Linkage and segregation analysis of black and brindle coat color in domestic dogs

Julie A. Kerns1,*, Edward J. Cargill2,†, Leigh Anne Clark2, Sophie I. Candille1, Tom Berryere4, Michael Olivier3,††, George Lust3, Sheila.M. Schmutz4, Keith E. Murphy2, and Gregory S. Barsh1

1Departments of Genetics and Pediatrics, Stanford University, Stanford, CA, USA, 2Departments of Pathobiology and Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, 3College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, and 4Department of Animal and Poultry Sciences, University of Saskatchewan, Saskatoon S7N5A8, Canada.

*Current address: Fred Hutchinson Cancer Research Center, Seattle, WA 98109
†Current address: Monsanto Corporation, St. Louis, MO, 63167
††Current address: Human and Molecular Genetics Center, Medical College of Wisconsin, Milwaukee, WI 53226

Address correspondence to Greg Barsh, Beckman Center B271A, Stanford University School of Medicine, Stanford, CA 94305, 650 723 5035, gbarsh@cmgm.stanford.edu
Running title: Dog Black and Brindle Genes

Keywords: Domestic Dog  
Melanocortin signaling  
Agouti gene  
Pigmentation  
Brindle coat color
ABSTRACT

Mutations of pigment type-switching have provided basic insight into melanocortin physiology and evolutionary adaptation. In all vertebrates that have been studied to date, two key genes, Agouti and Melanocortin 1 receptor (Mc1r), encode a ligand-receptor system that controls the switch between synthesis of red-yellow pheomelanin vs. black-brown eumelanin. However, in domestic dogs, historical studies based on pedigree and segregation analysis have suggested that the pigment type-switching system is more complicated and fundamentally different from other mammals. Using a genome-wide linkage scan on a Labrador x Greyhound cross segregating for black, yellow, and brindle coat colors, we demonstrate that pigment type-switching is controlled by an additional gene, the K locus. Our results reveal three alleles with a dominance order of black ($K^B$) > brindle ($k^{br}$) > yellow ($k'$), whose genetic map position on dog chromosome 16 is distinct from the predicted location of other pigmentation genes. Interaction studies reveal that Mc1r is epistatic to variation at Agouti or K, and that the epistatic relationship between Agouti and K depends on the alleles being tested. These findings suggest a molecular model for a new component of the melanocortin signaling pathway and reveal how coat color patterns and pigmentary diversity have been shaped by recent selection.
INTRODUCTION

Morphologic variation among domestic dogs exemplifies the power of selective breeding to uncover a diversity of phenotypes from a relatively homogeneous founder population. Major questions posed by this phenomenon are the extent to which widely different phenotypes are caused by previously existing genetic variation or new mutations, and by epistatic interactions vs. single loci (reviewed in Barton and Keightley 2002; Falconer 1992). In several cases, line crosses between divergent populations, e.g. mice or chickens with high or low body weight (Carlborg et al. 2006; Hrbek et al. 2006), maize with high or low oil content (Laurie et al. 2004), Drosophila melanogaster with different numbers of bristles (Dilda and Mackay 2002), have been used to study selective breeding; for the most part, these approaches provide a genome-level view of genetic architecture, and are particularly useful if little is known about the underlying cell and molecular biology of the phenotypes, if there are a large number of candidate genes, or if one wishes to make no prior assumptions about the number or types of genes involved.

An alternative approach, taken here, is to consider a particular phenotype that has been subject to selection, and use classical transmission genetics to investigate questions of allelism and epistasis. This approach is particularly useful for color variation, which often exhibits patterns of inheritance that are consistent with Mendelian transmission, and for which the underlying biochemical and molecular genetic pathways have been investigated in laboratory animals (Jackson 1997; Searle 1968; Silvers 1979). The case of eumelanin vs. pheomelanin coloration is particularly intriguing, since available evidence points to a genetic system in domestic dogs that is distinct from that known to operate in other mammals (Little 1957).
In all mammals that have been studied to date, hair follicle melanocytes synthesize red-yellow pheomelanin or black-brown eumelanin depending on the balance between two key genes, *Agouti* and *Melanocortin 1 receptor (Mc1r)* (ANDERSSON 2003; KLUNGLAND and VAGE 2003). *Agouti* encodes a signaling molecule secreted from specialized cells in the dermis that acts as an inhibitory ligand for the *Mc1r* expressed on melanocytes (reviewed in BARSH 2006; CONE 2006). Mutations that constitutively activate the Mc1r cause a uniform black appearance, generally inherited in a dominant manner, while mutations that inactivate the *Mc1r* cause a uniform red or yellow appearance, generally inherited in a recessive manner. Conversely, because Agouti protein inhibits Mc1r activity, gain-of-function mutations yield dominant inheritance of a yellow coat, while loss-of-function mutations yield recessive inheritance of a black coat. Much of the classical genetics underlying the aforementioned relationships was summarized in a series of papers by Sewall Wright (1917a; 1917b; 1917c; 1917d; 1918a; 1918b), when *Mc1r* was known as the *Extension* locus (because different alleles could extend the amount of yellow vs. black pigment), and loss-of-function *Mc1r* mutations were known as *recessive yellow* (e).

Most dogs with a uniform black appearance, e.g. the Newfoundland, the Flat-Coated Retriever, black Labrador Retrievers, or black Poodles, exhibit dominant transmission of the black color, consistent with mutations that constitutively activate the Mc1r. However, pedigree and segregation analyses carried out by Clarence Cook Little (1957) indicated that dominant black was non-allelic with *recessive yellow*, leading to the suggestion that dominant black might be an unusual allele of the *Agouti* locus, *A*<sup>S</sup>. Recently, we examined a Labrador Retriever x Greyhound backcross with molecular probes for *Agouti* and *Mc1r*, and concluded that neither gene could account for the Labrador Retriever-derived black variant, which was inherited in an apparent
autosomal dominant manner, and to which we provisionally assigned the symbol $K$ (Kerns et al. 2003).

An additional aspect of coat color variation in domestic dogs that appears distinct from most other mammals is the phenotype known as brindle, in which stripes of red-yellow hair alternate with black-brown hair. Brindle stripes form an irregular pattern, typically with a "V" shape over the dorsum, and an "S" shape over flanks and ventrum, and are somewhat reminiscent of a dermatologic phenomenon in humans known as lines of Blaschko, thought to be caused by mosaicism of gene expression in keratinocyte clones (Bolognia et al. 1994; Jackson 1976; Widelitz et al. 2006). Brindle segregates as a single gene in a variety of dog breeds such as the Boxer, Greyhound, and French Bulldog, and has been thought by some authors to be caused by variation in Agouti, but by others to be caused by variation in Mc1r (Little 1957; Willis 1989; Winge 1950).

To better understand the genetic mechanisms responsible for coat color diversity among domestic dogs, we carried out a genome-wide linkage scan on pedigrees segregating dominant black, brindle, or both. Our results reveal a single locus with three alleles—yellow ($k^y$), brindle ($k^{br}$), and black ($K^B$)—whose genetic map position is clearly distinct from pigmentation genes known in other mammals. Interactions between alleles of the $K$ locus and those of Agouti and Mc1r uncover a simple genetic architecture that explains all known eumelanic—pheomelanic variation, and helps to reveal how selection has shaped morphologic diversity among different breeds of domestic dogs.

MATERIALS AND METHODS

DNA samples and pedigrees
Genomic DNA from blood or cheek swab samples was isolated according to standard procedures. Pedigrees in Figures 1 and 2 were established by two of us (G.L. and M.O.) at Cornell University to study hip dysplasia; pedigrees in Figure 3 were ascertained by one of us (S.M.S.) as part of a series of ongoing studies on dog coat color genetics, and were donated by private breeders. In all cases, pedigree relationships were verified by determining that multiple markers exhibited Mendelian segregation in accord with expectations.

Genotyping, statistical analysis, and genomics

Genotyping for the minimal screening set I panel (MSSI) of simple sequence length repeat (SSLP) markers described by Richman et al. (2001) was carried out using multiplex PCR as previously described (CARGILL et al. 2002; CLARK et al. 2004). Fluorescently labeled PCR products were separated on an automated laser fluorescence DNA sequencer ABI377 (Perkin-Elmer), using GENESCAN (version 2.1) fragment analysis software, and alleles identified using the GENOTYPER program (version 2.0; Perkin Elmer).

Prior to linkage analysis, Mendelian error-checking was performed. The data were then analyzed under a model of autosomal dominant inheritance for black vs. non-black assuming complete penetrance. Two-point linkage analyses were carried out using the MLINK (to generate LOD scores at different theta values) and ILINK programs (to maximize LOD) from the LINKAGE 5.1 package (LATHROP and LALOUEL 1984). Given the small number of animals in the scan, further analysis of additional markers was done manually, to determine the critical region and to infer haplotypes, as depicted in Figures 1, 2, and 3.

To infer the epistasis relationships between Agouti, Mc1r, and K alleles, we determined the Agouti and Mc1r coding sequence by sequencing PCR-amplified fragments of genomic DNA.
Primer sequences have been described previously (BERRYERE et al. 2005; KERNS et al. 2004; NEWTON et al. 2000; SCHMUTZ et al. 2003) and are available upon request. We determined $K$ genotypes using linkage and pedigree analysis (as described below) and, in some cases, by using additional markers that are in linkage disequilibrium with $K$ locus alleles and which will be described elsewhere. Genotypes for all three loci were determined (by resequencing Agouti and Mc1r, or by genotyping flanking markers for $K$ as described above) for every individual depicted in Figures 1, 2, and 3. This included 35 animals from the Cornell Labrador Retriever x Greyhound cross, 10 Afghan Hounds, 8 Great Danes, and 10 Staffordshire Bull Terriers. At least 5 animals from each of 4 additional breeds: German Shepherd Dogs, French Bulldogs, Boxers, and Poodles were also genotyped for all three loci as described in Table 3.

The physical location of markers and consideration of candidate genes is based on the CanFam1.0 assembly of the dog whole genome sequence (LINDBLAD-TOH et al. 2005) available through the UCSC Genome Browser (KAROLCHIK et al. 2003).

RESULTS

Nomenclature

Historically, Little and others (LITTLE 1957; WILLIS 1989) recognized that at least two different genes could give rise to a uniform pheomelanic coat, and used the term "fawn" to refer to the phenotype caused by the $a^v$ allele of Agouti, distinguished from a similar phenotype caused by a loss-of-function Mc1r allele, originally known as recessive yellow or $e$, and now known to represent $Mc1r^{R306ter}$. In some cases, differential gene action was inferred on the basis of the phenotype, with homozygosity for $Mc1r^{R306ter}$ giving rise to a clear or diluted yellow color, and the $a^v$ allele of Agouti referring to a deeper, often dark-tinged shade of red-yellow. In hindsight,
the effects of Agouti and Mc1r alleles cannot always be distinguished by virtue of their phenotype; also the \( K \) locus genotype is equally important in determining the balance and distribution of pheomelanin vs. eumelanin. To reconcile the historical terms with both common usage and modern genetics, we propose that alleles of the \( K \) locus be designated as yellow \((k^y)\), brindle \((k_{br})\), and black \((K^B)\). A summary of this nomenclature that relates the historical terms to those used here and the underlying genetics (described further below) is given in Table 1.

**Genome-wide linkage scan and fine mapping for dominant black**

In a Labrador Retriever \( \times \) Greyhound cross that was established at Cornell University to study hip dysplasia, a subset of kindreds exhibit transmission of coat color variation in a pattern that is consistent with inheritance of dominant black as originally suggested by Little. Black Labrador Retrievers crossed to yellow or brindle Greyhounds invariably yield black F1 offspring; when an F1 animal is backcrossed to the Greyhound parent, backcross progeny exhibit 1:1 segregation of black to non-black.

In previous studies of the EB and GB kindreds from this cross (Kerns et al. 2003), we observed that variation at neither Mc1r nor Agouti could account for dominant black (as an allele of the putative \( K \) locus); therefore we carried out a genome-wide linkage scan of the same kindreds using a dense panel of highly informative SSLP markers. For the initial screen of 19 animals (Figure 1), 125 of 155 markers from the “minimal screening set” described by Richman et al. were informative. We analyzed the results by two point linkage analysis under a model of dominant inheritance with complete penetrance, and observed that three loci on chromosomes 4 and 16 (CFA4, CFA16) exceeded a LOD score of 2 (Table 2).
The strongest evidence for linkage was obtained on CFA16 with marker FH2155 ($Z_{\text{max}}=3.6$ at $\theta=0$). We used the same marker, FH2155, to analyze four additional kindreds from the Cornell pedigree (Figure 2, and data not shown), and observed no recombinants between FH2155 and the $K$ locus, yielding a LOD score of 6 at $\theta=0$.

To refine the map location, we examined three additional markers surrounding FH2155 that span a distance of ~24 Mb, FH3592, REN275L19, and FH2175. In the EB and GB kindreds, recombinant chromosomes in two animals, EB57 and GB17, define a critical region between FH2175 and FH3592 of 23.7 Mb (33.7 – 57.4). An additional marker that lies between FH2175 and FH2155, REN292N24, was only informative for the FB and HB kindreds (Figure 2), but exhibited the same segregation pattern as FH2175 (3/10 recombinants), and therefore narrows the critical region to a 12 Mb segment (45.4 – 57.4, Figure 2B).

This region of the dog genome contains two human homology segments, 4q34 – 4q35 and 8p12, and has been annotated with more than 250 genes, mostly from other mammalian genomes (Figure 2C). Notably, none of those genes has been previously implicated in pigmentation, i.e. as a cause of human albinism or a mouse coat color mutation. Thus, the dog $K$ locus is likely to represent a previously unappreciated component of the Agouti—Melanocortin pathway.

**Allelism of yellow ($k^y$) and brindle**

As depicted in Figures 1 and 2, the FB, GB, and HB litters contain only black and yellow animals, whereas the EB litter and several of the parents in the Cornell cross are brindle. These observations are consistent either with brindle being an intermediate allele of the $K$ locus, recessive to black ($K$) and dominant to yellow ($k^y$), or with brindle being caused by another gene
whose effects are hypostatic to those of the $K$ allele. Based on previous studies in which brindle x brindle crosses often yielded a mixture of brindle and yellow (but never black) progeny, many dog breeders and geneticists assumed that brindle is caused by an intermediate allele of the

*extension* locus, $e^{br}$, that is dominant to *recessive yellow* ($e$ or $Mc1r^{R306ter}$) but hypostatic to *dominant black* (originally assigned to $A^5$).

To investigate whether allelism or epistasis was more likely to explain the relationship between brindle and the $K$ locus, we ascertained several kindreds in which brindle and yellow were segregating, and asked whether FH2155, which cosegregated perfectly with black vs. yellow (Figures 1 and 2), might also cosegregate with brindle vs. yellow. As depicted in Figure 3, there was perfect cosegregation between FH2155 and brindle vs. yellow in 5 phase-known meioses (in an Afghan pedigree) and 14 phase-unknown meioses (across a Great Dane and a Staffordshire Bull Terrier pedigree), corresponding to a LOD score of 3.6. We also re-examined all kindreds in the entire Cornell cross and a Boxer pedigree (data not shown), and found that, in every case, transmission of brindle was consistent with an intermediate allele of the $K$ locus with the following dominance relationships: *dominant black* ($K^B$) > *brindle* ($k^{br}$) > *yellow* ($k^y$).

**Epistatic interactions between alleles of the Agouti, K, and Mc1r loci**

As indicated above, loss-of-function for $Mc1r$ (*recessive yellow, e*) causes a yellow coat color that may appear very similar or even indistinguishable from that caused by homozygosity for *yellow* ($k^y$). Likewise, loss-of-function for *Agouti* (*nonagouti, a*) causes a black coat color that is indistinguishable from that caused by heterozygosity for *black* ($k^B$). To investigate the epistatic relationships between $K$ locus alleles and those of *Agouti* and $Mc1r$, we determined the genotype for all three loci in key animals from the pedigrees depicted in Figures 1 – 3, and additional
animals described in previous studies (Berryere et al. 2005; Kerns et al. 2004; Schmutz et al. 2003). For Agouti, we used the predicted cDNA sequence to distinguish among the \( a', a' \), and \( a \) alleles (Berryere et al. 2005); for \( Mclr \), we used the predicted cDNA sequence to distinguish between the \( R306ter (e) \) allele and all others (referred to below as \( Mclr^+ \)).

The consequent genotype-phenotype relationships provide a coherent view of epistatic interactions. For example, the Labrador Retrievers Andy (Figure 1A) and A14 (Figure 2A) have a genotype of \( a'/a'; K^B/K^B; e/e \); both animals have a yellow coat demonstrating that loss-of-function for \( Mclr \) is epistatic to both the black-and-tan (\( a' \)) allele of Agouti, and the black (\( K^B \)) allele of the \( K \) locus. In fact, Labrador Retrievers are fixed for the black (\( K^B \)) allele of the \( K \) locus and the black-and-tan (\( a' \)) allele of the Agouti locus; thus, black Labrador Retrievers demonstrate that the ability of the \( K \) locus to produce black pigment is epistatic to that of the \( Agouti \) locus to produce yellow pigment (because \( a'/a'; K^B/K^B \) animals are black rather than black-and-tan).

Observations for an additional two breeds are particularly demonstrative. Traditionally marked German Shepherd Dogs are fixed for the yellow (\( k^y \)) allele of the \( K \) locus and the + allele of \( Mclr \); the difference between black and black-and-tan German Shepherd Dogs is determined solely by the nonagouti (\( a \)) vs. the \( a' \) allele of Agouti. Thus, the ability of Agouti to prevent production of yellow pigment is epistatic to that of the \( K \) locus to allow yellow pigment. (Stated differently, the yellow allele (\( k^y \)) of the \( K \) locus can only give rise to yellow pigment in the presence of a functional \( Agouti \) allele). Finally, Afghan Hounds with a \( K \) genotype that would ordinarily yield brindle (\( k^{br}/k^{br} \) or \( k^{br}/k^y \)) may vary at both \( Agouti \) (\( a' \) or \( a' \)) and \( Mclr \) (+ or \( R306ter \)). In all cases, the interactions between \( k^{br} \) and \( Agouti \) or \( Mclr \) alleles can be predicted based on what happens for \( k^y \) and for \( K^B \). In \( a'/a'; k^{br}/k^{br}; +/+ \) animals, brindling is restricted to
the areas of the coat that would otherwise be tan ("black and brindle"); in e/e animals, brindling is not apparent because Mc1r is epistatic not only to K^B but also to k^{br}.

These relationships together with specific examples in which we have directly determined the genotype for Agouti, Mc1r, and K are summarized in Table 3, and their implications for understanding the underlying biochemical pathways are depicted in Figure 4. There are several key points. First, the relationship between Mc1r and Agouti in dogs is identical to that which occurs in other mammals where Agouti acts to antagonize melanocortin signaling in a manner that is completely dependent on a functional receptor. Second, Mc1r is epistatic to all K locus variation, and the K gene product behaves similar to Agouti protein in this way; each requires a functional Mc1r in order to modulate melanocortin signaling. Finally, the epistatic relationship between Agouti and K depends on the alleles being tested: "black alleles" of K are epistatic to "yellow alleles" of Agouti, but "black alleles" of Agouti are epistatic to "yellow alleles" of K. Thus, the relationship between Agouti and K is fundamentally different from the relationship between Mc1r and either Agouti or K.

These considerations suggest two alternative models (Figure 4). The K gene product may lie genetically upstream of Agouti and inhibit its function, either as a negative regulator of Agouti mRNA expression, or as a post-translational inhibitor that reduces the levels of active Agouti protein at the Mc1r (Figure 4A). Alternatively, the K gene product may act directly at the Mc1r to stimulate melanocortin signaling and thereby oppose the action of Agouti protein indirectly (Figure 4B).

DISCUSSION
A general theme of pigmentary genetics for the last century is that patterns of Mendelian variation within one species frequently display apparent homology to those in other species. For example, similar segregation and dominance relationships are observed among mice, guinea pigs, rabbits, and cats for the phenotypic series full color > chinchilla > acromelanic > albino, leading to the suggestion that mutations in the same gene—now known as Tyrosinase—are responsible. These types of observations, first made by Sewall Wright (1917c, and later by Clarence Cook Little (1957) and A.G. Searle (1968), foreshadowed the field of comparative genomics. Indeed, comparison of genome sequences not only clarified the evolutionary relationships among mammals (and most other organisms), but also provided the tools to identify molecular alterations responsible for the Tyrosinase color series in mice (KWON et al. 1989), cats (LYONS et al. 2005; SCHMIDT-KUNTZEL et al. 2005), cattle (SCHMUTZ et al. 2004), and rabbits (AIGNER et al. 2000) (though, ironically, not yet in guinea pigs).

Dominant black and brindling in dogs have been curious and somewhat confusing exceptions to the aforementioned theme. Historically, the allelic relationships for the Agouti locus—to which dominant black was assigned as the AS allele—were thought to be opposite to what pertains in other mammals, where "yellow alleles" are dominant to "black alleles" (LITTLE 1957). In the case of brindle, assigned to the Mc1r locus as ebr, epistasis relationships were confusing, with ebr epistatic to the a" allele but not to the AS allele (a"/a"; ebr/embr animals would be brindle but AS/AS; ebr/embr animals would be black).

The work described here resolves this confusion by demonstrating that both dominant black and brindling are due to alleles of a previously unappreciated pigmentation gene that we have named the K locus. Although the K locus is an apparent exception to the idea that the same set of molecular tools are used in all mammals, its recognition reinforces the general theme that genetic
interactions and pathways for orthologous genes are conserved. Thus, interactions both within and between *Agouti* and *Mclr* alleles in dogs are identical to those observed in other mammals: "black" *Mclr* alleles are dominant to "yellow" *Mclr* alleles, "yellow" *Agouti* alleles are dominant to "black" *Agouti* alleles, and double mutants for *Mclr* and *Agouti* always exhibit the phenotype of single *Mclr* mutants.

Our original survey of *Mclr* variation among domestic dogs (Newton et al. 2000) was motivated by the idea that dominant black might be due to a gain-of-function *Mclr* allele, as described in many other vertebrates (Eizirik et al. 2003; Hoekstra et al. 2006; Klungland and Vage 2003, Mundy, 2003 #189; Nachman et al. 2003; Rosenblum et al. 2004). Although we and others have identified a number of *Mclr* polymorphisms among domestic dogs (Everts et al. 2000; Newton et al. 2000; Schmutz et al. 2003), the only one for which there is an unequivocal effect on function is R306ter, responsible for the loss-of-function allele originally described as recessive yellow (*e*). Given the diversity of coat colors and patterns selected in modern breeds, it is perhaps surprising that an *Mclr* mutation that causes dominant black has not been found in dogs. However, a likely explanation is that variation at the *K* locus is relatively old among the canid lineage, since preexisting polymorphism for *k* vs. *K* would make it less likely that a new dominant black mutation at *Mclr* would be noticed.

Because the black (*K^B*) allele is epistatic to variation at *Agouti*, the yellow (*k^r*) allele probably represents the ancestral state, otherwise the Agouti phenotype (and other aspects of *Agouti*-induced variation such as white-bellied Agouti and black-and-tan) would have been cryptic in the ancestral population where variation at *K* first occurred. According to this hypothesis, wolf populations from which dogs were domesticated some 15,000 – 40,000 years ago were Agouti-colored or a gray modification of Agouti, similar to the appearance of modern wolves.
(Savolainen et al. 2002; Vila et al. 1999). Mutation from $k^v$ to $K^B$ is likely to have occurred prior to the origin of modern breeds several hundred years ago, and could even have been present in wolves as an adaptive polymorphism prior to domestication. An alternative scenario—positing that brindle ($k^{br}$) is the ancestral allele, with yellow ($k^y$) and black ($K^B$) as derivative alleles—is less likely given that the brindle phenotype is not present in modern canids other than domestic dogs.

Superficially, the brindle phenotype in domestic dogs shares some features with tabby striping in domestic cats (Lomax and Robinson 1988). Both involve patches or stripes of eumelanin vs. pheomelanin hairs, and both require the presence of a functional Agouti gene. However, the allelic system for tabby striping is probably opposite to brindle: the presence of black tabby stripes ($t^b$) is recessive to the absence of such stripes associated with the Abyssinian ($T^a$) allele in cats, while the presence of black brindle stripes ($k^{br}$) is dominant to the absence of such stripes associated with the yellow ($k^y$) allele in dogs. Equally important, the pattern of tabby striping is alternating and regular, consistent with an underlying pattern based on a Turing-like reaction-diffusion mechanism (Jiang et al. 2004; Suzuki et al. 2003; Widelitz et al. 2006). By contrast, the pattern of brindle stripes is irregular and variegated, most consistent with an epigenetic mechanism (discussed further below). These considerations are consistent with the view based on phylogenetic distribution of color patterns that tabby striping and brindling have independent evolutionary histories (Searle 1968).

The evolutionary history of variation at the $K$ locus will also have an impact on strategies for its molecular identification. The 12 Mb region to which $K$ has been mapped contains approximately 250 genes, many of which might plausibly be involved in melanocortin signaling (but none of which are obvious candidates). While additional pedigree-based linkage analysis could further
narrow the critical interval, a potentially more effective strategy is based on genetic association. Additional genotyping of SSLP and SNP markers within the 12 Mb interval should reveal whether the $K$, $k^{br}$, and $k$ alleles have specific haplotypes with which they are associated. If so, comparing the length of those haplotypes among unrelated animals may delineate a small candidate region. Success of this approach will depend on the degree to which $K$ locus alleles are identical by descent.

The epistasis relationships between $K$ and Agouti or $Mclr$ may also help to prioritize candidate genes. A functional $Mclr$ is required to "visualize" variation at $K$ and at Agouti, e.g. animals homozygous for the $Mclr$ R306ter (e) allele are yellow regardless of their genotype at $K$ or Agouti. Furthermore, a functional Agouti gene is required to "visualize" variation at $K$. This latter point is especially apparent from interactions between the black-and-tan ($a'$) and the brindle ($k^{br}$) mutations. The $a'$ mutation affects transcriptional regulation of Agouti coding sequences, limiting their expression to the dorsum or saddle areas; thus, the tan areas in black-and-tan animals (of genotype $a'/a'; k^v/k^v; Mclr^{+/+}$) represent locations of Agouti expression. In $a'/a'; k^{br}/k^{br}; Mclr^{+/+}$ animals, the effects of $k^{br}$ are restricted to the areas of Agouti expression, producing the phenotype known as "black-and-brindle" or "black with brindle points". Taken together, these considerations suggest that the $K$ gene product functions outside rather than within melanocytes, either as a negative regulator of Agouti protein levels, or as an alternative Mc1r ligand that activates melanocortin signaling (Figure 4).

A corollary of this argument is that the stripes in a brindle animal are likely to represent clones of skin cells that behave genetically as either $K^B$ or $k^v$, in which the irregular and unpredictable distance between stripes reflects a stochastic event that initially "sets" the apparent genotype for each clone. The brindle stripe pattern is similar to Blaschko lines in humans, thought to be
caused by mosaicism of gene expression in keratinocyte clones (BOLOGNIA et al. 1994; WIDELITZ et al. 2006). From this perspective, the fascinating pattern caused by the $k^{br}$ mutation is most likely explained by an unstable allele—between yellow ($k^y$) and black ($K^b$)—that acquires one or the other state by chance, and then maintains that state epigenetically as keratinocytes divide and migrate during embryonic development. An epigenetic event acting on keratinocyte clones would also explain why the brindle pattern in dogs is qualitatively different from variegation observed in X-inactivation mosaics or embryonic stem cell chimeras, where the relevant cell type is usually a neural crest-derived melanocyte, as with chimeras involving the albino mutation, or dermal papilla cells, as with chimeras involving Agouti (MILLAR et al. 1995; MINTZ 1971a; MINTZ 1971b; WILKIE et al. 2002). Thus, a likely candidate for the $K$ gene product is a secreted protein produced primarily by keratinocytes, but which, like Agouti protein, has a short radius of action.

Molecular identification of the Agouti and Mc1r genes provided much of the molecular groundwork for understanding the role of melanocortin signaling in a variety of physiologic processes, including regulation of energy balance, sexual behavior, and adrenocortical homeostasis. Additional studies of the $K$ locus in domestic dogs may allow similar opportunities.

Acknowledgements

We thank J. Longmire for his support of JAK, and Elaine Ostrander for helpful discussions. We are grateful to the dog breeders who generously submitted DNA samples from their litters, and to the DogMap and the NHGRI dog genome project for providing public access to the canine map at http://www.dogmap.ch/index.html and http://research.nhgri.nih.gov/dog_genome/. This work was supported by funds from the National Institutes of Health.
LITERATURE CITED


LITTLE, C. C., 1957 The inheritance of coat color in dogs. Comstock, Ithaca, N.Y.


MILLAR, S. E., M. W. MILLER, M. E. STEVENS and G. S. BARSH, 1995 Expression and transgenic studies of the mouse agouti gene provide insight into the mechanisms by which mammalian coat color patterns are generated. Development 121: 3223-3232.


WILLIS, M. B., 1989 *Genetics of the dog*. Howell, New York, N.Y.


WRIGHT, S., 1917a Color Inheritance in Mammals: II. The Mouse—Better Adapted to Experimental Work than Any Other Mammal—Seven Sets of Mendelian Allelomorphs Identified—Factorial Hypothesis Framed by Cuenot on Basis of His Work with Mice. J. Hered. 8: 373-378.

WRIGHT, S., 1917b Color Inheritance in Mammals: IV. The Rabbit—Has Three Sets of Multiple Allelomorphs Which, as in Six Other Cases in Mammals, Determine Linear Series of
Physiological Effects Not to be Explained as Mere Linkage of Factors in the Germ-cells. J Hered 8: 473-475.

WRIGHT, S., 1917c Color Inheritance in Mammals: Results of Experimental Breeding Can Be Linked up With Chemical Researches on Pigments--Coat Colors of All Mammals Classified as Due to Variations in Action of Two Enzymes. J Hered 8: 224-235.


Table 1. Phenotype—genotype relationships for *Agouti*, *K*, and *Mc1r*

<table>
<thead>
<tr>
<th>Common name</th>
<th>Phenotype and breed example</th>
<th>Historical names (symbols)</th>
<th>Possible genotypes based on current work$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant black or “Self-colored”</td>
<td>Uniformly black, can be modified to brown or by white spotting: Newfoundland, black or brown Labrador Retriever</td>
<td>$^\text{dominant}$ black, <em>Agouti</em>-Self ($A^s$)</td>
<td>$^\text{Agouti}$ $a^s/a$ $K^B/K^B$ $+/+$ $+/R306ter$</td>
</tr>
<tr>
<td>Recessive yellow</td>
<td>Uniformly red-yellow, can be modified to pale yellow or cream: yellow Labrador Retriever, Irish Setter, Samoyed</td>
<td>$^\text{recessive}$ yellow, extension ($e$) (all combinations)</td>
<td>$K^B$, $k^br$, $k^e$ $R306ter/R306ter$</td>
</tr>
<tr>
<td>Fawn</td>
<td>Red-tan, can be dark-tinged: Great Dane, yellow Boxer</td>
<td>$^\text{dominant}$ yellow, golden sable ($a^s$)</td>
<td>$a^s/a$ $k^e/k^e$ $+/+$ $+/R306ter$</td>
</tr>
<tr>
<td>Black-and-tan</td>
<td>Dobermann Pinscher</td>
<td>$^\text{tan points}$ ($a^s$)</td>
<td>$a^s/a$ $k^e/k^e$ $+/+$ $+/R306ter$</td>
</tr>
<tr>
<td>Brindle</td>
<td>Black- and yellow-colored stripes: brindle French Bulldog, brindle Boxer</td>
<td>$^\text{brindle, partial}$ extension ($e$) ($a^s$)</td>
<td>$k^br/k^br$, $k^e/k^e$ $+/+$ $+/R306ter$</td>
</tr>
<tr>
<td>Recessive black</td>
<td>Uniformly black: black German Shepherd Dog</td>
<td>$^\text{recessive}$ black ($a$)</td>
<td>$a/a$ $K^B$, $k^br$, $k^e$ $+/+$ $+/R306ter$</td>
</tr>
</tbody>
</table>

$^1$Explanations and references for names and symbols are given in the text.

$^2$Possible genotypes according to epistasis relationships as described in the text and in Table 3. Only 3 *Agouti* alleles are considered for the sake of simplicity; the $a^W$ allele would behave identically to the $a^s$ allele. Also, the "+" allele at *Mc1r* is used to designate any *Mc1r* allele other than R306ter (also known as recessive yellow or $e$).
Table 2. Two-point LOD scores for selected markers from genome wide linkage analysis\(^1\)

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Chromosome</th>
<th>Theta 0.1</th>
<th>Theta 0.2</th>
<th>Theta 0.3</th>
<th>Theta 0.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH2309</td>
<td>1</td>
<td>-2.66</td>
<td>-1.163</td>
<td>-0.45</td>
<td>-0.106</td>
</tr>
<tr>
<td>FH2598</td>
<td>1</td>
<td>-6.48</td>
<td>-3.57</td>
<td>-1.93</td>
<td>-0.81</td>
</tr>
<tr>
<td>FH2294</td>
<td>1</td>
<td>-2.98</td>
<td>-1.58</td>
<td>-0.82</td>
<td>-0.33</td>
</tr>
<tr>
<td>CO2.342</td>
<td>2</td>
<td>-3.8717</td>
<td>-1.9691</td>
<td>-0.96843</td>
<td>-0.36165</td>
</tr>
<tr>
<td>CO2.864</td>
<td>2</td>
<td>-4.57067</td>
<td>-2.36704</td>
<td>-1.19028</td>
<td>-0.45856</td>
</tr>
<tr>
<td>C02.608</td>
<td>2</td>
<td>-4.57</td>
<td>-2.37</td>
<td>-1.19</td>
<td>-0.459</td>
</tr>
<tr>
<td>FH2302</td>
<td>3</td>
<td>-2.98431</td>
<td>-1.58146</td>
<td>-0.81699</td>
<td>-0.32619</td>
</tr>
<tr>
<td>FH2107</td>
<td>3</td>
<td>-3.62</td>
<td>-1.76</td>
<td>-0.822</td>
<td>-0.282</td>
</tr>
<tr>
<td>FH2531</td>
<td>3</td>
<td>-2.66219</td>
<td>-1.16292</td>
<td>-0.45432</td>
<td>-0.10637</td>
</tr>
<tr>
<td>AHT128</td>
<td>4</td>
<td>2.11</td>
<td>1.85</td>
<td>1.39</td>
<td>0.774</td>
</tr>
<tr>
<td>FH2534</td>
<td>4</td>
<td>2.11</td>
<td>1.85</td>
<td>1.39</td>
<td>0.774</td>
</tr>
<tr>
<td>GLUT4</td>
<td>5</td>
<td>-4.57067</td>
<td>-2.36704</td>
<td>-1.19028</td>
<td>-0.45856</td>
</tr>
<tr>
<td>CPH18</td>
<td>5</td>
<td>-2.47</td>
<td>-1.17</td>
<td>-0.52</td>
<td>-0.168</td>
</tr>
<tr>
<td>TAT</td>
<td>5</td>
<td>-2.984</td>
<td>-1.58</td>
<td>-0.82</td>
<td>-0.326</td>
</tr>
<tr>
<td>FH2119</td>
<td>6</td>
<td>-3.61643</td>
<td>-1.76498</td>
<td>-0.8223</td>
<td>-0.28246</td>
</tr>
<tr>
<td>CPH3</td>
<td>6</td>
<td>-2.66219</td>
<td>-1.16292</td>
<td>-0.45432</td>
<td>-0.10637</td>
</tr>
<tr>
<td>FH2396</td>
<td>7</td>
<td>-2.66219</td>
<td>-1.16292</td>
<td>-0.45432</td>
<td>-0.10637</td>
</tr>
<tr>
<td>CO8.618</td>
<td>8</td>
<td>-3.93855</td>
<td>-2.18352</td>
<td>-1.18496</td>
<td>-0.50228</td>
</tr>
<tr>
<td>FH2138</td>
<td>8</td>
<td>-4.57067</td>
<td>-2.36704</td>
<td>-1.19028</td>
<td>-0.45856</td>
</tr>
<tr>
<td>FH2186</td>
<td>9</td>
<td>-2.66</td>
<td>-1.16</td>
<td>-0.454</td>
<td>-0.106</td>
</tr>
<tr>
<td>FH2537</td>
<td>10</td>
<td>-2.66</td>
<td>-1.16</td>
<td>-0.454</td>
<td>-0.106</td>
</tr>
<tr>
<td>FH2293</td>
<td>10</td>
<td>-2.00838</td>
<td>-0.85516</td>
<td>-0.35447</td>
<td>-0.11861</td>
</tr>
<tr>
<td>FH2422</td>
<td>10</td>
<td>-2.54061</td>
<td>-1.38764</td>
<td>-0.74127</td>
<td>-0.30846</td>
</tr>
<tr>
<td>FH2319</td>
<td>11</td>
<td>-2.66219</td>
<td>-1.16292</td>
<td>-0.45432</td>
<td>-0.10637</td>
</tr>
<tr>
<td>FH2096</td>
<td>11</td>
<td>-3.49485</td>
<td>-1.9897</td>
<td>-1.10924</td>
<td>-0.48455</td>
</tr>
<tr>
<td>AHT137</td>
<td>11</td>
<td>-4.57067</td>
<td>-2.36704</td>
<td>-1.19028</td>
<td>-0.45856</td>
</tr>
<tr>
<td>C12.852</td>
<td>12</td>
<td>-3.49</td>
<td>-1.99</td>
<td>-1.12</td>
<td>-0.484</td>
</tr>
<tr>
<td>CXX.391</td>
<td>13</td>
<td>-3.49</td>
<td>-1.2</td>
<td>-1.12</td>
<td>-0.485</td>
</tr>
<tr>
<td>FH2060</td>
<td>14</td>
<td>-5.08122</td>
<td>-2.77528</td>
<td>-1.48253</td>
<td>-0.61692</td>
</tr>
<tr>
<td>FH2547</td>
<td>14</td>
<td>-5.5</td>
<td>-2.7</td>
<td>-1.56</td>
<td></td>
</tr>
<tr>
<td>CPH5</td>
<td>15</td>
<td>-2.21849</td>
<td>-0.9691</td>
<td>-0.3786</td>
<td>-0.08864</td>
</tr>
<tr>
<td>Marker</td>
<td>Chromosome</td>
<td>Two-point LOD Scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>------------</td>
<td>----------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2321</td>
<td>15</td>
<td>-2.66219 -1.16292 -0.45432 -0.10637</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COS15</td>
<td>15</td>
<td>-2.03006 -0.9794 -0.44901 -0.1501</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHT139</td>
<td>15</td>
<td>-4.2 -2.4 -1.33 -0.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2171</td>
<td>15</td>
<td>-6.48 -3.6 -1.9 -0.81</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2278</td>
<td>15</td>
<td>-2.00897 -0.86147 -0.38114 -0.17759</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2175</td>
<td>16</td>
<td>2.109028 1.84738 1.38556 0.774084</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2155</td>
<td>16</td>
<td>3.0632 2.449 1.754 0.950175</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHTK209</td>
<td>20</td>
<td>-2.98431 -1.58146 -0.81699 -0.32619</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2312</td>
<td>21</td>
<td>-3.62 -1.76 -0.822 -0.282</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2233</td>
<td>21</td>
<td>-2.98431 -1.58146 -0.81699 -0.32619</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2538</td>
<td>22</td>
<td>-2.66219 -1.16292 -0.45432 -0.10637</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REN49F22</td>
<td>22</td>
<td>-2.66 -1.16 -0.454 -0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2079</td>
<td>24</td>
<td>-2.96262 -1.45722 -0.72245 -0.2947</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2261</td>
<td>24</td>
<td>-5.52491 -2.9691 -1.55826 -0.63465</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C26.733</td>
<td>26</td>
<td>-2.54061 -1.38764 -0.74127 -0.30846</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REN48E01</td>
<td>26</td>
<td>-2.54061 -1.38764 -0.74127 -0.30846</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEZ6</td>
<td>27</td>
<td>-2.03006 -0.9794 -0.44901 -0.1501</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CXX.176</td>
<td>28</td>
<td>-2.47 -1.17 -0.525 -0.168</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2208</td>
<td>28</td>
<td>-2.66219 -1.16292 -0.45432 -0.10637</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2585</td>
<td>28</td>
<td>-2.66219 -1.16292 -0.45432 -0.10637</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2305</td>
<td>30</td>
<td>-4.57067 -2.36704 -1.19028 -0.45856</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2199</td>
<td>31</td>
<td>-4.57067 -2.36704 -1.19028 -0.45856</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2239</td>
<td>31</td>
<td>-2.54061 -1.38764 -0.74127 -0.30846</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2238</td>
<td>32</td>
<td>-3.61643 -1.76498 -0.8223 -0.28246</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPH2</td>
<td>32</td>
<td>-3.61643 -1.76498 -0.8223 -0.28246</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REN41D20</td>
<td>32</td>
<td>-3.61643 -1.76498 -0.8223 -0.28246</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHT133</td>
<td>37</td>
<td>-2.98431 -1.58146 -0.81699 -0.32619</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2532</td>
<td>37</td>
<td>-3.61643 -1.76498 -0.8223 -0.28246</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH2587</td>
<td>37</td>
<td>-2.66219 -1.16292 -0.45432 -0.10637</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1A complete set of the 155 markers used for the linkage scan and the results are available upon request. The table shows those markers for which two-point LOD scores between the marker and dominant black were either >2.0 or < -2.0 at theta=0.1. The former category is shown in bold.
Table 3. Epistasis relationships for *Agouti*, *K*, and *Mc1r*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>(a^1/a^1) k/k +/+ black-and-tan German Shepherd Dog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^1/a^1) k/k e/e yellow Afghan Hound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^1/a^1) (k^br/k^br) +/+ black, brindle points Staffordshire Bull Terrier</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^1/a^1) (k^br/k^br) e/e yellow French Bulldog</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^1/a^1) (K^B/K^B) +/+ black black Labrador Retriever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^1/a^1) (K^B/K^B) e/e yellow yellow Labrador Retriever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^2/a^2) k/k +/+ yellow Boxer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^2/a^2) k/k e/e yellow Afghan Hound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^2/a^2) (k^br/k^br) +/+ brindle Boxer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^2/a^2) (k^br/k^br) e/e yellow Afghan Hound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^2/a^2) (K^B/K^B) +/+ black Great Dane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a^2/a^2) (K^B/K^B) e/e yellow Poodle</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[^1]: Nomenclature similar to Table 1, with the R306ter allele of *Mc1r* indicated as *e*. For each category, only homozygous genotypes are shown for the sake of simplicity; more genotypes are possible according to dominance relationships for each locus as indicated in Table 1.

[^2]: These designations refer only to the distribution of eumelanin and pheomelanin, and ignore the effects of modifiers that affect spotting and/or pigment quality. For example, black-and-tan in a Cocker Spaniel homozygous for the *b* allele of the *Tyrp1* locus would be modified to liver-and-tan; brindle in a French Bulldog carrying an *s* mutation would appear white with brindle spots.

[^3]: Examples are based on genotyping studies of dogs from the indicated breeds as described in the text, Berryere et al. (Berryere et al. 2005), or Newton et al. (2000).
Legends to Figures

Figure 1. Segregation of CFA16 haplotypes in the EB and GB litters. A, Haplotypes based on the four SSLP markers shown in panel B are indicated with vertical bars, just above genotypes for $K$ locus alleles. (As described in the text, brindle and yellow were considered in the same class, "non-black", for analysis of the genome scan; genotypes given here for $k^{br}$ and $k^y$ are based on information presented in Figure 3). Black-colored haplotypes originate from the Labrador Retriever grandparent carrying dominant black (B53 or Andy); white-colored haplotypes originate from the non-black Greyhound parent or grandparent (Esther or Isis). SSLP alleles are numbered arbitrarily according to increasing size for each marker. B, Physical location of SSLP markers used in panel A indicated in megabases (Mb) from the centromere. To the right of each marker name is given the number of animals recombinant between that marker and the $K$ allele, over the total number of animals that were informative for that marker. Recombinant chromosomes are carried by EB57 and GB17, and define a critical region for $K$ between FH2175 and FH3592. In addition to the $K$ locus genotypes depicted in the figure, *Agouti* and *Mc1r* genotypes were determined for every dog as described in Materials and Methods.

Figure 2. Segregation of CFA16 haplotypes in the FB and HB litters. A, B, Symbols are as in Figure 1. Recombinant chromosomes are carried by FB27, FB67, and HB27, and indicate that $K$ must lie centromere-distal to REN292N24. In addition to the $K$ locus genotypes depicted in the figure, *Agouti* and *Mc1r* genotypes were determined for every dog as described in Materials and Methods. C, Diagram of the $K$ critical region from REN292N24 to FH3592, indicating the location of RefSeq genes in the region (blue), and evolutionarily conserved regions in the human genome. Annotation is based on the CanFam1.0 dog genome assembly (Lindblad-Toh *et al.*).
2005) as displayed by UCSC Genome Browser (KAROLCHIK et al. 2003) using the "Human Net" comparative genomics track, in which red and yellow indicate sequence similarity to human chromosomes 8 and 4, respectively.

Figure 3. Segregation of FH2155 alleles in three kindreds with brindle and yellow. As described in the text, there is perfect cosegregation of FH2155 with brindle vs. yellow under a model of dominant inheritance with $K^B > k^{br} > k^r$, corresponding to a LOD score of 3.6.

Figure 4. Models for gene action at the $K$ locus. Both models must account for the observations that (1) the dominance order of Agouti is opposite to that of $K$; (2) $Mc1r$ alleles are epistatic to both Agouti and $K$ locus alleles; (2) "black alleles" of $K$ are epistatic to "yellow alleles" of Agouti; and (3) "black alleles" of Agouti are epistatic to "yellow alleles" of $K$. These observations are consistent with a model in which (A) the $K$ gene product functions to inhibit Agouti function, but are also consistent with a model in which (B) the $K$ gene product acts directly at the $Mc1r$ to stimulate melanocortin signaling and thereby oppose the action of Agouti protein indirectly.
A

**Figure 1**

**A**

- **B53**
  - **Esther**
    - Anubis
    - **GX95**
    - **EB27**
      - **EB37**
      - **EB47**
      - **EB57**
      - **EB67**
      - **GB17**
      - **GB27**
      - **GB37**
      - **GB77**
      - **GB87**
      - **GB97**
      - **GB107**

- **Andy**
  - **Isis**
  - **FX16**

**Symbols**:
- **yellow**
- **brindle**
- **black**
- yellow Labrador Retriever homozygous for \(Mc1r^{R306ter}\)

**B**

- **FH2175 (1/12)**
- **REN275L19 (0/7)**
- **FH2155 (0/12)**
- **FH3592 (1/12)**

Mb from centromere on CFA16 SSLP (No. recombinants with **dominant black**)

---

**Details**:
- Mb from centromere on CFA16
- SSLP (No. recombinants with **dominant black**)

---

**Mc1r** gene

- **Mc1r** (Melanocortin 1 receptor)
- **R306ter** mutation

---

**Genotypes**:
- **K**
- **k**
- **Brindle**
- **Yellow**
- **Black**

---

**Parental Genotypes**:
- **B53**
  - **Esther**
    - **Anubis**
    - **GX95**
    - **EB27**
      - **EB37**
      - **EB47**
      - **EB57**
      - **EB67**
    - **GB17**
      - **GB27**
      - **GB37**
      - **GB77**
      - **GB87**
      - **GB97**
      - **GB107**

---

**Isis**

- **Andy**
  - **FX16**

---

**Pedigree**

- **K**
- **k**
- **Brindle**
- **Yellow**
- **Black**

---

**SLLP**

- **FH2175 (1/12)**
- **REN275L19 (0/7)**
- **FH2155 (0/12)**
- **FH3592 (1/12)**

Mb from centromere on CFA16 SSLP (No. recombinants with dominant black)**
Figure 2

A

- **Pollux**
  - **AX26**
  - **AX56**
  - **Poly**

- **Castor**
  - **A14**

Key:
- yellow
- brindle
- black
- yellow Labrador Retriever homozygous for \( Mc1r^{R306ter} \)

B

- **REN292N24** (3/10)
- **FH2175** (3/10)
- **FH2155** (0/10)

Mb from centromere on CFA16

SSLP (No. recombinants with dominant black)

C

- **REN292N24** (45.4 Mb)
- **FH2155** (52.7 Mb)
- **FH3592** (57.4 Mb)

Dog RefSeq

Other RefSeq

Human Alignment Net (hg17)

8p12

4q34.2 - 4q35.2
Figure 3

Afghans

Great Danes

Staffordshire Bull Terriers

black

yellow

brindle
Figure 4

A

Agouti

K

---

dopaquinone

Mc1r

(E)

eumelanin

(Brown/black)

B

Agouti

K

dopaquinone

Mc1r

(E)

eumelanin

(Brown/black)