Coat Color Mutations, Animals

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History

Discrete variation in external morphology or appearance that segregates in pedigrees provides the cornerstone of genetics in all organisms, and therefore it is no surprise that coat color variation has played a crucial role in animal genetics.

Pioneering studies in this field were carried out at the turn of the century by William Castle and two of his students, Clarence Cook Little and Sewall Wright. In particular, Wright published a series of manuscripts in 1917 and 1918 in which he argued that coat color genetics was a useful way "to assist embryology and biochemistry in filling in the links between germ cell and adult in specific cases," because many coat color mutations were available for comparative study, and because a rudimentary knowledge of pigment chemistry and biochemistry provided a foundation with which to interpret genetic interaction experiments. For example, in crosses between rabbits that were black or yellow, Wright remarked that there was a reciprocal biochemical and genetic relationship, indicative of different alleles acting at the same locus. By contrast, in crosses to albino rabbits, it was found that albinism could mask, or in genetic parlance, "be epistatic to," either black or yellow. This led to the hypothesis, verified biochemically nearly 50 years later, that the biochemical process responsible for determining whether hairs were black or yellow acted on a single substrate produced by the product of the albino locus.

In the early 1900s, availability of many coat color mutations for comparative study was driven primarily by cultural rather than scientific factors, as new variants of spontaneous origin had been collected and maintained by communities of animal enthusiasts, so-called fanciers, for several hundred years. However, the value of mutants to biomedical research became increasingly apparent and, by the 1920s, systematic attempts were initiated at several academic institutions to catalog and preserve different mutations and, in addition, to develop inbred strains of animals so that the effects of different mutations could be studied on a consistent genetic background. Although much of Wright's early work was with guinea pigs, the house mouse rapidly became favored due to smaller size, ready availability, and rapid generation time. The Jackson Laboratory, in Bar Harbor, Maine, founded in 1923 by C.C. Little, has played and continues to fulfill an especially prominent role, providing a repository and distribution center for different mutations and strains of mice to scientists around the world. Thus, most of our knowledge regarding coat color gene action has come from mice, although in some cases studies in other mammals have confirmed or refined our principles of color inheritance.

Many coat color mutations in mice and virtually all those in other mammals are of spontaneous origin. However, a special class of mouse mutations at a small number of loci have been induced in experiments designed to measure and characterize genotoxic effects of radiation or chemicals. Supported at large national laboratories such as Harwell (UK), Neuherberg (Germany), or Oak Ridge, Tennessee (USA), most of these experiments have been designed to detect loss-of-function mutations at one of seven different loci: *Agouti* (*a*), *Brown* (*b*), *Albino* (*c*), *Dilute* (*d*), *Short ear* (*se*), *Pink-eyed dilution* (*p*), and *Piebald* (*s*), of which all but one, *Short ear*, affect coat color. Typically, mutagenized wild-type animals are crossed to *a/a, b/b, c/c, d, se/d, se, p/p, s/s* animals, allowing new recessive mutations for each of the seven "specific loci" to be recognized in the F_1 progeny, along with new dominant mutations at other loci. The availability of multiple alleles at a single locus can be a powerful tool in any genetic system; consequently experimental results based on the specific locus test have played an important role in the history of coat color gene action.

Discussing genes, mutations, and loci can be confusing, since the meaning of these terms has changed somewhat as the era of molecular genetics has matured. Experimental geneticists use the term 'locus' to describe a specific heritable trait whose map position can be compared with other heritable traits that produce a similar effect. For example, oculocutaneous albinism in humans is a recessive condition, but, rarely, a mating between albino individuals may produce children that all have normal pigmentation, indicating that albinism in the parents is caused by two different loci. (One is homologous to the mouse albino locus, while the other is homologous to the mouse pink-eyed dilution gene.) Although the term 'mutation' is frequently used to describe an alteration in DNA sequence, here we use 'mutation' to describe phenotypic variation in a heritable trait. Finally, the term 'gene' may be used to describe a unit

of heritable variation (similar to locus) but, in a molecular context, usually refers to a contiguous DNA sequence required for production of a specific RNA or protein product.

Number of Coat Color Mutations and Coat Color Genes

Experiments based on the specific locus test have produced a large number of recessive mutations for the *a*, *b*, *c*, *d*, *p*, and *s* loci, as well as a large number of dominant or semidominant mutations for the *White spotting* (*W*) and *Steel* (*Sl*) loci. The types of mutations produced are usually loss-of-function, and the different inheritance patterns reflect instrinsic differences between gene action at the two groups of loci. In most circumstances, the *a*, *b*, *c*, *d*, *p*, and *s* loci are not very sensitive to gene dosage such that the phenotypes of *A/a*, *B/b*, *C/c*, *D/d*, *P/p*, or *S/s* mice are identical to the phenotypes of *A/A*, *B/B*, *C/C*, *D/D*, *P/P*, or *S/S* mice, respectively. By contrast, *W/w* or *Sl/sl* mice are easily distinguished from *W/W* or *Sl/Sl* loci (in genetic parlance, these loci are "haploinsufficient").

In addition to *a*, *b*, *c*, *d*, *p*, *s*, *W*, *Sl*, spontaneous coat color mutations have been observed for approximately 90 additional loci in mice (generally with a small number of mutations per locus) for a total of approximately 100 different coat color genes.

The terminology used here, up to this point, for the different loci is historical and reflects the fact that the genes were identified originally by virtue of their phenotype. However, in modern nomenclature, most of the genes have been renamed to reflect their protein product. Thus, the *b*, *c*, *d*, *s*, *W*, and *Sl* genes are now referred to as *tyrosinase-related* protein 1 (*Tyrp 1*), t*yrosinase* (*Tyr*), m*yosin* 5a (*Myo5a*), e*ndothelin receptor B* (*Ednrb*), c-*Kit* protooncogene (*Kit*), and m*ast cell growth factor* (*Mgf*), respectively (*Agouti* and *Pink-eyed dilution* have retained their original names).

In most mammals other than mice, a small number of loci (fewer than 10) have been recognized as coat color mutations. While only a few have been characterized at a molecular level, in many cases it has been possible to assign homologies among different mammals in the absence of molecular information. For example, a temperature-sensitive loss-of-function mutation in *Tyrosinase* produces a distinctive phenotype known as the '*Himalayan* mutation' in rabbits, mice, or guinea pigs, and is also responsible for the characteristic appearance of Siamese cats.

Different Types of Coat Color Mutations

Coat color mutations are usually classified on the basis of cellular and/or developmental processes that are disrupted: pigment cell differentiation/migration/survival, biochemical synthesis of melanin, intracellular trafficking/membrane sorting of pigment granules, or pigment type-switching. An alternative approach to classification is based on whether the effects of a particular mutation are limited to coat color or are pleiotropic, affecting multiple processes in tissues of different embryonic origins. For example, several coat color mutations that disrupt intracellular pigment granule sorting also affect the sorting of intracellular contents in platelets, leading to prolonged bleeding time.

Mutations that Cause White Spotting

During embryonic development, pigment cell precursors, melanoblasts, differentiate from a specialized region of the neural tube, the neural crest, which also gives rise to the peripheral nervous system, connective tissue of the head and neck, and a portion of the adrenal gland. The melanoblasts proliferate and migrate from the middorsal region in a lateral direction to meet at the ventral midline. In general, melanoblasts are restricted from migrating along the rostrocaudal axis, but probably produce paracrine factors that diffuse beyond the boundaries of migration, which may explain why death of a melanoblast during the migration process can cause an irregular, localized white spot in the adult animal. The developmental history of pigment cells also helps to explain why white spots are especially common on the ventral body surface, and why individual spots never cross the ventral midline. By contrast, the loss of pigment cells that appears in juvenile or adult life, also known as 'vitiligo', is caused not by a developmental abnormality, but instead by destruction of pigment cells, often by an autoimmune process.

The action of white-spotting mutations is representative of many developmental processes that are stochastic. For example, in animals heterozygous for a loss-of-function mutation at *Ednrb*, which encodes a receptor on melanoblasts that helps stimulate migration and proliferation, every cell in the animal has reduced gene dosage for *Ednrb*, which lowers the threshold for additional factors – environmental, genetic, or random – that may cause the death of an individual melanoblast. Thus, animals with identical *Ednrb* mutations have different amounts of spotting, and their spots are located in different regions of the body. In an extreme case, white-spotting mutations cause a completely white coat with preservation of

pigment in the back of the eye, since retinal pigment epithelial cells are not derived from the neural crest and do not depend on many of the molecular processes used during melanocyte development. This phenotype of "one big spot" is easily recognizable in many different animals, e.g., white horses, white cows, or white cats.

Many white-spotting mutations are pleiotropic, because the molecular process disrupted by the mutation is used in tissues other than pigment cells. For example, *Kit* encodes a receptor that is required for proliferation, migration, and/or survival not only for melanoblasts but also for developing blood cells and germ cells, therefore some *Kit* mutations cause not only white spotting, but also anemia and sterility. Neural crest-derived melanocytes (though not pigment) are also required for proper function of the inner ear, therefore some white-spotting mutations also cause deafness.

While most white-spotting mutations produce localized deficiency of melanocytes in an irregular pattern that varies among genetically identical animals, mutations that produce a regular and stereotypic pattern of spotting are easily recognized in certain species, e.g., panda bears or weasels. Although the underlying mechanisms are uncertain, similar phenotypes in mice are due to unusual molecular alterations that cause components of the melanoblast migration machinery to be overexpressed in certain regions of the body. In some cases, e.g., racoons and zebras, regular patterns of white spotting are probably caused not by melanocyte deficiency, but instead by genes that affect pigment type-switching. Regardless of the underlying mechanism, rare genetic variation in coat color genes has provided a substrate for environmental adaptation and selective advantage in certain species during mammalian evolution.

Mutations that Affect Melanin Biosynthesis and Different Pigment Types

In mammals, melanin is a complex polymer, derived from oxidized derivatives of tyrosine, and is deposited in an organized fashion within subcellular organelles known as 'melanosomes.' Melanin biosynthesis, which takes place within these organelles, requires a series of enzymes for different oxidation steps, structural proteins to make up the melanosome matrix, and transporters to maintain the appropriate levels of constituents inside the melanosomes. By contrast to white spotting, mutations that impair melanin biosynthesis affect the entire animal, often including retinal pigment. The best-known mutation of this type, albino, is a complete loss-of-function for tyrosinase, which catalyzes the initial step in melanin biosynthesis, oxidation of tyrosine to dopaquinone.

Further enzymatic oxidation of dopaquinone provides precursors for brown/black eumelanin, whereas cysteinyl derivatives of dopaquinone provide precursors for red/yellow pheomelanin. Thus, tyrosinase is required for synthesis of both types of pigment, whereas additional melanin biosynthetic genes are generally used either for eumelanin or pheomelanin, but not both. Genes required for eumelanin but not pheomelanin synthesis have been especially well characterized; loss of function in some, e.g., *Pink-eyed dilution*, blocks nearly all eumelanin synthesis, while loss of function in others, e.g., *Tyrosinase-related protein 1*, alters the quality of eumelanin, causing it to appear brown instead of black.

In general, genes required for eumelanin biosynthesis are not used outside of pigment cells, therefore their primary effects are limited to pigmentation. However, retinal pigment is required for axons of retinal ganglion cells to project to their proper locations in the brain. In addition, while neural crest-derived melanocytes may produce eumelanin or pheomelanin, retinal pigment cells make only eumelanin. Thus, absence of eumelanin may have variable effects on coat color (depending on whether or not pheomelanin is synthesized), but always causes a loss of retinal pigment and secondary defects in visual perception. Furthermore, while genetic variation in melanin biosynthetic components is probably responsible for a wide range of coat color phenotypes seen in nature, complete loss of pigmentation as in the albino phenotype is generally limited to animals in captivity.

Mutations that Affect Pigment Granule Trafficking or Membrane Sorting

In mice, mutations in a large class of coat color genes produce a generalized pigmentary dilution, platelet storage pool deficiency, and abnormal lysosomal trafficking. Among those whose molecular identity is known are several that encode components of membrane-sorting pathways. A related class of genes encodes components of molecular motors required for the intracellular transport of melanosomes. The identification and analysis of both types of genes has provided both a useful resource for, and molecular insight into, basic aspects of cell biology. Because mutations in most of these genes have nonpigmentary effects, there is little genetic variation outside of laboratory animals or human patients. However, in some cases, mutations in homologous genes have been identified among several domesticated species. For example, Chediak-Higashi syndrome, characterized by pigmentary dilution, abnormal membrane trafficking,

and immunodeficiency, is found in humans, mice, cats, mink, and cattle.

Mutations that Affect Pigment Type-Switching

As described above, hair follicle melanocytes may switch between the two basic pigment types, red/yellow pheomelanin and brown/back eumelanin. Depending on genetic background, switching between pigment types occurs at specific times during hair growth and in particular regions of the body, allowing genetic control of pigment type-switching to give rise to a diversity of coat color patterns.

A paracrine signaling molecule that plays a key role in pigment type-switching, Agouti protein, is produced by specialized dermal cells underneath each hair follicle, and causes overlying melanocytes to switch from the synthesis of eumelanin to pheomelanin. A commonly observed pattern in many animals, including a group of South American rodents after which Agouti protein is named, is the presence of a subapical band of pheomelanin on a hair that is otherwise eumelanic. The presence of such a band on most or all body hairs gives the entire animal a brushed golden appearance that can provide camouflage in some circumstances.

Mutations in several genes can alter pigment type-switching, including the *Agouti* gene itself, and the *Melanocortin receptor 1* (*Mc1r*) gene, which encodes the receptor for Agouti protein expressed on melanocytes. Genetic variation in *Agouti* and *Mc1r* are an important source of natural coat color polymorphisms that alter the balance between eumelanin and pheomelanin, and have been found in several domesticated species including dogs, pigs, horses, cows, as well as humans.

The intracellular signaling events responsible for switching from the synthesis of eumelanin to pheomelanin are not completely understood, but one important component associated with the switch is downregulation of Tyrosinase activity, since pheomelanin synthesis apparently requires less Tyrosinase activity than does eumelanin synthesis. However, in some genetic backgrounds, *Agouti* signaling reduces Tyrosinase activity to a level no longer sufficient to maintain pheomelanin synthesis, causing a switch from production of black/brown pigment to almost no pigment. This phenomenon is probably responsible for the difference between the appearances of brushed golden and brushed gray, the latter being characteristic of animals such as the chinchilla or the gray wolf.

Among the most interesting group of coat color mutations are those that cause regular patterns of stripes or spots, as in zebras, tigers, leopards, or giraffes. Although chemical or biochemical studies have not been carried out, the components of such patterns are likely to be eumelanin alternating with pheomelanin (as in tigers or leopards), or eumelanin alternating with no pigment (as in zebras).

Thus, the mechanisms operative in pigment type-switching – *Agouti* and *Mc1r* signaling – may also be responsible, in part, for regular pigmentation patterns. However, in contrast to most coat color variants, an ordered pattern of stripes or spots have not been identified in laboratory mice or other rodents, which has hampered molecular genetic insight into the underlying mechanisms. Nonetheless, some limited conclusions can be drawn from genetic studies in domestic cats, where different alleles of a single gene, *Tabby*, modify pigment type-switching in regular patterns that may resemble tiger stripes or leopard spots. Because a single *Tabby* genotype can produce patterns that are either yellow alternating with black or white alternating with black, the white areas probably represent pigment type-switching rather than the absence of pigment cells. Whether a similar phenomenon explains alternating patterns of black and white in ungulates, i.e., zebras, is less clear, however, since the *Tabby* gene is clearly recognized only in the carnivora.

Insight from Coat Color Mutations into Human Pigmentation

As an increasing number of genomes are sequenced, it is becoming clear that the genomes of different mammals show relatively little variation in gene content or gene identity. It is no surprise, then, that many of the coat color mutations identified in mice or other furred animals have also been found in humans. Genetic variation in human pigmentation genes can be classified into rare, disease-causing variants such as albinism or piebaldism, or common variation in eye, hair, and skin color that may distinguish individuals of different ancestry.

In medical genetics, albinism refers to a generalized dilution or loss of pigmentation and is broadly grouped into conditions that affect eyes, skin, and hair (oculocutaneous albinism) or just the eyes (ocular albinism). In both cases, defects in retinal pigmentation frequently lead to visual impairment. Approximately 10 different genes have been identified that, when mutated, cause human albinism, including some involved in melanin biosynthesis such as *Tyrosinase*, *Tyrosinase-related protein 1*, *Pink-eyed dilution*, and others involved in vacuolar sorting or transport, i.e., Hermansky–Pudlak or Chediak–Higashi syndrome. In addition, several genes identified because of white spotting in mice are also sources

of mutations that cause localized loss of melanocytes in humans, occasionally associated with deafness, a condition termed 'Waardenburg syndrome.'

Mutations that affect pigment type-switching are also found in humans but, in contrast to the conditions described previously, are relatively common and a source of normal variation in many human populations. In particular, loss-of-function mutations in the human *Mc1r* gene account for the majority of individuals in populations of European ancestry that have carrot-red hair, fair skin, and freckling.

The genetic causes of blond versus brown versus black hair, or those responsible for skin pigment phenotypes characteristic for individuals of African, Asian, or white ancestry, have not been identified. However, biochemical and histological studies suggest these determinants are likely to have a relatively minor effect on pigment type-switching, and instead are more likely to modulate overall levels of melanogenesis. Identifying and understanding how these genes act remains a challenge for the future.

Further Reading

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See *also:* 0017 (Agouti), 0020 (Albinism), 0997 (Piebald Trait), 1001 (Pleiotropy), 1390 (W (White Spotting) Locus)