphisms show that pigmentation can influence mate choice. In feral pigeons, irrespective of the female phenotype, the most desired male phenotype is that of blue checker males (reviewed in

Müllerian mimicry:

phenomenon in which an unpalatable organism mimics the appearance of another unpalatable one

Batesian mimicry:

phenomenon in which a palatable organism mimics the appearance of an unpalatable one

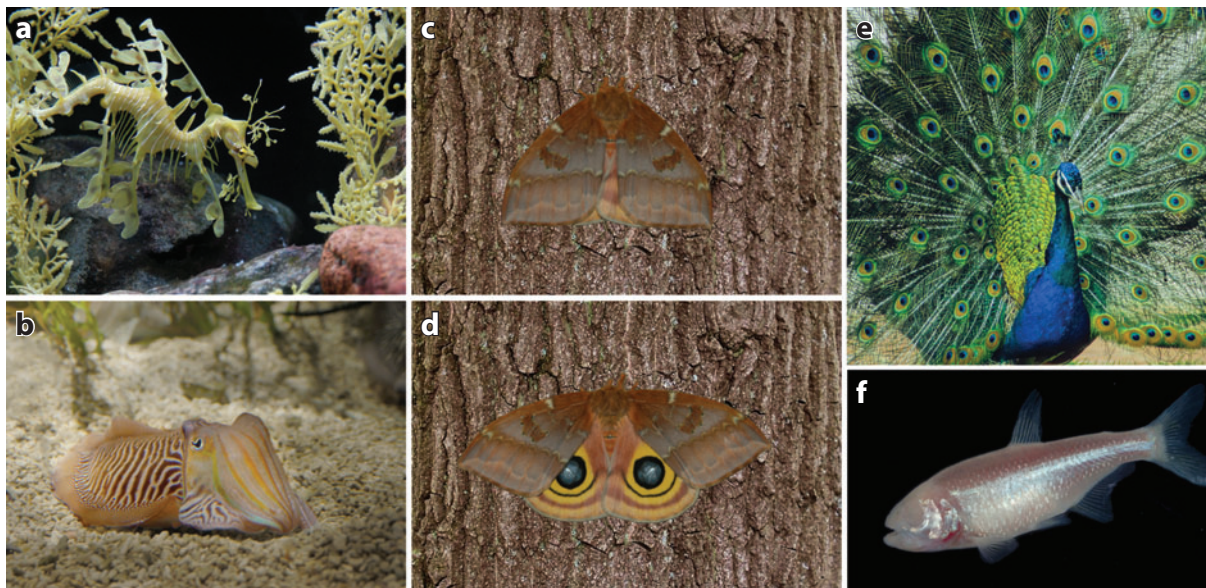


Figure 3

Examples of the many functions of coloration. (a) Both the coloration and shape of the leafy sea dragon help it to blend in perfectly with its surroundings. (b) A cuttlefish can quickly change its colors either to blend in or to communicate. (c) While at rest, the moth *Automeris io* is well camouflaged. (d) Shown is the same moth as in panel c, but after being disturbed. By raising its forewings, it displays the brightly colored eyespots of the hindwings, which are thought to startle a would-be predator. (e) A male peacock, whose brilliant coloration is thought to be due to sexual selection. (f) The cavefish *Astyanax mexicanus*, which lacks the pigmentation found on its surface relatives (image courtesy of R. Borowsky).

Roulin 2004). Additionally, in guppies, females prefer males with the most orange color, and this preference is strongest in populations that contain males with a large amount of orange

color (Houde & Endler 1990). There is a balance, however, between the advantage of being conspicuous to females and the disadvantage of being conspicuous to predators.

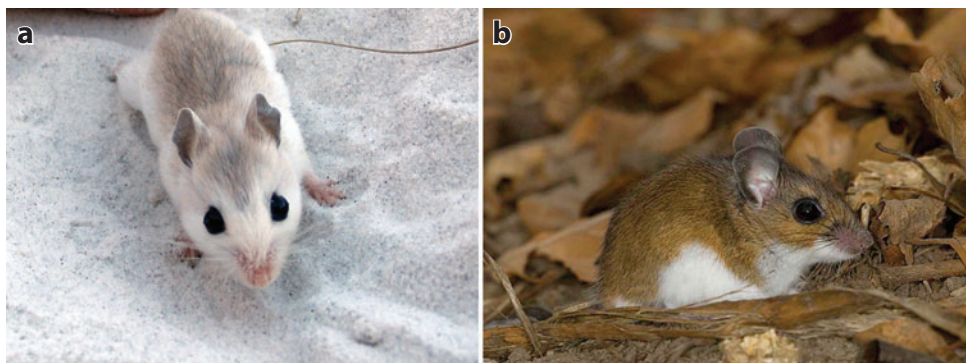


Figure 4

Intraspecific pigmentation differences in oldfield mice, *Peromyscus polionotus*. (a) A beach-dwelling mouse whose coloration matches its light-colored sandy habitat (image courtesy of C. Steiner). (b) A mainland-dwelling mouse whose brown color matches its habitat (image courtesy of S. Cary).



Figure 5

Examples of Batesian and Müllerian mimicry. In the left-most column are heliconid and ithomid butterflies that are thought to be unpalatable. In the middle column are pericopid moths that are also thought to be unpalatable. In the right-most column are pierid butterflies of the genus *Dismorphia* that are thought to be palatable. Thus, within each row, the left and middle Lepidoptera are examples of Müllerian mimics, whereas the right-most specimen is a Batesian mimic.

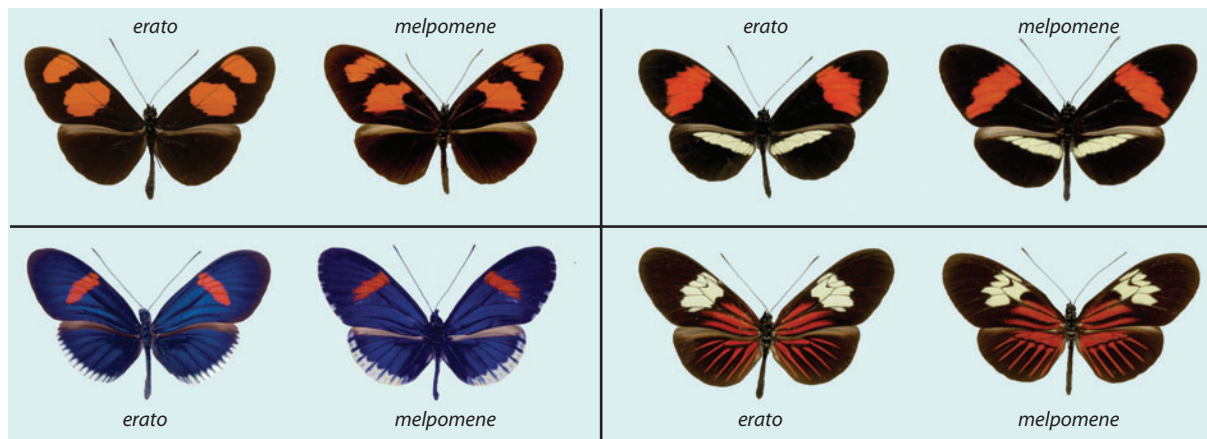


Figure 6

Heliconius mimicry. Each quadrant shows two *Heliconius* butterflies that look strikingly similar, but within each quadrant, the left specimen is *Heliconius erato*, whereas the right specimen is *Heliconius melpomene*. Thus, the two species show incredible variation, but where their geographic ranges overlap, the two species have evolved to be comimics (Müllerian mimics). For example, the *erato/melpomene* specimens shown in the upper left quadrant are found in the Chanchamayo region of Peru, whereas the ones in the lower left quadrant are found in Ecuador.

A particular pattern may not have an adaptive function. One potential example is the loss of pigmentation in cave animals (**Figure 3f**). Because of the dark habitat of these animals, pigmentation cannot provide typical functions, e.g., protection from ultraviolet radiation, concealment from predators and prey, and mate choice. Indeed, one of the theories for pigmentation loss in cavefish is that the cave environment predicts no selective advantage for this trait and therefore the trait is lost (Culver 1982). There are many other examples for which there appears to be no obvious adaptive function of a particular coloration pattern, but it is difficult to know what additional characteristics are affected by, or linked to, coloration.

All these possible functions give us some idea of the evolutionary forces that underlie the remarkable diversity of coloration patterns in animals. In recent years, it has been possible to move beyond observation and begin exploring the evolution of this amazing diversity at the genetic and molecular levels. By the use of both model and nonmodel species, it is now possible to begin addressing the following kinds of questions: What is the molecular basis of coloration patterns? How many genes are involved in orchestrating differences in coloration between species or between individuals within a population? What types of mutations cause phenotypic changes? If the same coloration pattern occurs repeatedly across taxa, will the same genes be responsible?

EARLY GENETIC STUDIES OF COLORATION AND STUDIES OF COLORATION IN MODEL SYSTEMS

Genetic methods have been used for many years to study coloration differences. More than a thousand years ago in China, selective breeding was used to create various colors of goldfish. Mice breeding for coat color variation started in China and Japan as early as the eighteenth century. Later, in the nineteenth century, Mendel's laws of inheritance were formulated by the use of traits in peas that included flower, seed, and

pod color. Mendel's rules were then applied in the early 1900s to examine the genetic basis of coat coloration in rodents (reviewed in Russell 1985).

Since these early days of genetic studies of coloration, extensive work has been performed to study coloration in various model species, including the fly *Drosophila melanogaster*, the mouse *Mus musculus*, and the zebrafish *Danio rerio*. Traditionally, large-scale mutagenesis screens for color mutants were carried out, the mutations were mapped, and the causative (i.e., mutated) genes were identified and cloned. We briefly discuss some of the major players in the pigmentation pathways of these three model systems (focusing on those that have been further studied in nonmodel systems) and then discuss nonmodel systems.

In *D. melanogaster*, epidermal cells produce and secrete cuticular pigments that give the body its characteristic brownish color with black bristles and dark abdominal stripes. Several structural genes that function in pigment synthesis include *yellow*, *pale*, *Ddc* (*dopa decarboxylase*), *ebony*, *black*, *tan*, and *aaNAT* (*N-acetyl transferase*), whereas regulatory genes, such as *optomotor-blind* and *bric-a-brac*, function in establishing the pigment pattern in *D. melanogaster* (reviewed in Wittkopp et al. 2003a).

Pigmentation in mice is also a vast field of research. There are more than 127 loci in mice affecting coloration (Bennett & Lamoreux 2003). Mammalian melanocytes, or pigment cells, are derived from the neural crest and migrate to various areas of the body (Bennett & Lamoreux 2003). Just as in *D. melanogaster*, there are several classes of genes that affect pigmentation in mice. For example, there are the spotting loci that affect melanocyte development. Examples of these genes are *Kit* and *Mitf*. Other genes such as *Tyrp1* (*Tyrosinase-related protein 1*), *Tyrosinase*, *Oca2* (*Ocular and cutaneous albinism 2*), and *Matp* (*Membrane-associated transporter protein*) affect the synthesis of melanin. Yet another group of genes affecting pigment includes those that control the switch between eumelanin and phaeomelanin production.

Melanophores, melanocytes: brown pigment- or black pigment-containing cells

Chromatophore: a cell in amphibians, reptiles, and fish that contains pigment or reflects light

Xanthophore: a yellow pigment-containing cell

Iridophore: an iridescent pigment cell

Complementation test: mating two individuals that have similar phenotypes to each other to ascertain if they have mutations in the same or different genes

Linkage map: the arrangement of genetic markers into groups that correspond to either entire chromosomes or pieces of chromosomes

Eumelanin produces a dark (brown to black) color, and pheomelanin produces a light (red to yellow) color. Major genes involved in this switch are *Melanocortin-1 receptor (Mclr)*, *Melanocyte-stimulating hormone (MSH)*, and *Agouti signaling protein (ASP)*.

Pigment cells in *D. rerio* also derive from the neural crest. There are three different types of pigment cells or chromatophores: black melanophores; yellow or orange xanthophores; and blue, silver, or gold iridophores (Kelsh 2004, Kelsh et al. 1996, Parichy 2006). Mutagenesis screens have generated many different phenotypes that fall into various classes: no chromatophores, fewer melanophores, fewer xanthophores, fewer iridophores, patterning defects, and ectopic chromatophores (Kelsh et al. 1996). Several of the genes responsible for these mutations have been cloned, and many of the genes involved in pigmentation in mice and humans are conserved in zebrafish (Camp & Lardelli 2001, Quigley & Parichy 2002).

When one is studying natural color variation, however, it is important to keep in mind the ways in which natural variants differ from the mutants uncovered by mutagenesis screens. First, coloration mutants created by mutagenesis may be viable in the controlled environment of a laboratory but may not be capable of surviving in the natural environment. As a caveat, however, it is important to remember that the phenotype of null alleles may be most obvious in a screen, but natural variation may still utilize weaker alleles of the same gene. Second, the timescale, population size, and genetic background in a genetic screen are very different from those that exist during the evolutionary processes that generate natural variation. One potential consequence of this is that phenotypes that can result from the mutation of a single gene in the lab may be created by the sum of several mutations in the natural world. Thus, to truly understand the evolution of coloration, it is necessary to examine color variation within naturally occurring species.

In some ways, studying coloration patterns in domestic animals provides an intermediate between genetic model systems and natu-

ral populations (Andersson & Georges 2004, Schmutz & Berryere 2007). The insights from these studies are highly informative for questions of the evolution of coloration and coloration pattern, but owing to space constraints, this review discusses only examples of naturally occurring populations.

NATURAL COLOR VARIATION IN NONMODEL SPECIES

Certain species are better suited than others for investigating questions of natural variation. For example, there is an enormous difference in the color and pattern of a giraffe versus those of a zebra. However, it is difficult to compare these two animals because they differ in so many other ways. But it is useful, at least initially, to examine coloration on a microevolutionary scale—within single species that are polymorphic in coloration or between related species that have diverged in coloration but are still close enough to interbreed. This kind of intraspecific variation in coloration is fairly common; for example, such variation exists in 3.5% of all bird species (Roulin 2004). Data from such microevolutionary studies can then be applied to understand coloration changes on a macroevolutionary scale.

The types of methods used to study coloration on a microevolutionary scale include genetic crosses, gene expression analyses (through *in situ* hybridization and microarray analysis), complementation studies, and linkage mapping. First, we describe each of these methods along with examples in which color patterns have been investigated. Then, we return to the question of how pigment patterns evolve, and we discuss the insights that recent studies have provided.

Among the first methods used to study coloration in nonmodel species were genetic crosses between individuals from populations with distinct coloration differences. One classic example comes from the snail *Cepea nemoralis*. The lip of the shell can be black, dark brown, pink, or white, whereas the rest of the shell is light tan, pink, orange, or red with one to five

dark bands (Cain & Sheppard 1954). Crosses between individuals with different coloration patterns indicated that a single locus is responsible for shell and lip color, the presence or absence of bands, and band pigmentation (reviewed in Jones et al. 1977). The number of bands, however, appears to be unlinked to this gene.

Crossing individuals with different phenotypes allows investigators to ask how many genes are responsible for a certain phenotype, which alleles are recessive and which are dominant, and what phenotypes are linked to each other. The next step is to delve into the pathways, and ultimately the mutations, that are modified to create variation in coloration. To accomplish this, one can use information known about pigmentation and coloration in model species and apply that information to nonmodel species.

CANDIDATE GENE APPROACH: GENETIC ASSOCIATIONS IN WILD POPULATIONS

The most common method employed in recent years to study the genetic basis of evolutionary variation in color is a candidate gene approach. As discussed above, pigmentation pathways in model organisms are generally well studied, and some of the major genes are conserved. These genes then become candidates for study in non-model systems. The disadvantages of using the candidate gene approach include selection bias and potentially long (and, ultimately, unsuccessful) lists of numerous candidate genes. A candidate gene approach, however, is sometimes the only option if one is working with a nonmodel species with few genetic resources.

The most studied coloration gene in non-model vertebrates is *Mcl1r*, a transmembrane receptor expressed in melanocytes that, when modified, generally produces changes in coloration of the entire body. *Mcl1r* is thought to be responsible for intraspecies coloration differences in at least four different bird species (Doucet et al. 2004, Mundy et al. 2004, Theron et al. 2001). For example, Caribbean

bananaquits have melanic morphs, which generally live in forests, and paler morphs, which usually live in drier lowland areas. *Mcl1r* was sequenced from pale and melanic individuals, and the melanic form of the bird has an amino acid substitution also found in melanic forms of chicken and mice (Theron et al. 2001). In Arctic skuas, another polymorphism in *Mcl1r* correlates with the coloration phenotype (Mundy et al. 2004). The lesser snow goose has six different classes of pigmentation, and the degree of melanism is associated with a different amino acid substitution in *Mcl1r* (Mundy et al. 2004). One final example, in birds, is that of the fairy wren. Male mainland fairy wrens have bright blue nuptial coloring, whereas island populations have black nuptial coloring, a phenotypic difference also associated with polymorphisms in *Mcl1r* (Doucet et al. 2004).

Pocket mice are another example in which the candidate gene approach was used to test if there was an association between coloration and *Mcl1r*. As mentioned above, the sandy-colored mice live in an area that has pale rocks, whereas the melanic form lives on dark lava flows. An association was found between four linked amino acid polymorphisms in *Mcl1r* and coloration in one lava-dwelling population of melanic rock pocket mice (Hoekstra & Nachman 2003). Furthermore, *Mcl1r* is associated with variation in coloration in jaguars, jaguarundis, and reptiles (Eizirik et al. 2003, Rosenblum et al. 2004).

Most of these studies used the approach of sequencing the coding region of *Mcl1r* from wild individuals and comparing genotypes with coloration. This allows for the discovery of associations while avoiding laborious and time-consuming interspecific and intraspecific breeding programs. The primary disadvantage of this approach is that it is often difficult to determine experimentally that the changes in the *Mcl1r* sequence actually cause the coloration differences. Even when the results from this approach are combined with genetic mapping data (see below), the observed association between an *Mcl1r* genotype in the coding sequence and a melanic phenotype may instead be due to

a regulatory mutation in *Mc1r* or a mutation in a different gene very closely linked to *Mc1r*.

CANDIDATE GENE APPROACH: COMPARATIVE EXPRESSION ANALYSIS

Above, we discuss the candidate gene approach in the context of testing whether genetic polymorphisms are associated with a certain phenotype. Another method used to investigate a particular candidate gene is to see if the gene's expression pattern is correlated with a coloration phenotype. Such an association does not necessarily mean that a particular gene is the locus modified to produce the genetic change; e.g., changes in a gene's expression pattern may also be the result of regulatory changes in upstream regulators of that gene. An example of the utility of the expression analysis method is a wing spot study done in *Drosophila biarmipes* (Gompel et al. 2005). *D. biarmipes* has a dark pigmented spot on its wing that is absent from the closely related *D. melanogaster*. The gene, *yellow*, is expressed at very low levels throughout the wing of *D. melanogaster* but highly expressed in the area that forms the pigmented spot in *D. biarmipes* (Gompel et al. 2005). The advantage to working with species closely related to a model system (such as *D. melanogaster*) is the myriad of ways to functionally test hypotheses about coloration patterns. Indeed, Gompel et al. (2005) were able to provide evidence that changes in the *yellow* gene itself, as opposed to changes in a different gene that acts as a regulator of *yellow* expression, are responsible for the expression differences. These authors made transgenic *D. melanogaster* that contained the upstream regulatory region of *yellow* from *D. biarmipes* driving expression of the fluorescent protein GFP. These investigators found that this reporter construct was expressed in *D. melanogaster* in a similar manner to that seen for *yellow* in *D. biarmipes*. This provides good evidence that the expression pattern differences seen for *yellow* between the two species are due to evolutionary changes in the regulatory re-

gions of the *yellow* gene. However, when Gompel et al. (2005) rescued *D. melanogaster yellow* mutants with a construct of the *D. biarmipes yellow* gene that presumably drives expression in the wing, no pigmented wing spot was observed. Because of this, the authors argue that other genetic changes, in addition to a regulatory change in *yellow*, are responsible for the observed coloration difference.

Another example of the candidate gene expression analysis approach was taken with the gene *bric-a-brac* in *Drosophila willistoni* and *D. melanogaster*. *D. melanogaster* has sexually dimorphic pigmentation patterns, whereas *D. willistoni* does not; expression of *bric-a-brac* is correlated with these coloration differences, and the gene maps to a locus accounting for the coloration differences (Kopp et al. 2000). Finally, within cichlid fish, a correlation was seen between the expression of *fms*, a type III receptor tyrosine kinase thought to be a duplicated form of *kit*, and the presence of egg dumplings (pigmentation spots that look like eggs) on the anal fins of male cichlids (Mellgren & Johnson 2002, Salzburger et al. 2007).

This sort of expression analysis is a powerful tool, but it is sometimes difficult to perform on nonmodel organisms. Also, coloration occurs relatively late during embryonic development, and it is not always possible to conduct these analyses on late-stage embryos or juvenile animals for technical reasons. As an alternative, other methods, including microarray analysis and quantitative polymerase chain reaction (PCR), can also be used to look at expression levels in different parts of the animal during development (Reed et al. 2008).

COMPLEMENTATION CROSSES WITH MUTANTS IN A MODEL SPECIES

Another clever way to figure out the genetic basis of a coloration pattern is by a complementation test, a method that can be used only to detect recessive genetic determinants. Complementation tests allow one to ask if the same gene is affected in two species (that have similar

phenotypes) by crossing them. If the variation is in the same gene, the offspring will have the same pattern as both parents. If, however, the affected genes are not the same, complementation will occur, and the offspring will look different from either parent.

A modified version of this method has been used to investigate the genetic makeup of coloration in species of the *Danio* genus. By crossing different *Danio* species to *D. rerio* mutants, investigators could assay whether certain genes were responsible for the difference in coloration between different *Danio* species. *D. rerio* has four to five horizontal stripes on its body. The closely related *Danio albolineatus* has no stripes and a more dispersed pattern of pigment cells (Parichy & Johnson 2001). Numerous screens have been performed in *D. rerio* to isolate coloration mutants. Seventeen *D. rerio* mutants in genes of the coloration pathway or neural crest development were crossed to *D. albolineatus* (Quigley et al. 2005). All the mutants complemented except for *fms* mutants (zebrafish *fms* and *kit* are thought to have resulted from an ancient duplication of *kit*). This indicates that the *fms* gene or *fms* pathway underlies the difference in appearance in the two species.

Further experiments addressed whether characteristics of pigment cell development in the *D. rerio fms* mutant were similar to those of *D. albolineatus*. *D. albolineatus* had fewer melanophores, increased melanophore death, and aberrant melanophore migration, all similar characteristics of the *D. rerio fms* mutant (Quigley et al. 2005). However, *D. rerio fms* mutants also have fewer xanthophores than does *D. albolineatus*. Because of this discrepancy, Quigley et al. (2005) suggest that genetic changes in the *fms* gene are not responsible for the difference in coloration between *D. rerio* and *D. albolineatus*. Instead, changes in the *fms* pathway are responsible for the observed phenotypic differences. This would imply that the reduced functions of two different genes in the *fms* pathway combined cause the cross between *D. albolineatus* and the *D. rerio fms* mutant not to complement.

MAPPING APPROACH

The candidate gene approach is very powerful for systems in which many of the genes involved are known and well conserved in nonmodel organisms. Although the candidate gene approach often works well, novel gene(s) may be responsible for the observed coloration differences. For example, candidate genes for coloration differences may include members of the biosynthetic pathway of a pigment. However, previously uncharacterized genes that are upstream of this pathway or that regulate the pathway may also affect the coloration of an animal. A mapping approach is a less biased method that can also uncover novel genes. Such an approach involves examining a cross of individuals with a number of genetic markers, phenotyping the members of the cross, and from this determining regions of the genome where loci responsible for these phenotypes occur. The initial results of such an approach generally narrow down the search significantly, but hundreds of genes can be within the mapped region. Often a candidate gene approach may then further narrow the analysis to a small handful of genes. It is starting to become possible, however, to use a completely unbiased approach (positional cloning) on nonmodel organisms in which sequence information from BAC or whole-genome sequencing is available (e.g., Colosimo et al. 2005, Miller et al. 2007).

Mapping approaches have been used to examine morphological changes in the extremely diverse group of East African cichlid fishes (**Figure 7**) (Albertson et al. 2003). One coloration phenotype examined is the orange blotch phenotype (**Figure 7a**), which is found mainly in females and is of unknown function (Streelman et al. 2003). Another coloration phenotype found in the same species is blue with black bars (**Figure 7b**). A blue-with-black-bar male was crossed to an orange blotch female to generate F1 hybrids that were then crossed to generate F2s (Streelman et al. 2003). Multiple genetic markers were used to genotype the F2s, and then these genetic data were compared with phenotypes to define a region containing a gene or genes responsible for

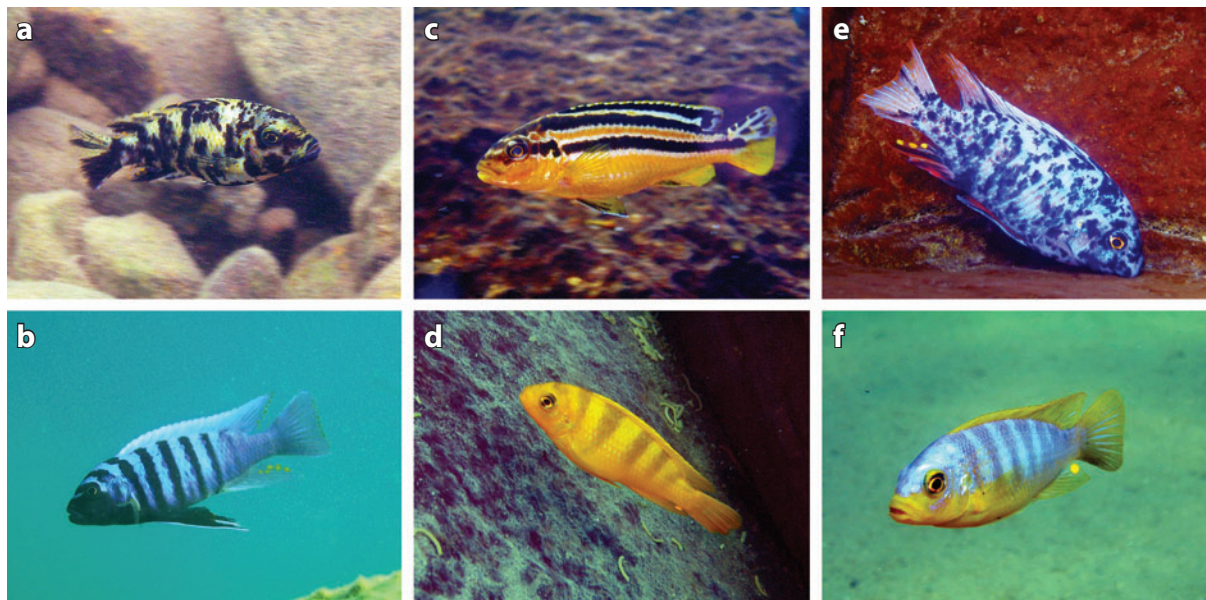


Figure 7

Coloration differences in cichlids. In the Great Lakes of East Africa, there are almost 2000 species of cichlids that have evolved relatively recently with a wide variety of color patterns. (a) Orange blotch phenotype in a female *Metriaclima zebra*. (b) Blue-with-black-bar phenotype in a male *M. zebra*. (c) *Metriaclima aurora*. (d) *Metriaclima lombardoi*. (e) *Labetropheus fueelleborni*. (f) *Metriaclima auratus*. (All cichlid images are courtesy of R. Roberts.)

Monogenic: a trait that is encoded by one gene

Polygenic: a trait that is encoded by more than one gene

Quantitative trait locus (QTL): an area on a genetic linkage map that is responsible for a certain amount of variance in a measured trait

the orange blotch phenotype. Then, the researchers genotyped approximately 65 wild-caught individuals for the markers flanking this region to see whether this region was associated with the coloration phenotype in the wild. The same genetic marker that showed the highest association to coloration phenotype in the lab-raised cross also showed the highest association to coloration in the wild-caught individuals. Because there was little genomic information available for this species, the markers flanking the orange blotch locus were compared with sequence information from the pufferfish *Takifugu rubripes*. From this, the researchers identified a syntenic region that contained several genes, some of which are promising candidate genes. Ideally, one would now attempt to test these candidate genes within the cichlids by transgenesis, knockdown, or overexpression. Indeed, in other nonmodel fish species, some of these techniques have already been described (Colosimo et al. 2005, Hosemann et al. 2004, Yamamoto et al. 2004).

Figuring out the genetic basis of phenotypic variation is easiest for monogenic traits. However, some coloration variation appears to be polygenic. One recent example is the old-field mouse, *Peromyscus polionotus*, which has darkly colored populations and lightly colored populations (Figure 4) (Hoekstra et al. 2006, Steiner et al. 2007). The lightly colored beach mouse lives in dunes and islands, and it is thought that the light color protects the animals from predators by providing camouflage. A large F2 cross between the beach and mainland populations was used to generate a linkage map, which identified three quantitative trait loci (QTLs) (regions where genes responsible for a certain trait reside). The coloration pathway of both mice and humans has been well studied. Ten coloration candidate genes were placed on the map, and of these, *Agouti*, *Mcl1r*, and *Kit* mapped to the three QTLs. Mapping to a QTL does not prove that the gene is responsible for a particular QTL because there are many other unidentified genes in that region. However, a

previous paper examined the sequence of *Mc1r* in beach and mainland mice and found that there was an amino acid change in a critical part of the protein (Hoekstra et al. 2006). Comparing the beach and mainland MC1R in an in vitro assay to test MC1R function showed that the beach MC1R had a significantly lower activity level than did the mainland MC1R. The coding sequence of *Agouti* in the beach and mainland mice was also compared, but no differences were observed (Steiner et al. 2007). The expression level of *Agouti* was examined, comparing beach and mainland mice by RT-PCR, and it was found that the level of *Agouti* was higher in the beach versus the mainland mice—consistent with the observation in laboratory mouse strains that higher levels of *Agouti* lead to light pigmentation (Steiner et al. 2007). The authors hypothesize that a regulatory mutation in *Agouti*, rather than a coding mutation, is responsible for the phenotypic difference between the two populations.

As outlined above, mapping combined with the candidate gene approach has potential for successfully investigating the genetic basis of coloration pattern. However, it is always possible that no matter how many candidate genes are added to the map, few, if any, will ever coincide with the loci responsible for coloration differences. For example, extensive genetic crosses and mapping have been done between different species and populations of *Heliconius* to map loci responsible for the position, size, and shape of patterns on the wings (**Figure 6**) (Jiggins et al. 2005, Kapan et al. 2006, Töbler et al. 2005). Many different genes known to be involved in *Drosophila* wing patterning or pigment biosynthesis, such as *apterous*, *distal-less*, *hedgehog*, *patched*, *cubitus interruptus*, *vermillion*, and *cinnabar*, have been placed on the map, and none of them map to the pattern loci being studied (Jiggins et al. 2005, Kapan et al. 2006, Töbler et al. 2005). Therefore, it remains to be determined whether novel wing-patterning genes or candidate genes other than those studied are responsible for the varied coloration phenotypes in *Heliconius* butterflies.

Similar to the difficulties experienced in predicting the genes responsible for coloration differences in *Heliconius* butterflies, the majority of studies in drosophilid species have not observed associations between candidate genes and coloration phenotypes (Wittkopp et al. 2003a). However, an association was seen between variation in coloration and the gene *ebony* in the offspring of crosses between *Drosophila americana* and *Drosophila novamexicana* (Wittkopp et al. 2003b), and as mentioned above, sexually dimorphic pigmentation in *D. melanogaster* is associated with the gene *bric-a-brac* (Kopp et al. 2003). In cases in which the candidate gene method is not able to predict the genes responsible for a coloration trait, the ideal method to use is a positional cloning approach. The difficulty is that to use this approach one must have either the genome sequence of the species of interest or a large amount of sequence information from BAC sequencing. Within this sequence, it is possible to identify a large number of polymorphic sites that can then be used for high-resolution mapping.

Recently, a coloration difference in sticklebacks was investigated by high-resolution mapping and a candidate gene approach (Miller et al. 2007). Some freshwater populations have lightly melanized gills and ventral surfaces, whereas marine populations have heavily melanized gills and ventral surfaces. A major QTL was found for this phenotype and narrowed down by genotyping a total of 1182 F2 fish. The recombination data from these individuals and a draft of the stickleback genome allowed the area to be narrowed down to a 4.5-mb region. Additional genetic markers were designed within this region. By typing the recombinants with the new markers, Miller et al. (2007) narrowed the gene responsible for this QTL to an interval of 315 kb. Fifteen genes resided within this region, and only one had been previously implied to function in pigmentation, *Kit ligand*. If there had been no promising candidate genes within the 15 genes, the authors would have had to take a functional approach to test each gene and see if each gene affected gill pigmentation. As this example shows,

it is possible to use high-resolution mapping to narrow down a mapped region to a small number of genes that contain the loci responsible for genetic variation in a naturally occurring vertebrate population.

THE GENETIC BASIS OF ENVIRONMENTAL EFFECTS ON COLORATION

The environment can play a large part in determining an individual's coloration. Arctic foxes have a brownish coat during the summer and a white coat during the winter (Vage et al. 2005). The different seasonal forms of the butterfly *Precis octavia* are so different from each other that one might easily assume that they were actually different species (Figure 8). Another example is *Manduca quinquemaculata*, a species closely related to *Manduca sexta*, the tobacco hornworm moth (Suzuki & Nijhout 2006). *M. quinquemaculata* larvae are black when raised at 20°C and green when raised at 28°C. In *M. sexta*, a species that has only green larvae, a mutation causing a reduction of juvenile hormone secretion results in only black larva. Heat shocking these black larvae often resulted in a color change to green. The individuals that showed the most marked color change upon heat shock were selected and bred to one another. After 13 generations of such selection, the larvae mimicked *M. quinquemaculata* in that they were green when raised at 28°C without heat shock. The researchers were able to generate environmentally plastic coloration through reduction of juvenile hormone production and subsequent selection of the uncovered phenotypic variation (Suzuki & Nijhout 2006). Further studies determined that one major gene and several modifier genes were responsible for the artificially selected phenotype (Suzuki & Nijhout 2008). It is unclear how the coloration system evolved in *M. quinquemaculata*, but this system may have taken a path similar to the artificial selection in *M. sexta*. This example demonstrates a more complicated example of coloration differences and shows how the field is adapting



Figure 8

Example of environmentally influenced coloration. These two butterflies are both males of the species *Precis octavia* collected in South Africa. The only difference is that they were collected at different times of the year and represent the two very different seasonal forms of this species. It appears that both temperature and day/night length during the larval period determine the coloration pattern of the adult.

methods to address even these more complex phenomena.

Having examined the many different approaches for studying the genetic basis of pigmentation patterns, and having sampled some of the results, we can now return to the evolutionary questions we hope to address by studying coloration systems.

MANY GENES OR FEW GENES?

How many genes are responsible for the evolution of new forms of coloration? One factor to consider is that there is a bias in examining

species with a simple genetic basis of coloration because it is easier to study such systems. With that in mind, there are many examples of single genes responsible for coloration differences (Brown et al. 2001, Curtis 2002, Nachman et al. 2003, Trapezov 1997, Wittkopp et al. 2003a). It is easy to see how an overall change in body pigmentation could be affected by modification of one gene in a pigment synthesis pathway. However, differences in the coloration pattern may be easier to explain via a polygenic mode of inheritance. Polygenic modes of inheritance have been identified for wing spots, abdomen, and overall pigmentation in various *Drosophila* species as well as pigmentation in the oldfield mouse (Carbone et al. 2005, Steiner et al. 2007, Wittkopp et al. 2003a, Yeh et al. 2006). In some cases, observed so far mainly in *Lepidoptera*, it is unclear whether a mode of inheritance is monogenic or polygenic with a tightly linked cluster of genes (Clarke & Sheppard 1959, 1960a,b; Joron et al. 2006b). Many recent studies looking at noncoloration variability have also documented that a few genes of large effect account for a large amount of phenotypic difference (Albertson et al. 2003; Colosimo et al. 2004, 2005; Peichel et al. 2001; Shapiro et al. 2004).

REGULATORY VERSUS CODING CHANGES?

What kinds of mutations are responsible for phenotypic changes—regulatory or coding changes? Again, there is bias in the studies that address this question because it is very difficult to find regulatory changes in nonmodel species when there is a lack of knowledge of structure of the gene regulatory elements or when detailed gene expression studies are not possible. Most often, regulatory changes are not identified but are hypothesized when no coding changes are found. An example of a gene that appears prone to coding changes in pigment evolution is *Mc1r*. Potential coding changes have been found in the rock pocket mouse, bananaquit, lesser snow goose, arctic skua, beach mouse, jaguars, jaguarundis, and Neanderthals (Baião et al. 2007, Eizirik et al.

2003, Hoekstra et al. 2006, Lalueza-Fox et al. 2007, Mundy et al. 2004, Nachman et al. 2003, Rosenblum et al. 2004, Theron et al. 2001). Most of these studies show an association between *Mc1r* changes and coloration phenotype. Often the changes are in conserved amino acids, implying that the function of the receptor is affected, and recently an in vitro test was devised to test the function of variants of beach mouse *Mc1r* (Hoekstra et al. 2006). Examples of predicted coding changes in other genes causing pigmentation differences include *Agouti* in domestic cats, *Tyrp1* in Soay sheep, and *Oca2* in the Mexican cave tetra (Eizirik et al. 2003, Gratten et al. 2007, Protas et al. 2006).

Examples of regulatory changes causing pigmentation differences are much fewer in number. However, likely examples are *yellow* and the wing pigmentation spot and male-specific pigmentation in drosophilids (Gompel et al. 2005, Jeong et al. 2006, Prud'homme et al. 2006). Another predicted regulatory change is in *Agouti* in beach mice (Steiner et al. 2007), and a regulatory mutation in *Kit ligand* is probably responsible for the gill pigmentation differences between marine and freshwater sticklebacks (Miller et al. 2007). Coding changes involved in the evolution of coloration may be prevalent because genes involved in pigment biosynthesis are relatively free to alter biochemical function without causing lethality. Genes involved in other processes during development often have pleiotropic effects, and coding changes would likely result in lethality. Whether coding or regulatory sequences are more common agents of evolutionary change is still hotly debated (Hoekstra & Coyne 2007).

SAME GENES OR DIFFERENT GENES?

The third question that can be addressed by genetic studies of coloration is, If the same phenotype comes up multiple times, is it the same or different genes that are responsible? Above we see that *Mc1r* appears to be involved in pigmentation differences in multiple cases. However, we must keep in mind that *Mc1r* is

Parallel evolution:
the independent
evolution of similar
characteristics coming
from a similar starting
point

a highly conserved gene with only one exon and is therefore relatively easy to amplify from a nonmodel organism. Regardless of the bias, *Mc1r* seems to be involved in pigmentation differences between closely related species or populations in many different types of vertebrates. However, *Mc1r* polymorphisms are not always associated with pigmentation differences. For example, pocket gophers with coat colors corresponding to the color of the substrate that they live on do not show an association of coloration phenotype with *Mc1r* genotype (Wlasiuk & Nachman 2007). Also, leaf warblers with small, unmelanized patches and multiple nonhuman primates do not show an association of coloration with *Mc1r* (MacDougall-Shackleton et al. 2003, Mundy & Kelly 2003). Other examples can be found in populations of rock pocket mice, as well as in beach mice, in which one population does show an association between *Mc1r* variants and the melanic phenotype, whereas another population does not (Hoekstra & Nachman 2003, Hoekstra et al. 2006). This implies that the melanic phenotype evolved by a different mechanism in the separate populations. So, if there is a bias for mutation in *Mc1r*, it is not a complete bias.

In the Mexican cave tetra (**Figure 3f**), the genetic basis of albinism was studied in three different cave populations: Molino, Japonés, and Pachón (Protas et al. 2006). The gene *Oca2* was linked to the phenotype of albinism in crosses from the Molino and Pachón populations. These two cave populations both had deletions, but in different parts of the *Oca2* coding region. To test whether the cave forms of *Oca2* were functional, constructs of the surface *Oca2* and *Oca2* with the two deletions were transfected into a mouse melanocyte cell line deficient in *Oca2*. The surface form was able to rescue pigmentation in the cell line, but the two cave forms were unable to rescue pigmentation. Therefore, it appears that these two deletions cause the protein to be nonfunctional. As for the Japonés population, complementation tests were performed with the Molino and Pachón cave populations, and the offspring did not complement, indicating that the Japonés

population was likely also deficient in *Oca2*. A construct containing the coding sequence of *Oca2* in the Japonés population was able to rescue pigmentation in the *Oca2*-deficient melanocyte cell line, suggesting a possible regulatory change. Therefore, it is likely that albinism arose three times separately, in an example of parallel evolution, and that, in each case, the same gene was targeted. This is different from the previously discussed role of *Mc1r* in the evolution of pigmentation phenotypes in beach mice and rock pocket mice. In this latter case, some mouse populations utilized *Mc1r*, whereas others used something else. It is unclear why this bias is seen with *Oca2*, perhaps because the gene is very large and therefore more vulnerable to mutation. It is also important to keep in mind that the different types of coloration changes, such as loss of coloration, gain of coloration, and change in coloration pattern, are quite different from one another and may come about by the alteration of very different genetic programs.

Another example of parallel evolution is coloration patterns from *Heliconius* butterflies (**Figure 6**). As discussed above, *Heliconius* butterflies are a classic example of Müllerian mimicry. Each species has many different geographical races with varied pigmentation patterns, but where races of different species coexist they have matching pigmentation patterns. This provides a unique scenario in which several species have evolved virtually the same pigmentation pattern, allowing investigators to ask whether the same genetic path or different genetic paths were taken to achieve this result. This scenario is different from that of *Mc1r* in rock pocket mice and *Oca2* in cavefish because here we have multiple species—rather than multiple populations of species—evolving the same phenotypes.

Heliconius erato and *Heliconius melpomene* are two of the better-studied *Heliconius* species—they are distantly related but have very similar coloration patterns for 23 geographical races (**Figure 6**) (Joron et al. 2006a). Initial genetic experiments suggest that approximately a dozen loci are responsible for the variation seen within

each species. Genetic linkage maps have been generated, and the positions of a number of coloration loci, including four to five alleles of major effect (Joron et al. 2006a), have now been mapped for both *H. erato* and *H. melpomene* (Jiggins et al. 2005, Kapan et al. 2006). In another species, *Heliconius numata*, most of the variation in wing patterning appears to be controlled by a single genomic locus (possibly one or more tightly linked genes). By investigating the placement of flanking genetic markers relative to the position of each of the color pattern loci in *H. erato*, *H. melpomene*, and *H. numata*, Joron et al. (2006b) determined that the loci responsible for specific color patterns were located in homologous chromosomal regions. Similarly, three color patterning loci were compared between the *Heliconius bimera/H. erato* group and the *Heliconius cydno/Heliconius pacheinus* group and were also found to be located in homologous chromosomal regions (Kronforst et al. 2006). Given the current resolution of the mapping data, these results do not yet prove that the same genes are affected but do support the idea of parallel evolution.

Although it is striking to see similar phenotypes mapping to similar genomic regions in *H. erato* and *H. melpomene* (something we see in examples above), the mapping of very different phenotypes to the same location (e.g., when comparing *H. erato/H. melpomene* with *H. numata*) is remarkable. Future work will hopefully show how the same region can encode such different phenotypes and uncover the specifics of the genetic architecture controlling these color patterns.

A very detailed example of convergence and parallel evolution is that of wing spot evolution in drosophilids. We discuss above one instance of wing spot difference between *D. melanogaster* and *D. biarmipes* (Gompel et al. 2005). Another example of wing spot gain is *Drosophila tristis* (Prud'homme et al. 2006). A *D. tristis* sequence homologous to that responsible for wing spot gain in *D. biarmipes* did not cause a wing spot pattern when inserted into *D. melanogaster*. Only when a *D. tristis* yellow intronic sequence was included in the reporter construct did the

sequence drive wing spot-specific expression. Therefore, the same gene seems to be involved in wing spot gain in two species of *Drosophila*, but different regulatory elements are involved (Prud'homme et al. 2006). Prud'homme et al. (2006) suggest that the evolution of these novel patterns came about by co-option of regulatory elements with preexisting functions.

STANDING VERSUS NOVEL VARIATION?

Our last question regarding the evolution of morphological change is whether change comes about by standing variation in the ancestral population or novel variation. Answering this type of question requires a system for which one knows the direction of morphological change. For example, in the Mexican cave tetra, we know that the albino cave form evolved from the pigmented surface form. The example of *Oca2* in cavefish showed that albinism was likely caused by novel variation in the cave populations because, although the same gene was affected, there were at least two different mutations (Protas et al. 2006). Another system in which the question of standing versus novel variation is being examined is with old-field mice. Again, we know the direction of change—the lightly pigmented beach mice are derived from the more highly pigmented mainland mice. As stated above, three QTLs were found for pigmentation in this species, and two of them mapped to the genes *Agouti* and *Mc1r* (Steiner et al. 2007). *Agouti* is epistatic to *Mc1r*. Epistasis may allow the beach *Mc1r* allele to remain hidden in the mainland population as standing variation (Barrett & Schluter 2007). To examine this idea further, *Mc1r* needs to be sequenced from many individuals of the mainland population to see if the beach *Mc1r* alleles are preexisting in that population. Another likely evolution via standing variation is gill pigmentation in sticklebacks (Miller et al. 2007). Three freshwater populations of sticklebacks have *Kit ligand* alleles associated with light pigmentation. Even though the populations are geographically separate, they share the same

Standing variation:
variation that exists in
the population

alleles at *Kit ligand*. One possible explanation for this is that standing variation in the ancestral (marine) population was fixed in each of the freshwater populations. To investigate this hypothesis, *Kit ligand* was sequenced in 107 marine individuals; the light allele was present at a frequency of 12%, supporting the idea that the evolution of lightly colored gills occurred by the repeated fixation of standing variation.

CONCLUSION

There has been much progress in understanding the genetic basis of morphological change in the evolution of pigmentation and coloration patterns. The advantages to studying the genetic basis of coloration include the extreme

ease of observing this phenotype, the vast knowledge of pigment synthesis pathways in model organisms, and the great diversity of coloration and patterns within and between species. Pigmentation is hence a relatively simple system in which we can explore the possible processes by which phenotypes evolve. Although broad trends in the evolution of pigmentation patterns emerge from the discussion above, there are clearly multiple ways in which evolution of coloration occurs. Also, most of the examples we discuss above are relatively simple pigmentation differences that are more easily examined with existing tools. However, there is a huge diversity of more complicated pigmentation systems being studied that will yield a more complete picture as to how coloration differences evolve.

SUMMARY POINTS

1. There are many advantages to studying coloration and coloration patterns in nature, including ease of observation and great morphological diversity within and between species.
2. Molecular and genetic techniques can now be applied to nonmodel organisms to ask questions about evolution.
3. Variation in coloration can evolve by single gene changes or by multiple gene changes.
4. Differences in coloration can evolve by regulatory or coding changes.
5. The evolution of similar phenotypic changes can be accomplished by the same genes or by different genes.
6. Changes in coloration can evolve by selection on standing variation or on novel mutations.

FUTURE ISSUES

1. There is a need to identify genes responsible for more quantitative trait loci encoding a small-percent variance of a coloration trait as well as those responsible for a large-percent variance of a trait.
2. Systems that have a more complicated coloration phenotype, for example, those with partially environmentally determined coloration, should be examined.
3. More cases of parallel and convergent evolution of coloration variation require examination.
4. Regulatory changes responsible for coloration variation should be determined.

5. Investigators need to determine other characteristics that evolve in tandem with color variation and how the genetic architecture of these traits interacts with the genetic architecture of coloration traits.
6. The genetics behind examples in which alleles of a single locus can create many different coloration phenotypes merits investigation.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank C. Chaw, C. Grande, J. Gross, H. Hoekstra, M. Modrell, R. Parchem, E.J. Rehm, and A. VanHook for helpful comments. We also thank H. Hoekstra, S. Cary, and C. Steiner for providing the oldfield/beach mice images, R. Borowsky for supplying the cavefish images, and R. Roberts for providing the cichlid images. Thanks to K. Koenig for mounting and photographing some of the *Heliconius* butterflies used in this review and to Américo Bonkewitz for providing the specimens of *Precis octavia*.

LITERATURE CITED

- Albertson RC, Strelman JT, Kocher TD. 2003. Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. *Proc. Natl. Acad. Sci. USA* 100:5252–57
- Andersson L, Georges M. 2004. Domestic-animal genomics: deciphering the genetics of complex traits. *Nat. Rev. Genet.* 5:202–12
- Baião PC, Schreiber E, Parker PG. 2007. The genetic basis of the plumage polymorphism in red-footed boobies (*Sula sula*): a *melanocortin-1 receptor (MC1R)* analysis. *J. Hered.* 98:287–92
- Barbato M, Bernard M, Borrelli L, Fiorito G. 2007. Body patterns in cephalopods: “Polyphenism” as a way of information exchange. *Pattern Recognit. Lett.* 28:1854–64
- Barrett RD, Schluter D. 2007. Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23:38–44
- Bennett DC, Lamoreux ML. 2003. The color loci of mice—a genetic century. *Pigment Cell Res.* 16:333–44
- Brown TM, Cho SY, Evans CL, Park S, Pimprale SS, Bryson PK. 2001. A single gene (*yes*) controls pigmentation of eyes and scales in *Heliothis virescens*. *J. Insect Sci.* 1:1
- Cain AJ, Sheppard PM. 1954. Natural selection in *Cepaea*. *Genetics* 39:89–116
- Camp E, Lardelli M. 2001. Tyrosinase gene expression in zebrafish embryos. *Dev. Genes Evol.* 211:150–53
- Carbone MA, Llopert A, de Angelis M, Coyne JA, Mackay TF. 2005. Quantitative trait loci affecting the difference in pigmentation between *Drosophila yakuba* and *D. santomea*. *Genetics* 171:211–25
- Caro T. 2005. The adaptive significance of coloration in mammals. *BioScience* 55:125–36
- Clarke CA, Sheppard PM. 1959. The genetics of *Papilio dardanus*, Brown. I. Race *cenea* from South Africa. *Genetics* 44:1347–58
- Clarke CA, Sheppard PM. 1960a. The genetics of *Papilio dardanus*, Brown. II. Races *dardanus*, *polytrophus*, *meseres*, and *tibullus*. *Genetics* 45:439–57
- Clarke CA, Sheppard PM. 1960b. The genetics of *Papilio dardanus*, Brown. III. Race *antinorii* from Abyssinia and race *meriones* from Madagascar. *Genetics* 45:683–98
- Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G Jr, Dickson M, et al. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. *Science* 307:1928–33

Investigated the regulation of *yellow* in *D. melanogaster* and *D. biarmipes* and the involvement of *yellow* in a pigmented wingspot.

- Colosimo PF, Peichel CL, Nereng K, Blackman BK, Shapiro MD, et al. 2004. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. *PLoS Biol.* 2:E109
- Culver DC. 1982. *Cave Life*. Cambridge, MA: Harvard Univ. Press
- Curtis JT. 2002. A blond coat color variation in meadow vole (*Microtus pennsylvanicus*). *J. Hered.* 93:209–10
- Doucet SM, Shawkey MD, Rathburn MK, Mays HL Jr, Montgomerie R. 2004. Concordant evolution of plumage colour, feather microstructure and a melanocortin receptor gene between mainland and island populations of a fairy-wren. *Proc. Biol. Sci.* 271:1663–70
- Eizirik E, Yuhki N, Johnson WE, Menotti-Raymond M, Hannah SS, O'Brien SJ. 2003. Molecular genetics and evolution of melanism in the cat family. *Curr. Biol.* 13:448–53
- Gompel N, Prud'homme B, Wittkopp PJ, Kassner VA, Carroll SB. 2005. Chance caught on the wing: cis-regulatory evolution and the origin of pigment patterns in *Drosophila*. *Nature* 433:481–87**
- Gratten J, Beraldi D, Lowder BV, McRae AF, Visscher PM, et al. 2007. Compelling evidence that a single nucleotide substitution in TYRP1 is responsible for coat-colour polymorphism in a free-living population of Soay sheep. *Proc. Biol. Sci.* 274:619–26
- Hoekstra HE. 2006. Genetics, development and evolution of adaptive pigmentation in vertebrates. *Heredity* 97:222–34
- Hoekstra HE, Coyne JA. 2007. The locus of evolution: evo devo and the genetics of adaptation. *Evol. Int. J. Org. Evol.* 61:995–1016
- Hoekstra HE, Hirschmann RJ, Bunday RA, Insel PA, Crossland JP. 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science* 313:101–4
- Hoekstra HE, Nachman MW. 2003. Different genes underlie adaptive melanism in different populations of rock pocket mice. *Mol. Ecol.* 12:1185–94
- Hosemann KE, Colosimo PF, Summers BR, Kingsley DM. 2004. A simple and efficient microinjection protocol for making transgenic sticklebacks. *Behaviour* 141:1345–55
- Houde AE, Endler JA. 1990. Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science* 248:1405–8
- Ito F, Hashim R, Huei YS, Kaufmann E, Akino T, Billen J. 2004. Spectacular Batesian mimicry in ants. *Naturwissenschaften* 91:481–84
- Jeong S, Rokas A, Carroll SB. 2006. Regulation of body pigmentation by the Abdominal-B Hox protein and its gain and loss in *Drosophila* evolution. *Cell* 125:1387–99
- Jiggins CD, Mavarez J, Beltran M, McMillan WO, Johnston JS, Bermingham E. 2005. A genetic linkage map of the mimetic butterfly *Heliconius melpomene*. *Genetics* 171:557–70
- Jones JS, Leith BH, Rawlings P. 1977. Polymorphism in *Cepaea*: a problem with too many solutions? *Annu. Rev. Ecol. Syst.* 8:109–43
- Joron M, Jiggins CD, Papanicolaou A, McMillan WO. 2006a. Heliconius wing patterns: an evo-devo model for understanding phenotypic diversity. *Heredity* 97:157–67
- Joron M, Papa R, Beltran M, Chamberlain N, Mavarez J, et al. 2006b. A conserved supergene locus controls colour pattern diversity in *Heliconius* butterflies. *PLoS Biol.* 4:e303
- Kapan DD, Flanagan NS, Tobler A, Papa R, Reed RD, et al. 2006. Localization of Müllerian mimicry genes on a dense linkage map of *Heliconius erato*. *Genetics* 173:735–57
- Kelsh RN. 2004. Genetics and evolution of pigment patterns in fish. *Pigment Cell Res.* 17:326–36
- Kelsh RN, Brand M, Jiang YJ, Heisenberg CP, Lin S, et al. 1996. Zebrafish pigmentation mutations and the processes of neural crest development. *Development* 123:369–89
- Kopp A, Duncan I, Godt D, Carroll SB. 2000. Genetic control and evolution of sexually dimorphic characters in *Drosophila*. *Nature* 408:553–59
- Kopp A, Graze RM, Xu S, Carroll SB, Nuzhdin SV. 2003. Quantitative trait loci responsible for variation in sexually dimorphic traits in *Drosophila melanogaster*. *Genetics* 163:771–87
- Kronforst MR, Kapan DD, Gilbert LE. 2006. Parallel genetic architecture of parallel adaptive radiations in mimetic *Heliconius* butterflies. *Genetics* 174:535–39
- Lalueza-Fox C, Römpler H, Caramelli D, Stäubert C, Catalano G, et al. 2007. A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. *Science* 318:1453–55

- MacDougall-Shackleton EA, Blanchard L, Igdoura SA, Gibbs HL. 2003. Unmelanized plumage patterns in Old World leaf warblers do not correspond to sequence variation at the melanocortin-1 receptor locus (MC1R). *Mol. Biol. Evol.* 20:1675–81
- Mellgren EM, Johnson SL. 2002. The evolution of morphological complexity in zebrafish stripes. *Trends Genet.* 18:128–34
- Miller CT, Beleza S, Pollen AA, Schluter D, Kittles RA, et al. 2007. *cis*-regulatory changes in Kit ligand expression and parallel evolution of pigmentation in sticklebacks and humans. *Cell* 131:1179–89**
- Mundy NI. 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. *Proc. Biol. Sci.* 272:1633–40
- Mundy NI, Badcock NS, Hart T, Scribner K, Janssen K, Nadeau NJ. 2004. Conserved genetic basis of a quantitative plumage trait involved in mate choice. *Science* 303:1870–73
- Mundy NI, Kelly J. 2003. Evolution of a pigmentation gene, the melanocortin-1 receptor, in primates. *Am. J. Phys. Anthropol.* 121:67–80
- Nachman MW, Hoekstra HE, D'Agostino SL. 2003. The genetic basis of adaptive melanism in pocket mice. *Proc. Natl. Acad. Sci. USA* 100:5268–73
- Parchem RJ, Perry MW, Patel NH. 2007. Patterns on the insect wing. *Curr. Opin. Genet. Dev.* 17:300–8
- Parichy DM. 2006. Evolution of danio pigment pattern development. *Heredity* 97:200–10
- Parichy DM, Johnson SL. 2001. Zebrafish hybrids suggest genetic mechanisms for pigment pattern diversification in Danio. *Dev. Genes Evol.* 211:319–28
- Peichel CL, Nereng KS, Ohgi KA, Cole BL, Colosimo PF, et al. 2001. The genetic architecture of divergence between threespine stickleback species. *Nature* 414:901–5
- Protas ME, Hersey C, Kochanek D, Zhou Y, Wilkens H, et al. 2006. Genetic analysis of cavefish reveals molecular convergence in the evolution of albinism. *Nat. Genet.* 38:107–11
- Prud'homme B, Gompel N, Rokas A, Kassner VA, Williams TM, et al. 2006. Repeated morphological evolution through *cis*-regulatory changes in a pleiotropic gene. *Nature* 440:1050–53
- Quigley IK, Manuel JL, Roberts RA, Nuckels RJ, Herrington ER, et al. 2005. Evolutionary diversification of pigment pattern in *Danio* fishes: differential *fms* dependence and stripe loss in *D. albolineatus*. *Development* 132:89–104
- Quigley IK, Parichy DM. 2002. Pigment pattern formation in zebrafish: a model for developmental genetics and the evolution of form. *Microsc. Res. Tech.* 58:442–55
- Reed RD, McMillan WO, Nagy LM. 2008. Gene expression underlying adaptive variation in *Heliconius* wing patterns: nonmodular regulation of overlapping cinnabar and vermilion prepatterns. *Proc. Biol. Sci.* 275:37–45
- Rosenblum EB, Hoekstra HE, Nachman MW. 2004. Adaptive reptile color variation and the evolution of the *Mcl1r* gene. *Evol. Int. J. Org. Evol.* 58:1794–808
- Roulin A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. *Biol. Rev. Camb. Philos. Soc.* 79:815–48
- Russell ES. 1985. A history of mouse genetics. *Annu. Rev. Genet.* 19:1–28
- Salzburger W, Braasch I, Meyer A. 2007. Adaptive sequence evolution in a color gene involved in the formation of the characteristic egg-dummies of male haplochromine cichlid fishes. *BMC Biol.* 5:51
- Schmutz SM, Berryere TG. 2007. Genes affecting coat colour and pattern in domestic dogs: a review. *Anim. Genet.* 38:539–49
- Shapiro MD, Marks ME, Peichel CL, Blackman BK, Nereng KS, et al. 2004. Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* 428:717–23
- Steiner CC, Weber JN, Hoekstra HE. 2007. Adaptive variation in beach mice produced by two interacting pigmentation genes. *PLoS Biol.* 5:e219**
- Streelman JT, Albertson RC, Kocher TD. 2003. Genome mapping of the orange blotch colour pattern in cichlid fishes. *Mol. Ecol.* 12:2465–71
- Suzuki Y, Nijhout HF. 2006. Evolution of a polyphenism by genetic accommodation. *Science* 311:650–52**
- Suzuki Y, Nijhout HF. 2008. Genetic basis of adaptive evolution of a polyphenism by genetic accommodation. *J. Evol. Biol.* 21:57–66

Used high-resolution mapping to identify a gene involved in gill pigmentation variation in different populations of sticklebacks.

The authors performed a quantitative trait and candidate gene analysis of a polygenic pigmentation trait in the oldfield mice.

Investigated a pigmentation trait that changes with the environment by carrying out artificial-selection experiments in a related species.

- Theron E, Hawkins K, Bermingham E, Ricklefs RE, Mundy NI. 2001. The molecular basis of an avian plumage polymorphism in the wild: A melanocortin-1-receptor point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola*. *Curr. Biol.* 11:550–57
- Tobler A, Kapan D, Flanagan NS, Gonzalez C, Peterson E, et al. 2005. First-generation linkage map of the warningly colored butterfly *Heliconius erato*. *Heredity* 94:408–17
- Trapezov OV. 1997. Black crystal: a novel color mutant in the American mink (*Mustela vison Schreber*). *J. Hered.* 88:164–66
- Vage DI, Fuglei E, Snipstad K, Beheim J, Landsem VM, Klungland H. 2005. Two cysteine substitutions in the MC1R generate the blue variant of the arctic fox (*Alopex lagopus*) and prevent expression of the white winter coat. *Peptides* 26:1814–17
- Wittkopp PJ, Carroll SB, Kopp A. 2003a. Evolution in black and white: genetic control of pigment patterns in *Drosophila*. *Trends Genet.* 19:495–504
- Wittkopp PJ, Williams BL, Selegue JE, Carroll SB. 2003b. *Drosophila* pigmentation evolution: divergent genotypes underlying convergent phenotypes. *Proc. Natl. Acad. Sci. USA* 100:1808–13
- Wlasiuk G, Nachman MW. 2007. The genetics of adaptive coat color in gophers: Coding variation at Mc1r is not responsible for dorsal color differences. *J. Hered.* 98:567–74
- Yamamoto Y, Stock DW, Jeffery WR. 2004. Hedgehog signalling controls eye degeneration in blind cavefish. *Nature* 431:844–47
- Yeh SD, Liou SR, True JR. 2006. Genetics of divergence in male wing pigmentation and courtship behavior between *Drosophila elegans* and *D. gunungcola*. *Heredity* 96:383–95



Contents

Microtubule Dynamics in Cell Division: Exploring Living Cells with Polarized Light Microscopy <i>Shinya Inoué</i>	1
Replicative Aging in Yeast: The Means to the End <i>K.A. Steinkraus, M. Kaeberlein, and B.K. Kennedy</i>	29
Auxin Receptors and Plant Development: A New Signaling Paradigm <i>Keithanne Mockaitis and Mark Estelle</i>	55
Systems Approaches to Identifying Gene Regulatory Networks in Plants <i>Terri A. Long, Siobhan M. Brady, and Philip N. Benfey</i>	81
Sister Chromatid Cohesion: A Simple Concept with a Complex Reality <i>Itay Onn, Jill M. Heidinger-Pauli, Vincent Guacci, Elçin Ünal, and Douglas E. Kosbland</i>	105
The Epigenetics of rRNA Genes: From Molecular to Chromosome Biology <i>Brian McStay and Ingrid Grummt</i>	131
The Evolution, Regulation, and Function of Placenta-Specific Genes <i>Saara M. Rawn and James C. Cross</i>	159
Communication Between the Synapse and the Nucleus in Neuronal Development, Plasticity, and Disease <i>Sonia Cohen and Michael E. Greenberg</i>	183
Disulfide-Linked Protein Folding Pathways <i>Bharath S. Mamathambika and James C. Bardwell</i>	211
Molecular Mechanisms of Presynaptic Differentiation <i>Yishi Jin and Craig C. Garner</i>	237
Regulation of Spermatogonial Stem Cell Self-Renewal in Mammals <i>Jon M. Oatley and Ralph L. Brinster</i>	263
Unconventional Mechanisms of Protein Transport to the Cell Surface of Eukaryotic Cells <i>Walter Nickel and Matthias Seedorf</i>	287

The Immunoglobulin-Like Cell Adhesion Molecule Nectin and Its Associated Protein Afadin <i>Yoshimi Takai, Wataru Ikeda, Hisakazu Ogita, and Yoshiyuki Rikitake</i>	309
Regulation of MHC Class I Assembly and Peptide Binding <i>David R. Peaper and Peter Cresswell</i>	343
Structural and Functional Aspects of Lipid Binding by CD1 Molecules <i>Jonathan D. Silk, Mariolina Salio, James Brown, E. Yvonne Jones, and Vincenzo Cerundolo</i>	369
Prelude to a Division <i>Needhi Bhalla and Abby F. Dernburg</i>	397
Evolution of Coloration Patterns <i>Meredith E. Protas and Nipam H. Patel</i>	425
Polar Targeting and Endocytic Recycling in Auxin-Dependent Plant Development <i>Jürgen Kleine-Vehn and Jiří Friml</i>	447
Regulation of APC/C Activators in Mitosis and Meiosis <i>Jillian A. Pesin and Terry L. Orr-Weaver</i>	475
Protein Kinases: Starting a Molecular Systems View of Endocytosis <i>Prisca Liberali, Pauli Rämö, and Lucas Pelkmans</i>	501
Comparative Aspects of Animal Regeneration <i>Jeremy P. Brockes and Anoop Kumar</i>	525
Cell Polarity Signaling in <i>Arabidopsis</i> <i>Zhenbiao Yang</i>	551
Hunter to Gatherer and Back: Immunological Synapses and Kinapses as Variations on the Theme of Amoeboid Locomotion <i>Michael L. Dustin</i>	577
Dscam-Mediated Cell Recognition Regulates Neural Circuit Formation <i>Daisuke Hattori, S. Sean Millard, Woj M. Wojtowicz, and S. Lawrence Zipursky</i>	597

Indexes

Cumulative Index of Contributing Authors, Volumes 20–24	621
Cumulative Index of Chapter Titles, Volumes 20–24	624

Errata

An online log of corrections to *Annual Review of Cell and Developmental Biology* articles may be found at <http://cellbio.annualreviews.org/errata.shtml>