

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

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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^y), 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 eumelaninic vs. pheomelaninic 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^S . 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 pheomelanin coat, and used the term "fawn" to refer to the phenotype caused by the a^y 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^y 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 x 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 ($Z_{\max}=3.6$ 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^S).

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^y , a^t , and a alleles (BERRYERE *et al.* 2005); for *Mc1r*, we used the predicted cDNA sequence to distinguish between the *R306ter* (e) allele and all others (referred to below as $Mc1r^+$).

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^t/a^t ; K^B/K^B ; e/e ; both animals have a yellow coat demonstrating that loss-of-function for *Mc1r* is epistatic to both the *black-and-tan* (a^t) 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^t) 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^t/a^t ; 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 *Mc1r*; the difference between black and black-and-tan German Shepherd Dogs is determined solely by the *nonagouti* (a) vs. the a^t 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^t or a^y) and *Mc1r* (+ or *R306ter*). In all cases, the interactions between k^{br} and *Agouti* or *Mc1r* alleles can be predicted based on what happens for k^y and for K^B . In a^t/a^t ; 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 #171) and A.G. Searle (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 brindle 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 A^S 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 e^{br} , epistasis relationships were confusing, with e^{br} epistatic to the a^y allele but not to the A^S allele (a^y/a^y ; e^{br}/e^{br} animals would be brindle but A^S/A^S ; e^{br}/e^{br} 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^y) 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^y 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 $Mc1r$ may also help to prioritize candidate genes. A functional $Mc1r$ is required to "visualize" variation at K and at $Agouti$, e.g. animals homozygous for the $Mc1r$ *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^t) and the *brindle* (k^{br}) mutations. The a^t 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 $a^t/a^t; k^y/k^y; Mc1r^{+/+}$) represent locations of $Agouti$ expression. In $a^t/a^t; k^{br}/k^{br}; Mc1r^{+/+}$ 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^y , 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 *Mclr* 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.

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LITERATURE CITED

- AIGNER, B., U. BESENFELDER, M. MULLER and G. BREM, 2000 Tyrosinase gene variants in different rabbit strains. *Mamm Genome* **11**: 700-702.
- ANDERSSON, L., 2003 Melanocortin receptor variants with phenotypic effects in horse, pig, and chicken. *Ann N Y Acad Sci* **994**: 313-318.
- BARSH, G. S., 2006 Regulation of pigment type-switching by Agouti, Melanocortin signaling, Attractin, and Mahoganoid, pp. 395-410 in *The Pigmentary System*, edited by J. J. NORDLUND, R. E. BOISSY, V. J. HEARING, R. A. KING, W. S. OETTING *et al.* Blackwell.
- BARTON, N. H., and P. D. KEIGHTLEY, 2002 Understanding quantitative genetic variation. *Nat Rev Genet* **3**: 11-21.
- BERRYERE, T. G., J. A. KERNS, G. S. BARSH and S. M. SCHMUTZ, 2005 Association of an Agouti allele with fawn or sable coat color in domestic dogs. *Mamm Genome* **16**: 262-272.
- BOLOGNIA, J. L., S. J. ORLOW and S. A. GLICK, 1994 Lines of Blaschko. *J Am Acad Dermatol* **31**: 157-190; quiz 190-152.
- CARGILL, E. J., L. A. CLARK, J. M. STEINER and K. E. MURPHY, 2002 Multiplexing of canine microsatellite markers for whole-genome screens. *Genomics* **80**: 250-253.
- CARLBORG, O., L. JACOBSSON, P. AHGREN, P. SIEGEL and L. ANDERSSON, 2006 Epistasis and the release of genetic variation during long-term selection. *Nat Genet* **38**: 418-420.

CLARK, L. A., K. L. TSAI, J. M. STEINER, D. A. WILLIAMS, T. GUERRA *et al.*, 2004 Chromosome-specific microsatellite multiplex sets for linkage studies in the domestic dog. *Genomics* **84**: 550-554.

CONE, R. D., 2006 Studies on the physiological functions of the melanocortin system. *Endocr Rev* **27**: 736-749.

DILDA, C. L., and T. F. MACKAY, 2002 The genetic architecture of *Drosophila* sensory bristle number. *Genetics* **162**: 1655-1674.

EIZIRIK, E., N. YUHKI, W. E. JOHNSON, M. MENOTTI-RAYMOND, S. S. HANNAH *et al.*, 2003 Molecular genetics and evolution of melanism in the cat family. *Curr Biol* **13**: 448-453.

EVERTS, R. E., J. ROTHUIZEN and B. A. VAN OOST, 2000 Identification of a premature stop codon in the melanocyte-stimulating hormone receptor gene (MC1R) in Labrador and Golden retrievers with yellow coat colour. *Anim Genet* **31**: 194-199.

FALCONER, D. S., 1992 Early selection experiments. *Annu Rev Genet* **26**: 1-14.

HOEKSTRA, H. E., R. J. HIRSCHMANN, R. A. BUNDEY, P. A. INSEL and J. P. CROSSLAND, 2006 A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science* **313**: 101-104.

HRBEK, T., R. A. DE BRITO, B. WANG, L. S. PLETSCHER and J. M. CHEVERUD, 2006 Genetic characterization of a new set of recombinant inbred lines (LGXSM) formed from the inter-cross of SM/J and LG/J inbred mouse strains. *Mamm Genome* **17**: 417-429.

JACKSON, I. J., 1997 Homologous pigmentation mutations in human, mouse and other model organisms. *Hum Mol Genet* **6**: 1613-1624.

JACKSON, R., 1976 The lines of Blaschko: a review and reconsideration: Observations of the cause of certain unusual linear conditions of the skin. *Br J Dermatol* **95**: 349-360.

JIANG, T. X., R. B. WIDELITZ, W. M. SHEN, P. WILL, D. Y. WU *et al.*, 2004 Integument pattern formation involves genetic and epigenetic controls: feather arrays simulated by digital hormone models. *Int J Dev Biol* **48**: 117-135.

KAROLCHIK, D., R. BAERTSCH, M. DIEKHANS, T. S. FUREY, A. HINRICHS *et al.*, 2003 The UCSC Genome Browser Database. *Nucleic Acids Res* **31**: 51-54.

KERNS, J. A., J. NEWTON, T. G. BERRYERE, E. M. RUBIN, J. F. CHENG *et al.*, 2004 Characterization of the dog Agouti gene and a nonagouti mutation in German Shepherd Dogs. *Mamm Genome* **15**: 798-808.

KERNS, J. A., M. OLIVIER, G. LUST and G. S. BARSH, 2003 Exclusion of melanocortin-1 receptor (mc1r) and agouti as candidates for dominant black in dogs. *J Hered* **94**: 75-79.

KLUNGLAND, H., and D. I. VAGE, 2003 Pigmentary switches in domestic animal species. *Ann N Y Acad Sci* **994**: 331-338.

KWON, B. S., R. HALABAN and C. CHINTAMANENI, 1989 Molecular basis of mouse Himalayan mutation. *Biochem Biophys Res Commun* **161**: 252-260.

LATHROP, G. M., and J. M. LALOUEL, 1984 Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* **36**: 460-465.

- LAURIE, C. C., S. D. CHASALOW, J. R. LEDEAUX, R. MCCARROLL, D. BUSH *et al.*, 2004 The genetic architecture of response to long-term artificial selection for oil concentration in the maize kernel. *Genetics* **168**: 2141-2155.
- LINDBLAD-TOH, K., C. M. WADE, T. S. MIKKELSEN, E. K. KARLSSON, D. B. JAFFE *et al.*, 2005 Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* **438**: 803-819.
- LITTLE, C. C., 1957 *The inheritance of coat color in dogs*. Comstock, Ithaca, N.Y.
- LOMAX, T. D., and R. ROBINSON, 1988 Tabby pattern alleles of the domestic cat. *J Hered* **79**: 21-23.
- LYONS, L. A., D. L. IMES, H. C. RAH and R. A. GRAHN, 2005 Tyrosinase mutations associated with Siamese and Burmese patterns in the domestic cat (*Felis catus*). *Anim Genet* **36**: 119-126.
- 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.
- MINTZ, B., 1971a Clonal basis of mammalian differentiation. *Symp Soc Exp Biol* **25**: 345-370.
- MINTZ, B., 1971b Genetic mosaicism in vivo: development and disease in allophenic mice. *Fed Proc* **30**: 935-943.
- NACHMAN, M. W., H. E. HOEKSTRA and S. L. D'AGOSTINO, 2003 The genetic basis of adaptive melanism in pocket mice. *Proc Natl Acad Sci U S A* **100**: 5268-5273.

NEWTON, J. M., A. L. WILKIE, L. HE, S. A. JORDAN, D. L. METALLINOS *et al.*, 2000 Melanocortin 1 receptor variation in the domestic dog. *Mamm Genome* **11**: 24-30.

RICHMAN, M., C. S. MELLERSH, C. ANDRE, F. GALIBERT and E. A. OSTRANDER, 2001 Characterization of a minimal screening set of 172 microsatellite markers for genome-wide screens of the canine genome. *J Biochem Biophys Methods* **47**: 137-149.

ROSENBLUM, E. B., H. E. HOEKSTRA and M. W. NACHMAN, 2004 Adaptive reptile color variation and the evolution of the *Mc1r* gene. *Evolution Int J Org Evolution* **58**: 1794-1808.

SAVOLAINEN, P., Y. P. ZHANG, J. LUO, J. LUNDEBERG and T. LEITNER, 2002 Genetic evidence for an East Asian origin of domestic dogs. *Science* **298**: 1610-1613.

SCHMIDT-KUNTZEL, A., E. EIZIRIK, S. J. O'BRIEN and M. MENOTTI-RAYMOND, 2005 Tyrosinase and tyrosinase related protein 1 alleles specify domestic cat coat color phenotypes of the albino and brown loci. *J Hered* **96**: 289-301.

SCHMUTZ, S. M., T. G. BERRYERE, D. C. CIOBANU, A. J. MILEHAM, B. H. SCHMIDTZ *et al.*, 2004 A form of albinism in cattle is caused by a tyrosinase frameshift mutation. *Mamm Genome* **15**: 62-67.

SCHMUTZ, S. M., T. G. BERRYERE, N. M. ELLINWOOD, J. A. KERNS and G. S. BARSH, 2003 MC1R studies in dogs with melanistic mask or brindle patterns. *J Hered* **94**: 69-73.

SEARLE, A. G., 1968 *Comparative Genetics of Coat Color in Mammals*. Academic Press, New York, N.Y.

- SILVERS, W. K., 1979 *The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction*. Springer-Verlag, New York, N.Y.
- SUZUKI, N., M. HIRATA and S. KONDO, 2003 Traveling stripes on the skin of a mutant mouse. *Proc Natl Acad Sci U S A* **100**: 9680-9685.
- VILA, C., J. E. MALDONADO and R. K. WAYNE, 1999 Phylogenetic relationships, evolution, and genetic diversity of the domestic dog. *J Hered* **90**: 71-77.
- WIDELITZ, R. B., R. E. BAKER, M. PLIKUS, C. M. LIN, P. K. MAINI *et al.*, 2006 Distinct mechanisms underlie pattern formation in the skin and skin appendages. *Birth Defects Res C Embryo Today* **78**: 280-291.
- WILKIE, A. L., S. A. JORDAN and I. J. JACKSON, 2002 Neural crest progenitors of the melanocyte lineage: coat colour patterns revisited. *Development* **129**: 3349-3357.
- WILLIS, M. B., 1989 *Genetics of the dog*. Howell, New York, N.Y.
- WINGE, O., 1950 *Inheritance in Dogs*. Comstock, Ithaca, NY.
- 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.

WRIGHT, S., 1917d Color Inheritance in Mammals: V. The Guinea-pig--Great Diversity in Coat-pattern, Due to Interaction of Many Factors in Development--Some Factors Hereditary, Others of the Nature of Accidents in Development. J Hered **8**: 476-480.

WRIGHT, S., 1918a Color Inheritance in Mammals: IX, The Dog--Many Kinds of White Patterns Found--Albinism Resembles That of Other Mammals in Reducing Red More Than Black--Inheritance of Black-and-Tan Requires Further Data--Red and Liver Simple Recessives. J Hered **9**: 87-90.

WRIGHT, S., 1918b Color Inheritance in Mammals: X., The Cat--Curious Association of Deafness with Blue-eyed White Color and of Femaleness with Tortoise-shelled Color, Long Known--Variations of Tiger Pattern Present Interesting Features. J Hered **9**: 139-144.

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

Common name	Phenotype and breed example	Historical names (symbols)	Possible genotypes based on current work ²		
			<i>Agouti</i>	<i>K</i> locus	<i>Mc1r</i>
Dominant black or "Self-colored"	Uniformly black, can be modified to brown or by white spotting: Newfoundland, black or brown Labrador Retriever	<i>dominant black, Agouti-Self</i> (<i>A^S</i>)	<i>a^y/a</i> <i>a^y/a^t</i> <i>a^y/a</i>	<i>K^B/K^B</i> <i>K^B/k^y</i> <i>K^B/k^{br}</i>	<i>+/+</i> <i>+/R306ter</i>
Recessive yellow	Uniformly red-yellow, can be modified to pale yellow or cream: yellow Labrador Retriever, Irish Setter, Samoyed	<i>recessive yellow, extension</i> (<i>e</i>)	<i>a^y, a^t, a</i> (all combinations)	<i>K^B, k^{br}, k^y</i> (all combinations)	<i>R306ter/R306ter</i>
Fawn	Red-tan, can be dark-tinged: Great Dane, yellow Boxer	<i>dominant yellow, golden sable</i> (<i>a^y</i>)	<i>a^y/a</i> <i>a^y/a^t</i> <i>a^y/a</i>	<i>k^y/k^y</i>	<i>+/+</i> <i>+/R306ter</i>
Black-and-tan	Dobermann Pinscher	<i>tan points</i> (<i>a^t</i>)	<i>a^t/a</i> <i>a^t/a</i>	<i>k^y/k^y</i>	<i>+/+</i> <i>+/R306ter</i>
Brindle	Black- and yellow-colored stripes: brindle French Bulldog, brindle Boxer	<i>brindle, partial extension</i> (<i>e^{br}</i>)	<i>a^y/a</i> <i>a^y/a^t</i> <i>a^y/a</i>	<i>k^{br}/k^{br},</i> <i>k^{br}/k^y</i>	<i>+/+</i> <i>+/R306ter</i>
Recessive black	Uniformly black: black German Shepherd Dog	<i>recessive black</i> (<i>a</i>)	<i>a/a</i>	<i>K^B, k^{br}, k^y</i> (all combinations)	<i>+/+</i> <i>+/R306ter</i>

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

²Possible 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^t* 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 analysis¹

Marker Name	Chromosome	Theta			
		0.1	0.2	0.3	0.4
FH2309	1	-2.66	-1.163	-0.45	-0.106
FH2598	1	-6.48	-3.57	-1.93	-0.81
FH2294	1	-2.98	-1.58	-0.82	-0.33
CO2.342	2	-3.8717	-1.9691	-0.96843	-0.36165
CO2.864	2	-4.57067	-2.36704	-1.19028	-0.45856
C02.608	2	-4.57	-2.37	-1.19	-0.459
FH2302	3	-2.98431	-1.58146	-0.81699	-0.32619
FH2107	3	-3.62	-1.76	-0.822	-0.282
FH2531	3	-2.66219	-1.16292	-0.45432	-0.10637
AHT128	4	2.11	1.85	1.39	0.774
FH2534	4	2.11	1.85	1.39	0.774
GLUT4	5	-4.57067	-2.36704	-1.19028	-0.45856
CPH18	5	-2.47	-1.17	-0.52	-0.168
TAT	5	-2.984	-1.58	-0.82	-0.326
FH2119	6	-3.61643	-1.76498	-0.8223	-0.28246
CPH3	6	-2.66219	-1.16292	-0.45432	-0.10637
FH2396	7	-2.66219	-1.16292	-0.45432	-0.10637
CO8.618	8	-3.93855	-2.18352	-1.18496	-0.50228
FH2138	8	-4.57067	-2.36704	-1.19028	-0.45856
FH2186	9	-2.66	-1.16	-0.454	-0.106
FH2537	10	-2.66	-1.16	-0.454	-0.106
FH2293	10	-2.00838	-0.85516	-0.35447	-0.11861
FH2422	10	-2.54061	-1.38764	-0.74127	-0.30846
FH2319	11	-2.66219	-1.16292	-0.45432	-0.10637
FH2096	11	-3.49485	-1.9897	-1.10924	-0.48455
AHT137	11	-4.57067	-2.36704	-1.19028	-0.45856
C12.852	12	-3.49	-1.99	-1.12	-0.484
CXX.391	13	-3.49	-1.2	-1.12	-0.485
FH2060	14	-5.08122	-2.77528	-1.48253	-0.61692
FH2547	14	-5.5	-2.7	-1.56	
CPH5	15	-2.21849	-0.9691	-0.3786	-0.08864

FH2321	15	-2.66219	-1.16292	-0.45432	-0.10637
COS15	15	-2.03006	-0.9794	-0.44901	-0.1501
AHT139	15	-4.2	-2.4	-1.33	-0.58
FH2171	15	-6.48	-3.6	-1.9	-0.81
FH2278	15	-2.00897	-0.86147	-0.38114	-0.17759
FH2175	16	2.109028	1.84738	1.38556	0.774084
FH2155	16	3.0632	2.449	1.754	0.950175
AHTK209	20	-2.98431	-1.58146	-0.81699	-0.32619
FH2312	21	-3.62	-1.76	-0.822	-0.282
FH2233	21	-2.98431	-1.58146	-0.81699	-0.32619
FH2538	22	-2.66219	-1.16292	-0.45432	-0.10637
REN49F22	22	-2.66	-1.16	-0.454	-0.11
FH2079	24	-2.96262	-1.45722	-0.72245	-0.2947
FH2261	24	-5.52491	-2.9691	-1.55826	-0.63465
C26.733	26	-2.54061	-1.38764	-0.74127	-0.30846
REN48E01	26	-2.54061	-1.38764	-0.74127	-0.30846
PEZ6	27	-2.03006	-0.9794	-0.44901	-0.1501
CXX.176	28	-2.47	-1.17	-0.525	-0.168
FH2208	28	-2.66219	-1.16292	-0.45432	-0.10637
FH2585	28	-2.66219	-1.16292	-0.45432	-0.10637
FH2305	30	-4.57067	-2.36704	-1.19028	-0.45856
FH2199	31	-4.57067	-2.36704	-1.19028	-0.45856
FH2239	31	-2.54061	-1.38764	-0.74127	-0.30846
FH2238	32	-3.61643	-1.76498	-0.8223	-0.28246
CPH2	32	-3.61643	-1.76498	-0.8223	-0.28246
REN41D20	32	-3.61643	-1.76498	-0.8223	-0.28246
AHT133	37	-2.98431	-1.58146	-0.81699	-0.32619
FH2532	37	-3.61643	-1.76498	-0.8223	-0.28246
FH2587	37	-2.66219	-1.16292	-0.45432	-0.10637

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 *Mclr*

Genotype ¹			Phenotype ²	Example ³
<i>Agouti</i>	<i>K</i> locus	<i>Mclr</i>		
a^1/a^1	k/k	$+/+$	black-and-tan	German Shepherd Dog
a^1/a^1	k/k	e/e	yellow	Afghan Hound
a^1/a^1	k^{br}/k^{br}	$+/+$	black, brindle points	Staffordshire Bull Terrier
a^1/a^1	k^{br}/k^{br}	e/e	yellow	French Bulldog
a^1/a^1	K^B/K^B	$+/+$	black	black Labrador Retriever
a^1/a^1	K^B/K^B	e/e	yellow	yellow Labrador Retriever
a^y/a^y	k/k	$+/+$	yellow	Boxer
a^y/a^y	k/k	e/e	yellow	Afghan Hound
a^y/a^y	k^{br}/k^{br}	$+/+$	brindle	Boxer
a^y/a^y	k^{br}/k^{br}	e/e	yellow	Afghan Hound
a^y/a^y	K^B/K^B	$+/+$	black	Great Dane
a^y/a^y	K^B/K^B	e/e	yellow	Poodle

¹Nomenclature similar to Table 1, with the R306ter allele of *Mclr* 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.

²These 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.

³Examples 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 $K^B > k^{br} > k^y$, 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.

Figure 1

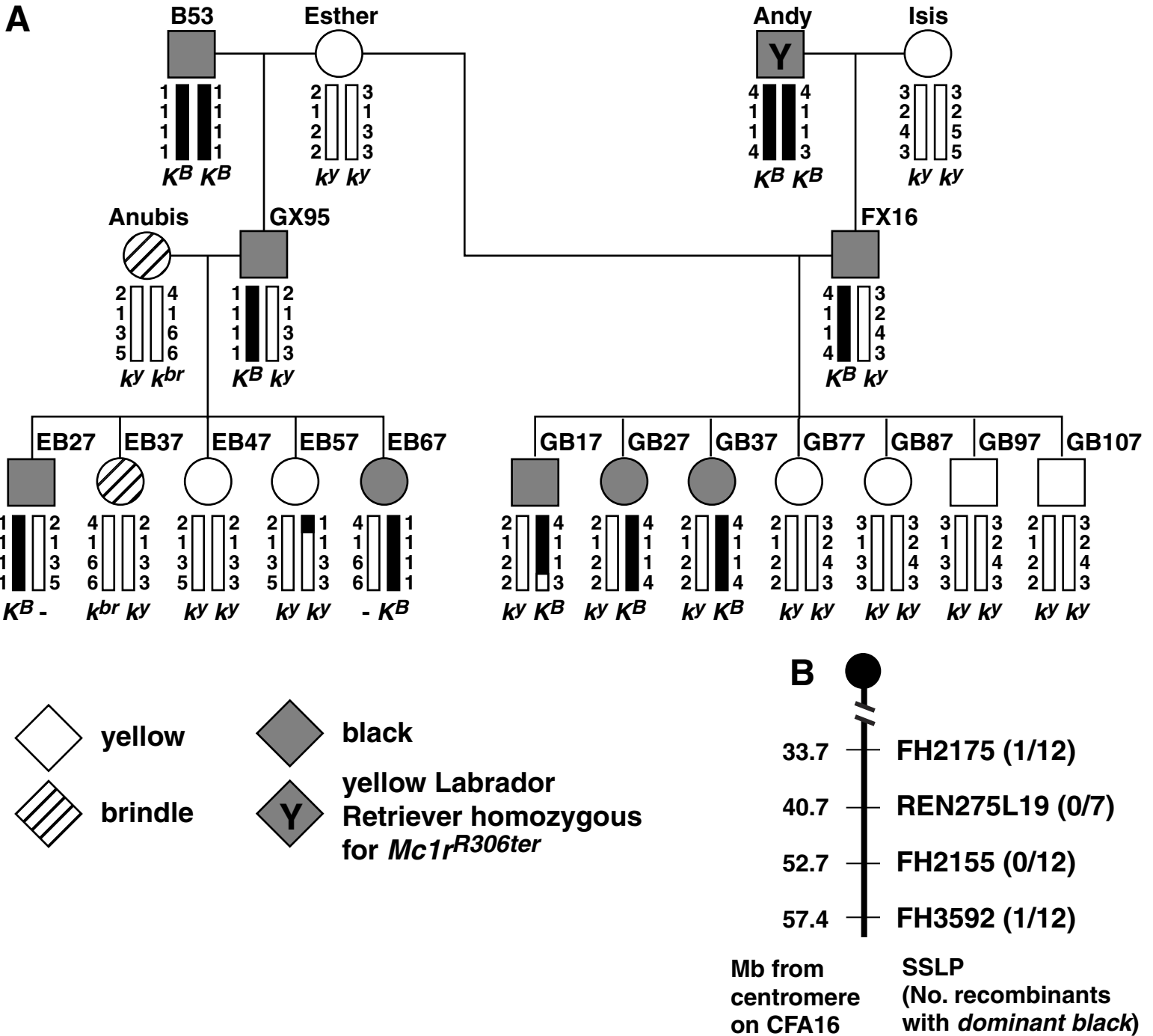
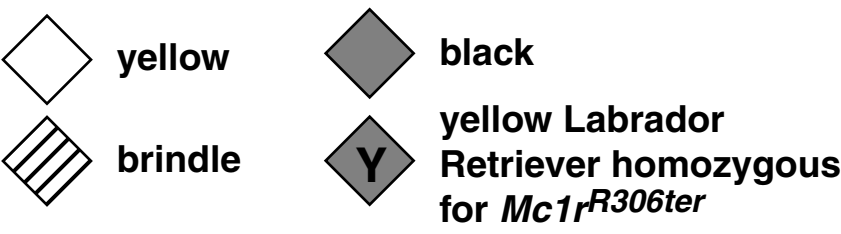
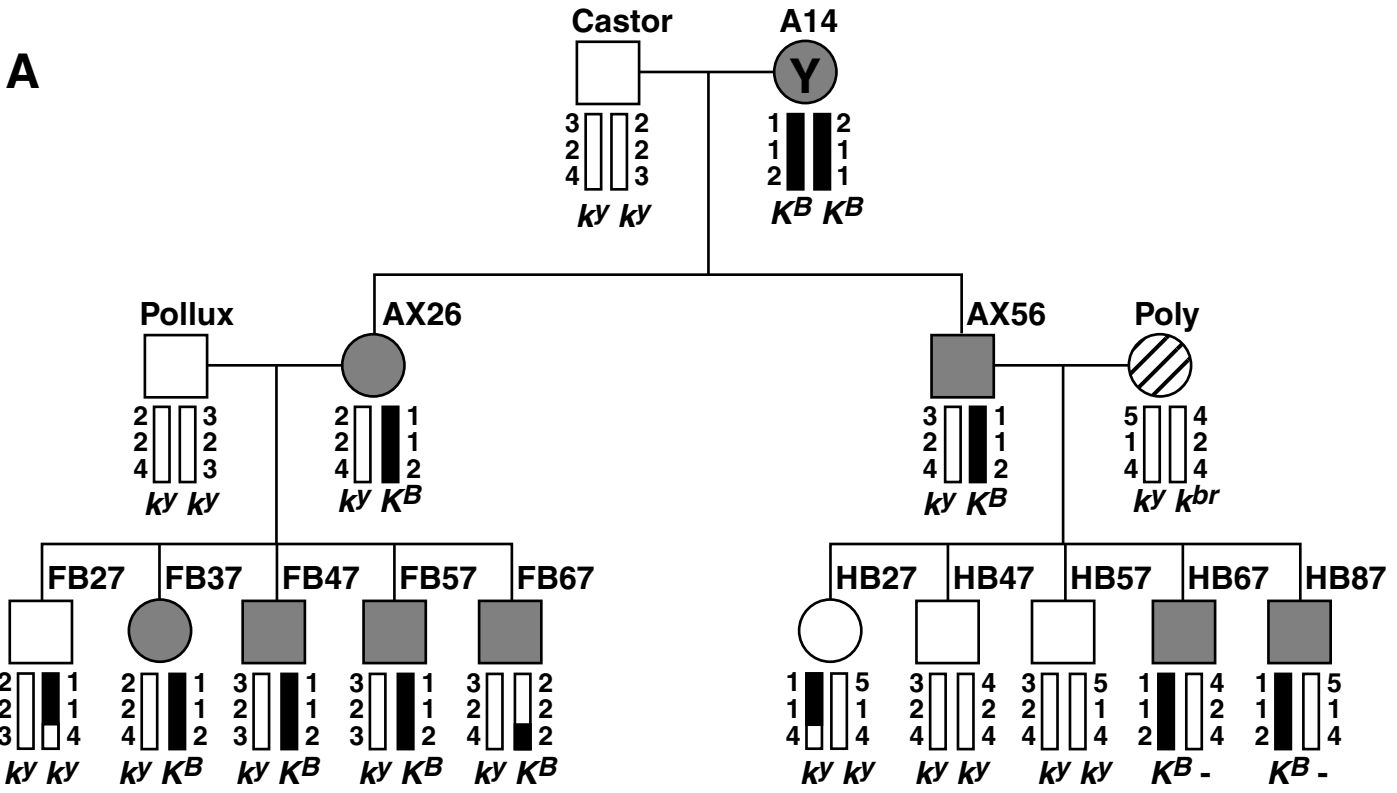
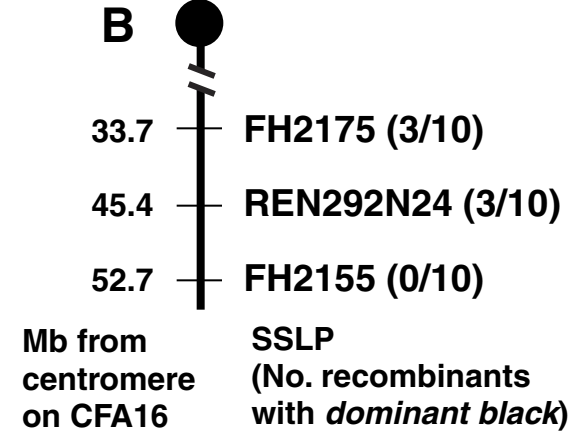


Figure 2

A



B



C

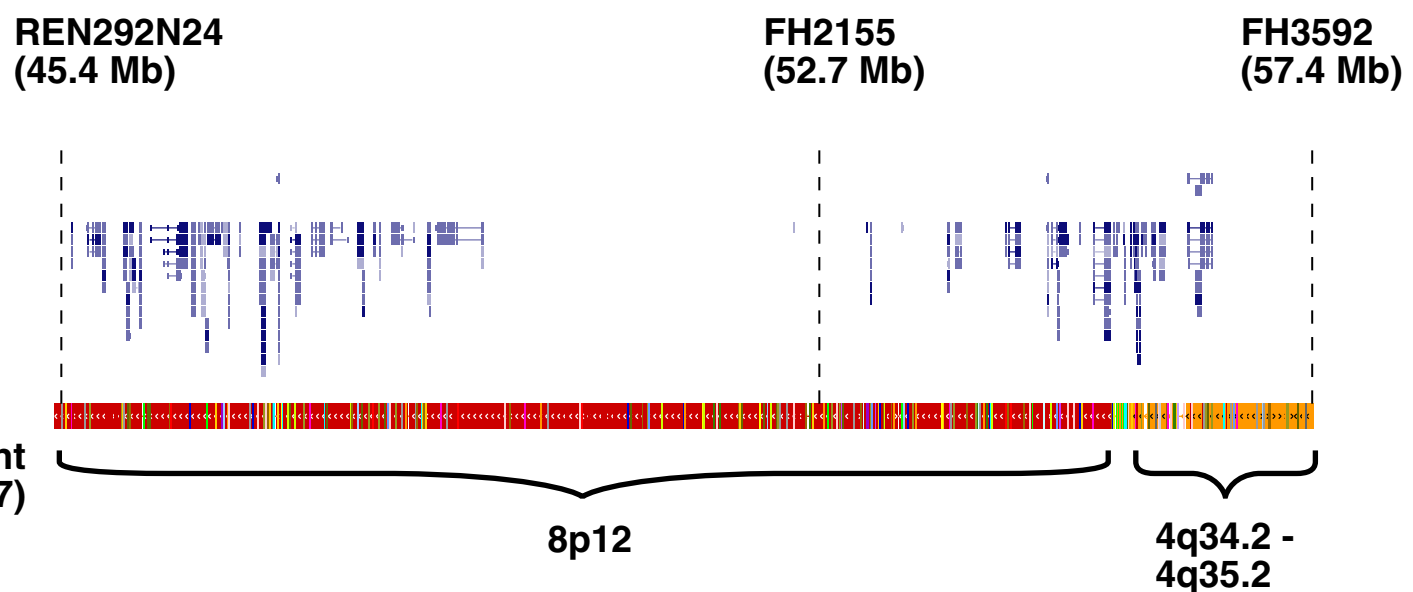


Figure 3

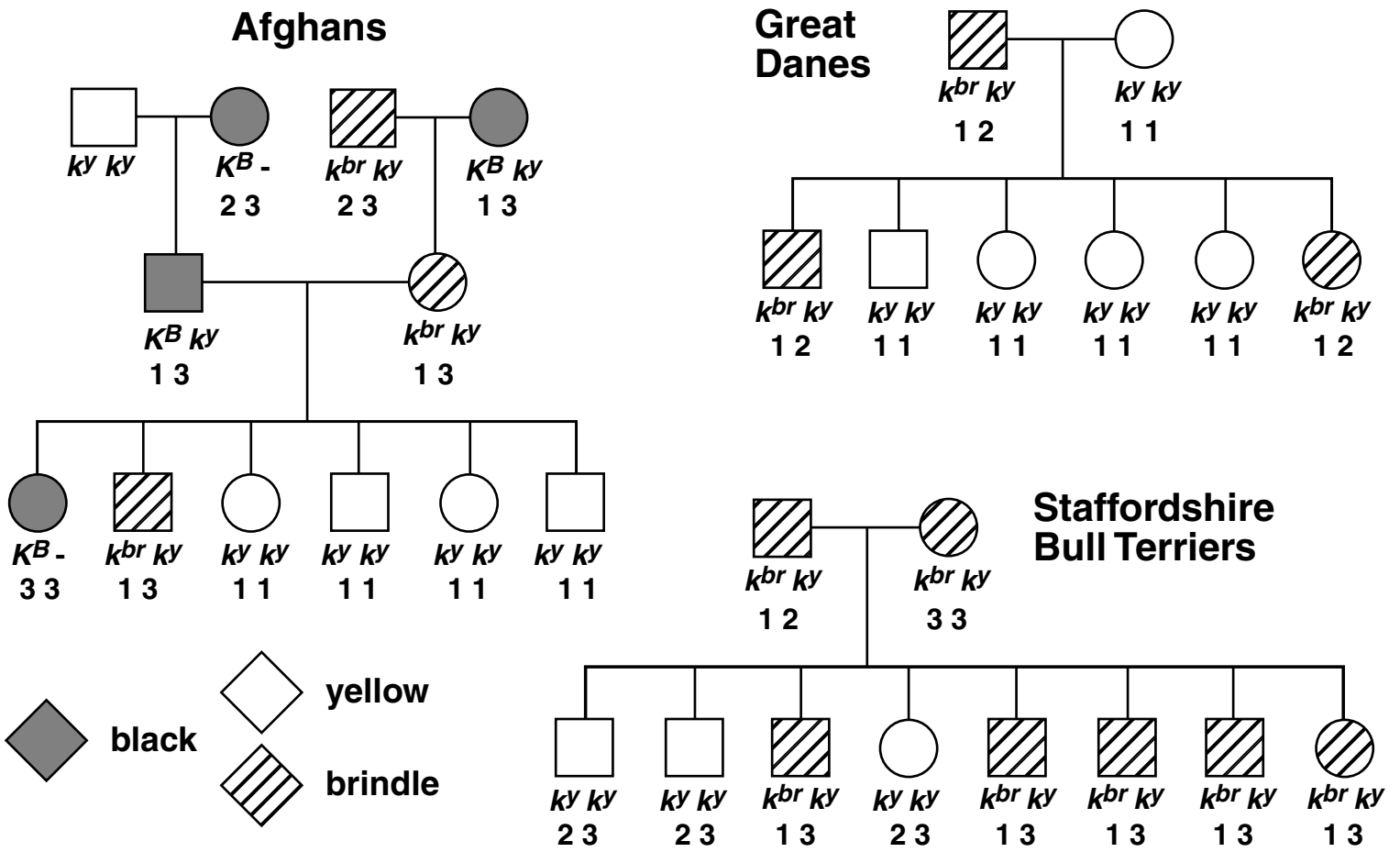
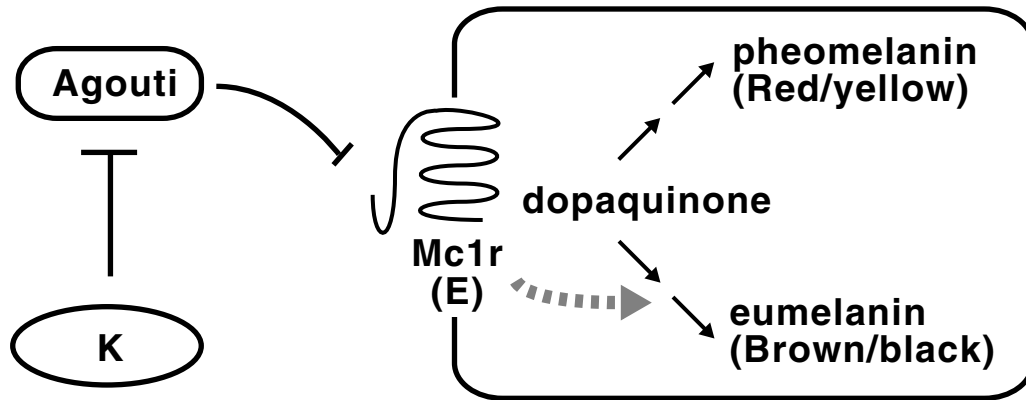


Figure 4

A



B

