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Genes affecting coat colour and pattern in domestic dogs: a review

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Summary

Tremendous progress has been made in identifying genes involved in pigmentation in dogs in the past few years. Comparative genomics has both aided and benefited from these findings. Seven genes that cause specific coat colours and/or patterns in dogs have been identified: *melanocortin 1 receptor*, *tyrosinase related protein 1*, *agouti signal peptide*, *melanophilin*, *SILV* (formerly *PMEL17*), *microphthalmia-associated transcription factor* and *beta-defensin 103*. Although not all alleles have been yet identified at each locus, DNA tests are available for many. The identification of these alleles has provided information on interactions in this complex set of genes involved in both pigmentation and neurological development. The review also discusses pleiotropic effects of some coat colour genes as they relate to disease. The alleles found in various breeds have shed light on some potential breed development histories and phylogenetic relationships. The information is of value to dog breeders who have selected for and against specific colours since breed standards and dog showing began in the late 1800s. Because coat colour is such a visible trait, this information will also be a valuable teaching resource.

Keywords black hair follicular dysplasia, canine, coat colour, colour dilution alopecia, dog breeding.

Introduction

Dogs have been selected to have more variation in size and body type than most species of domestic animals. Is it any wonder they also vary so much in coat colour (Fig. 1)? The genetics of coat colour in dogs, as well as other mammals, has been studied for many years. Little (1957) and Winge (1950) both wrote books postulating a number of genes that could explain the inheritance of coat colour and pattern in dogs. Each based their hypotheses on breeding data. Both authors used letters to indicate various loci, some with only two alleles, some with more. Although they did not use the same letters, in general their hypotheses regarding the number of genes with various effects were very similar.

Many dog breeders have tried to study the genetics of coat colour in their particular breed and most have relied on Little (1957). In most breeds, a relatively narrow range of colours is allowed within the standard and in a few breeds, all dogs have the same colour pattern. Certain colours have been considered a disqualification in some breeds, because either they were considered to suggest crossbreeding or the colour was considered to have deleterious side effects.

This review explains the research in molecular genetics that has occurred to both support and negate the hypotheses of Little (1957). For many dog breeders, these data will help explain why some litters did not fit their expectations in terms of colours of the pups. Because many people own dogs, the coat colour information provided herein can become a valuable resource to teach genetic principles of gene interaction and epistasis, compound mutations and pleiotropic effects. In addition to examples of autosomal dominant and recessive inheritance, there are also examples of co-dominant inheritance.

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Basic coat colour loci in dogs

The *melanocortin 1 receptor* gene

Melanocortin 1 receptor (*MC1R*) was the first gene studied using molecular approaches in dogs. *MC1R* was mapped to CFA5, 6 cM from *ZuBeCa*6 (Schmutz *et al.* 2001a). Both Newton *et al.* (2000) and Everts *et al.* (2000) published a loss-of-function mutation, 914C>T, which causes clear red coat colour in dogs (Fig. 1e,h,l). This mutation, which causes an arginine to be replaced by a premature stop codon (R306ter), is present in a wide range of dog breeds (Newton *et al.* 2000; Schmutz *et al.* 2002). Little (1957) called this

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Figure 1 Photographs and selected genotypes of various dogs illustrating the variety of coat colours and gene interactions. (a) Brown-and-tan French Brittany k^y/k^y, *E/E*, *b/b*, a^t/a^t; (b) (left) brown German Shorthaired Pointer K^B/K^B, *E/E*, *b/b* and (right) black Large Munsterlander K^B/K^B, *E/E*, *B/b*; (c) Landseer Newfoundland K^B/K^B, *E/E*, *B/B*, *s/s*; (d) fawn masked Chinese Shar-Pei k^y/k^y, E^M/E, *B/B*, a^y/a^y; (e) fawn French Bulldog k^{br}/k^y, *e/e*, *B/B*, a^y/a^y; (f) brindle Staffordshire Bull Terrier k^{br}/k^y, *E/E*, *B/B*, a^y/a^t; (g) merle Australian Shepherd k^y/k^y, *E/E*, *B/B*, a^t/a^t, *M/m*; (h) (left) clear red Miniature Dachshund k^y/k^y, *e/e*, *B/B* and (right) black-and-tan Miniature Dachshund k^y/k^y, *E/–*, *B/B*, a^t/a^t; (i) blue Great Dane $K^B/–$, *E/E*, *B/B*, a^y/a^y, *d/d*; (j) dilute fawn Italian Greyhound k^y/k^y, E^M/E, *B/B*, a^y/a^y, *d/d*; (k) young Kerry Blue Terrier K^B/K^B, E^M/E, *B/B*, *D/D*, *G/G*; (l) gold Viszla K^B/K^B, *e/e*, *b/b*. See Table 1 for allele abbreviations.

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the *E* or *extension* locus and therefore this allele was termed *e* and the wild type allele *E* (Table 1).

A third allele E^M (Table 1) is caused by a single nucleotide substitution (799A>G) resulting in a M264V amino acid change (Schmutz *et al.* 2003a). The melanistic mask caused by one copy of this allele is only visible on dogs that are fawn or brindle (Fig. 1d). Dogs that are solid black, brown, or blue do not have a mask that is distinguishable from their body colour. However, dogs that fade to grey with age do show their mask for a time (Fig. 1k). Similarly, dogs that have white muzzles do not produce melanin in that area of the body and so they may not exhibit a mask, even if they carry this allele (Fig. 1j).

The *tyrosinase related protein 1* gene

Tyrosinase related protein 1 (*TYRP1*) is the gene causing brown coat colour in dogs (Schmutz *et al.* 2002) (Fig. 1a,b). *TYRP1* was mapped to CFA 11 between microsatellites *C03109* and *FH2004* (Schmutz *et al.* 2002). Little (1957) referred to this as the *B* locus (Table 1) with brown coat colour inherited recessive to black. Three different new alleles were detected in *TYRP1*, a combination of any two of which will cause brown coat colour. One variant (b^s) contained a premature stop codon in exon 5 (Q331ter) (c.991C>T), the second variant (b^d) has a deleted proline residue in exon 5 (345delP) (c.1033-6 deleted) and the third variant (b^c) was a base-pair substitution in exon 2 that causes a serine to be changed to a cysteine (S41C) (c.121T>A) (Schmutz *et al.* 2002).

All three alleles have been detected in several of the 28 breeds that have been genotyped but some breeds with brown individuals do not exhibit all three alleles. It is possible that there are additional rare alleles of *TYRP1* causing brown that were not detected in our original survey of dogs but such alleles would likely be quite rare. In mice, there are also three alleles causing brown coat colour, each attributed to a different shade of brown (Jackson 1988; Zdarsky *et al.* 1990; Javerzat & Jackson 1998). Although the shade of brown also varies in dogs, both within and among breeds, there is no consistent shade associated with any of the possible six brown genotypes of *TYRP1* in dogs (unpubl. data).

In some breeds, such as Doberman Pinschers and Australian Shepherds, brown dogs are referred to as red. Such dogs that are called red have been confirmed as brown, because such dogs have *TYRP1* mutations, based on our research.

The *TYRP1* alleles interact with the *MC1R* alleles (Schmutz *et al.* 2002). A dog with an *e*/*e* genotype at *MC1R* has a cream, yellow or red coat colour but the nose leather, eye rims and pads which have keratinized epidermis are either black (Fig. 1e,h left) or brown (Fig. 1l), or a dilution of these, depending on the *TYRP1* genotype. Such a two-gene interaction affecting nose leather and hair colour differently was postulated by Templeton *et al.* (1977) but the mechanism for this is not yet understood, even though the genes and mutations have now been identified. Presumably *e*/*e* affects the melanocytes in keratinized skin differently than those in the hair follicles.

All dogs that have a coat colour of black, brown or grey, whether solid or spotted, inherited as a dominant, have at least one *E* or E^M allele. In addition such dogs also have at least one K^B allele (Kerns *et al.* 2005, 2007; Candille *et al.* 2007).

The *agouti signal peptide* gene

Agouti signal peptide (*ASIP*) has several alleles that are involved in coat colour in dogs. *ASIP* has been mapped to CFA24 between *AHT 118* and *AHT 125* (Kerns *et al.* 2004).

There are four alleles present in dogs in the dominance hierarchy $a^y > a^w > a^t > a$. The wild type allele (a^w) causes some hairs to have a band of eumelanin, phaeomelanin, eumelanin pigment from base to tip. These banded hairs are typically along the dorsal region of the torso. The *ASIP* sequence of this allele has complete homology to the wolf sequence (Berryere *et al.* 2005) and the amino acid sequence of the coyote is also the same (Schmutz *et al.* 2007).

As in horses (Rieder *et al.* 2001), there is a recessive allele which causes dogs to have a black coat. This R96C allele (*a*) (c.288C>T) occurs primarily, but not solely, in herding breeds. It is the only cause of black in the German Shepherd Dog and Shetland Sheepdog (Kerns *et al.* 2004; Berryere *et al.* 2005) but it occurs in some black Schipperke, Groenendael and Puli. This allele has also been identified in Samoyeds and American Eskimo Dogs, both of which are white (unpubl. data). The occurrence of this allele in Samoyeds was perplexing but a review of the history of this breed suggests they were originally used to herd reindeer and then later used additionally as sled dogs (Gardner 2002). The American Eskimo Dog may include Samoyed ancestors based on this finding, but not all breed clubs suggest this is the history of this breed's development.

One of the most common alleles in domestic dogs is known as the a^y allele, inherited as the dominant allele in this series. The allele has two amino acid changes in comparison to the wild type, A82S and R83H (c.246G>T and c.250G>A) (Berryere *et al.* 2005). The coat colour is called fawn in most breeds (Fig. 1d,e,j), but sable in a few. This allele has been identified in over 22 breeds. In all 45 dogs of this phenotype for which complete exon 4 sequence was obtained, both mutations were present. Neither mutation was found in the wolf or seven coyotes for which sequence was obtained. As residue 82 was conserved in more species than residue 83, the PCR-RFLP test to detect this allele was designed for this mutation (Berryere *et al.* 2005). There is considerable variation in the number of black hairs intermingled in dogs with this a^y allele. Although it has been

Table 1 Genes and loci discovered and predicted to be involved in dog pigmentation.

BASIC COLOURS	
A (agouti) = *agouti signalling protein* (***ASIP***) CFA24	
a^{y}	Fawn/sable (cream to yellow to red with darker tips) (some solid black hairs intermingled amongst reddish hairs in some breeds)
a^{w}	Wolf sable – wild type colour (many banded hairs – black-reddish-black)
a^{t}	Black-and-tan or brown-and-tan
a	Recessive black
B (brown) = *tyrosinase related protein 1* (***TYRP1***) CFA11	
B	Black eumelanin
b (***b^{s}***, ***b^{d}***, ***b^{c}***)	Brown eumelanin
E (extension) = *melanocortin receptor 1* (***MC1R***) CFA5	
E^{M}	Melanistic mask
E	Eumelanin (black, brown, blue) can be produced
e	Only phaeomelanin (red, yellow, cream) produced
K (from 'dominant blacK') = (***CBD103***) CFA16	
K^{B}	Black, brown or blue (eumelanin pigmentation only)
k^{br}	Brindle (on body region that would be phaeomelanin pigmented otherwise)
k^{y}	Expression of agouti alleles that express phaeomelanin possible
DILUTED COLOURS	
D (dilutes eumelanin) = *melanophilin* (***MLPH***) CFA25	
D	Not diluted
d	Diluted pigmentation
d2?	Diluted pigmentation with skin problems
C (coloured) = ? gene	
C	Full pigmentation
c^{ch}	'Chinchilla' which causes paler eumelanin and phaeomelanin
c^{a}	Complete albinism
P (dilutes both melanins) = *P* gene? (dominant/recessive)	
P	Not diluted
p	Diluted but not to completely white
I (dilutes only phaeomelanin) = ? gene (co-dominant)	
I	Intense, not diluted
i	Co-dominant decrease in intensity
G (progressive grey) = ? gene (dominant/recessive)	
G	Greying at early age, progressive greying
g	Not greying
WHITE MARKINGS	
M (merle) = (***SILV***) (*M*/*m* is the merle genotype) (co-dominant) CFA10	
M	Not coloured
m	Coloured
S (spotting) = (***MITF***) CFA20	
S	Solid coloured
s^{i}	Irish spotting
s^{p}	Piebald
s^{w}	Extreme white
T (ticking) = ? gene (dominant/recessive)	
T	Ticked
t	Not ticked
R (roan) = ? gene	
R	White in homozygote
r	Coloured in homozygote
H (Harlequin) = ? gene (dominant/recessive if *M* allele causing merle is present)	
H	Harlequin if also *M*/*m* or *M*/*M*
h	Not Harlequin

The alleles are listed in their 'predicted' dominance hierarchy. Those in bold have been confirmed at the DNA level.

postulated that this variation may be due to the *Mahogunin* gene (Berryere *et al.* 2005), this has not been proven.

A fourth allele, known as a^t, is believed to exist in dogs that are black-and-tan (Fig. 1a,h right). No differences have been observed in the coding sequence (exon 2–4) of dogs of this phenotype and dogs with the banded-hair wild-type phenotype. This putative allele must likely differ in one of the promoter regions. In other mammals such as mice, there are alternate dorsal and ventral promoters (Vrieling *et al.* 1994), but the *ASIP* promoters have not yet been identified in dogs. In many dog breeds, the extent of the ventral phaeomelanin varies amongst individuals, and also among breeds (Palacio, G.A. 2006, Pers. comm.). Candille *et al.* (2004) were able to find an association with *TBX15* mutations and variation in the relative proportion of black vs. tan in mice. Although some breeders have postulated a fifth allele, a^s for saddle tan, there is no evidence of such an allele at this time.

The *beta-defensin 103* gene

The symbol *K* was chosen to denote the locus causing black coat colour which is inherited as a dominant because some dog breeders had adopted this, choosing the *K* from the last letter of black. Little (1957) had postulated there was an allele '*A*', for solid colour, at the *agouti* locus, but as discussed above, this allele does not seem to exist in dogs. A few breeds do have a loss-of-function mutation in *agouti* that causes black to be inherited as a recessive trait, as discussed above.

In the vast majority of dog breeds, in order for solid eumelanin coat colour (black, brown and grey) (Fig. 1a–c,i,k) to occur the dog must have at least one *E* or E^M allele at *MC1R* and at least one dominant allele at the *K* locus. Research on this locus has been led by Greg Barsh in collaboration with others (Kerns *et al.* 2005, 2007; Candille *et al.* 2007). The gene is *beta-defensin 103* on CFA16. The gene at the *K* locus has not previously been described as having a function in the pigmentation pathway and therefore the mechanism by which it functions is of considerable interest (Candille *et al.* 2007).

There are also two other alleles at this locus. A single copy of the k^{br} allele in the presence of a k^y allele is sufficient to cause the dog to express the phenotype known as brindle (Kerns *et al.* 2007). Brindle in dogs consists of alternate stripes of phaeomelanin and eumelanin of various shades (Fig. 1f). In some dogs, there is such a preponderance of eumelanin that the dog appears virtually black whereas in other individuals the eumelanin stripes are very thin. Brindle occurs over the entire body in dogs with an a^y allele (Fig. 1f) but only on the ventral surfaces in dogs with an a^t/a^t genotype (Berryere *et al.* 2005). Dogs with a k^y/k^y genotype at this locus could be fawn (Fig. 1d,j), wolf sable or eumelanin-and-tan (Fig. 1a,h right) depending on their genotype at *ASIP*.

DNA testing has successfully predicted eumelanin vs. phaeomelanin pigmentation in some breeds, such as Labrador Retrievers, using only *MC1R* genotyping. The reason is likely that this breed is virtually fixed with a K^B/K^B genotype and *e*/*e* is epistatic to both K^B and k^{br} (Fig. 1e). However in other breeds, such as Great Danes (Fig. 1i), eumelanin vs. phaeomelanin pigmentation is controlled by $K^B/-$ vs. k^y/k^y, as this breed appears to be fixed for the a^y/a^y gentoype in the presence of *E* or E^M.

Shades of the basic coat colours

The *melanophilin* gene

Many dog breeds have individuals that are grey or dilute in coat colour (Fig. 1i,j). However, blue is used to denote other phenotypes in various breeds. In some breeds, these blue individuals are born grey, whereas in other breeds individuals take several months to turn from black to grey. The latter characteristic was referred to as 'progressive greying' by Little (1957) and attributed to the *G* locus. In some dog breeds this is called 'silver'. A few dog breeds have both types of grey occurring. Some Great Danes and all Weimaraners are born blue or dilute whereas Kerry Blue Terriers (Fig. 1k) and Old English Sheepdogs are born black and lighten as they grow into adulthood. Both of these inherited traits cause modification of both eumelanin and phaeomelanin pigmented areas to a paler shade, although the phaeomelanin change is not as dramatic as the eumelanin dilution. Fawn dogs with a melanistic mask (Schmutz *et al.* 2003a) are easier to observe because their mask is grey instead of black. Dilute fawn dogs have charcoal instead of black nose leather and pads (Fig. 1j). Dogs with an *e*/*e* genotype at *MC1R* (Newton *et al.* 2000) or clear red phenotype, such as the two Beagles in our previous study (Philipp *et al.* 2005) were very difficult to detect as dilute. Dogs that are brindle and dilute, such as some Whippets and Greyhounds, have grey stripes on a pale fawn background. Dogs, such as Weimaraners, that have two copies of the mutations in *TYRP1* causing brown (Schmutz *et al.* 2002) and two dilute alleles are a pale brown. The nose leather and pads of such dogs are a similar pale brown. In some breeds such as Chinese Shar-Pei, the dogs are called lilac and in Doberman Pinschers, they are called Isabella.

Recently, we reported that Doberman Pinschers, German Pinschers, Large Munsterlanders, and Beagles with a dilute phenotype, co-segregated with specific haplotypes of *melanophilin* (*MLPH*) (Philipp *et al.* 2005). A mutation in exon 2 of *MLPH* causes a splice junction problem in homozygous mice of the leaden phenotype (Matesic *et al.* 2001). The last seven amino acids of exon 2 are spliced out in leaden mice because a C-to-T transition introduces a premature stop codon. A human infant was reported to have Griscelli Syndrome Type III due to a R35W mutation near the end of exon 2 (Ménasché *et al.* 2003). The hair

colour of this child was not reported but this syndrome is considered a form of albinism.

We have now extended our study of *MLPH* to include approximately 20 dog breeds. Although a mutation that co-segregates with blue in some breeds has been found (unpubl. data), no single mutation has been found that explains the blue in all these breeds. A couple of common mutations occur only in blue dogs (unpubl. data). Our study to identify all the alleles causing blue is ongoing.

Progressive greying

We support Little's (1957) hypothesis that dogs with a progressive grey phenotype have a mutation distinct from the allele he refers to a '*d*' at the *D* or *dilute* locus. The greying begins on different parts of the body and at different ages in the breeds that show progressive greying. Some such dogs had a localized reaction to vaccination or skin injury that caused hair in the affected area to revert to its dark juvenile pigment. Over the course of several months, this darker hair would typically lighten again. This would fit with Little's (1957) suggestion that the progressive greying trait is controlled by a separate locus he designated as the *G* locus.

A progressive greying phenotype also occurs in horses. This trait has been mapped to a region of ECA25q (Pielberg *et al.* 2005) which corresponds to HSA9q. Based on reciprocal chromosome paints (Breen *et al.* 1999), the relevant section of HSA9, corresponds to canine chromosome 11. A search for the *G* locus in dogs, thought to be associated with progressive greying might be directed to this chromosome. Horses with the progressive greying phenotype show a propensity to develop skin tumours (Rieder *et al.* 2000). This pleiotropic effect was not reported in any of the dogs in our study with a progressive greying phenotype (unpubl. data).

Cream and/or white

There are several breeds of dogs that are born white or cream with pigmented nose leather and pads. This is another example demonstrating that pigment migration into hairs and keratinized skin is not entirely controlled by the same genes. One example of a breed fixed for white is the Samoyed. A limited number of Samoyeds were genotyped and all were *e*/*e* at *MC1R* and also *a*/*a* at *ASIP*. We have also genotyped white individuals in several other breeds and found that all were *e*/*e* at *MC1R* (Schmutz & Berryere 2007). These included the Akita, Shar-Pei, Poodle, Puli, German Shepherd Dog, Caucasian Mountain Dog and the Miniature Schnauzer. Some individuals might more appropriately be called cream as a hint of phaeomelanin pigmentation existed, often on the ears. In the Akita, no littermates that had an E or E^M allele were ever cream, although some appeared fawn red because of an $a^y/-$ genotype. This suggests that there is a gene that only pales individuals with an *e*/*e* genotype. In the Akita, Caucasian Mountain Dog, German Shepherd Dog, Miniature Schnauzer and Puli breeds, there are no individuals that are red, in turn, suggesting that these breeds are fixed for this allele that pales only and all individuals with an *e*/*e* genotype (Schmutz & Berryere 2007).

In some breeds, such as the Labrador Retriever and Golden Retriever, all dogs that are pigmented with phaeomelanin have an *e*/*e* genotype at *MC1R*. These dogs vary in shade from a golden yellow to a cream colour, but are not white. In a study of families which segregated for these shades, it would appear that the cream shade is inherited as an autosomal recessive. Cream did not co-segregate with polymorphisms in tyrosinase (*TYR*) nor with *SLC45A2*, formerly called *MATP* (unpubl. data). The first gene that is reported to dilute only phaeomelanin is *SLC7A11* (Chintala *et al.* 2005). Although Chintala *et al.* (2005) found abbreviated mRNA products in mice with dilute phaeomelanin pigmentation, we obtained mRNA sequence from pale yellow dogs that appeared to be full length (GenBank EF143580).

However, in some breeds such as Afghans and Poodles, there are also individuals that are cream/white that have an E or E^M allele at *MC1R*. The genetic mechanism underlying this form of white is unknown at this time.

Spotting patterns and white markings

There are many types of white markings in dogs. Until 2006, none of the genes causing these patterns had been elucidated in dogs.

Spotting

Metallinos & Rine (2000) were among the first to try to find a gene associated with spotting. They used a family of dogs from a study designed to map behaviour traits. They excluded both *EDNRB* and *KIT* as the gene causing the white underside markings seen in most Border Collies which segregated as an autosomal recessive trait in Newfoundland–Border Collie backcross families. Similarly, these genes were excluded for the white markings seen in Boxers (van Hagen *et al.* 2004).

The *microphthalmia-associated transcription factor* gene

Researchers at Iowa State University completed a candidate gene study of spotting in dogs and identified *MITF* as the candidate gene for random spotting, while excluding six other genes (Rothschild *et al.* 2006). Their study focused on beagle crossbreds and Newfoundlands (Fig. 1c). They found complete co-segregation with a SNP in intron 3 and recessively inherited, random or piebald spotting in these dogs.

In a simultaneous collaborative study between researchers at the Broad Institute and Swedish Agricultural

University, one major gene involved in white markings has been identified. A genome scan identified CFA20 as harbouring the gene for white markings in Boxers (Karlsson *et al.* 2007). The candidate gene in this region was *MITF*. Their study focused on Boxers but also included Bull Terriers, Dalmatians and Cavalier King Charles Spaniels. Little (1957) had predicted that Dalmatians would have the same allele for white markings as Boxers as they were born white and the small black spots developed at a few weeks of age, under the influence of another gene.

Mutations in *MITF* apparently can affect melanoblast survival in the lineage derived from neural crest cells that ultimately cause coat pigmentation (Bismuth *et al.* 2005). Therefore, a lack of pigmentation or white markings could be anticipated. Several isoforms of *MITF* have been described in species such as mouse and human (Udono *et al.* 2000). *MITF-M* has been considered the major isoform expressed in melanocytes in most species. We had obtained *MITF-M* mRNA sequence, with and without the 18-bp exon 6A, from dog skin (GenBank AY240952). The MITF protein is encoded by exons 5–9 and it is believed that the presence and absence of exon 6A in the product is related to different regulatory elements (Bismuth *et al.* 2005). Although some polymorphisms were detected in the coding region (GenBank DQ923322) and adjacent 3′-UTR sequence of exon 9, none of these occurred consistently in dogs with any particular form of white markings. However several mutations in *MITF* in mice cause spotting (reviewed by Goding 2000) and involve mutations outside the coding exons.

The markings in Boxers are often considered to fit a co-dominant inheritance pattern: no white (*S*/*S*), white undersides and facial blaze in the 'flashy' Boxers (*S*/*s*), predominantly white (*s*/*s*) and this was supported by the *MITF* data (Karlsson *et al.* 2007). Although the white markings of a flashy Boxer and a typical Border Collie are phenotypically very similar, Border Collies are rarely predominantly white and only occasionally have no white. Although Little (1957) postulated that there were four alleles at the locus for spotting (S, s^p, s^i, s^w), he also suggested that plus and minus modifiers caused a gradation of phenotypes such that distinct phenotypes of these alleles were not clear. Pape (1990) also suggested modifiers but implied these would be at separate genes. It is likely further research from Broad/Swedish collaborative group and the Iowa group, as well as others, will help determine how many alleles occur in *MITF* and which spotting patterns they explain, or if variability in spotting is affected by other genes.

Merle

Clark *et al.* (2006) have demonstrated that a SINE element and a run of thymidine nucleotides in intron 10 of the 11 exon *SILV* causes the merle pattern (Fig. 1g). This allele is typically known as the *M* allele since Little (1957). Sponenberg (1985) had postulated that merle would be caused by a transposable element because he had collected reports from breeders that some merle offspring were born to parents, neither of which were merle. He postulated that this was due to germinal reversion. Hedan *et al.* (2006) had also mapped the merle trait to CFA10 in the region where *SILV* is located.

We have obtained normal mRNA product from Australian Shepherds heterozygous for this SINE insertion with the accompanying run of thymidines (GenBank EF090519) for part of exons 9, 10 and 11 through the stop codon, with no evidence of a mixed product (unpubl. data). This suggests any improperly spliced product degrades quickly. It also illustrates how easy it would have been to miss this mutation if one studied only the coding sequence of dogs with the merle phenotype.

Several dog breeders consider they have a dog that produces merle pups, even though it is not merle and refer to such dogs as cryptic merles or phantom merles. Clark *et al.* (2006) suggest that the SINE element alone, with no accompanying string of thymidines is the explanation for these cryptic merles. We have observed this SINE element alone in several dogs that do not exhibit a merle phenotype. One such tricoloured Shetland Sheepdog has sired 7 litters but no pups that were merle.

Since the mutation was discovered, we have been able to test dogs for the merle mutation of various coat colour genotypes at other loci. Dogs that are fawn show minimal merle markings as Little (1957) postulated. This can occur in fawn Dachshunds where the trait is called dapple, as well as in fawn Great Danes. As merle is the presence of dark eumelanin areas swirled amongst a paler colour, and dogs with an *e*/*e* genoytpe at *MC1R* do not produce any eumelanin pigment in their hair, such a dog would not exhibit a merle phenotype even though it carried the merle mutation (unpubl. data).

We had excluded *KITLG* as the gene causing merle (Schmutz *et al.* 2003b) during a study of this phenotype in Australian Shepherds. Recently, we genotyped these dogs for the *SILV* mutations and 27 dogs were found to be homozygous for the merle mutation of the SINE and run of thymidines. All were deaf by breeder observation and/or BAER testing. Only five reported serious eye anomalies such as microphthalmia, although several dogs had blue or heterochromatic iris colour. Murphy *et al.* (2006) reported that deafness did not occur in all Catahoula Leopard dogs that were homozygous for the merle mutation, however. Perhaps Little's (1957) hypothesis that merle would have more effects in dogs that also carry white spotting is the explanation for this difference between breeds, as all the Australian Shepherds we studied had white markings. Baxter & Pavan (2003) demonstrated that mice with mutated *MITF* that lead to absence of melanoblasts, did not express *SILV* in their retinal pigmented epithelium either. This demonstrates that *MITF* and *SILV* interact.

Harlequin

Harlequin is the pattern that occurs in Great Danes that consists of ragged black patches on a white background. Authors such as Sponenberg (1985) have postulated that this pattern requires one copy of the merle mutation and one copy of a second mutation, designated *H*, at another locus. Clark *et al.* (2006) confirmed that the Harlequin Great Danes they tested all had at least one copy of the merle mutation. We have studied a few candidate genes in segregating litters and have excluded *MITF* and *EDNRB* but *PAX3* co-segregated with Harlequin in one small pedigree (unpubl. data).

Ticking

Little (1957) described another type of spotting that consists of very small spots on a white background (Fig. 1a,b right). These are not present at birth but begin to appear within a few weeks. At the present time, no study of the gene causing ticking is available.

The type of spotting seen in Dalmatians is considered to be unique to this breed by some, although Little (1957) provided some breeding data from a small number of litters which led him to conclude this was ticking. Safra *et al.* (2006) presented data to suggest that the gene associated with the atypical production of urea instead of allantoin by Dalmatians was in LD with the gene causing this pattern. It is possible that this gene is either closely linked to Dalmatian spotting, or even that this is the gene involved in this trait.

Roan

Roan is a pattern consisting of intermingled pigmented and unpigmented hairs (Fig. 1b left). Roan often occurs in dogs that also have ticking. Little (1957) questions whether roan is actually a separate pattern or if it is just a variation of ticking. Until such time as the gene causing ticking or roan is identified this cannot be supported or refuted. As roan in cattle was found to be caused by a mutation in *KITLG* (Seitz *et al.* 1999), we studied this as a candidate gene but it did not co-segregate with ticking or roan in families (unpubl. data).

Furthermore, some dogs are called blue belton which is a pattern of intermingled black and white hairs, often called roan in other animals. Little (1957) also suggested that this trait was called 'roan' and that it was inherited as an autosomal recessive. Australian Cattle Dogs, sometimes called Blue Heelers in North America often have this pattern.

Speculation regarding related phenotypes

Recently, the work of Belyaev *et al.* (1981) on the differences during the domestication of foxes has been re-discovered. One trait he mentioned is the presence of white markings that became more prominent with more generations of domestication. He attributed this to the 'star gene'. Is this *MITF*? Is there a chance that a difference in neuroblast migration or development caused by mutations in *MITF* might affect docility?

Some breeds of dogs, such as Siberian Husky, have a characteristic facial pattern in which white extends up the muzzle and over each eye. Is this caused by another allele of the *S* locus or is this pattern caused by another gene? Is it coincidental that Siberian Huskies often have blue eyes into adulthood when this trait is not typical in any other breed?

There are some additional phenotypes that are known to dog breeders, but not yet tackled by molecular geneticists. Chow Chow and Chinese Shar-Pei (Fig. 1d) typically have a melanistically pigmented tongue, whereas the tongue of most other breeds is pink or not pigmented (Fig. 1k). Although nose leather, pad and eye rim pigmentation is influenced by the alleles at *TYRP1* (Schmutz *et al.* 2002), the tongue was not affected in most breeds. Does this suggest that the tongue is more keratinized in these Asian breeds or is there a mutation in another pigmentation gene?

The effects of *TYRP1* mutations in the eumelanin pathway suggest that no dog should be able to simultaneously produce both brown and black pigment anywhere on their body. However, there is a colour called 'seal' in some breeds which gives the impression of a brown coat colour from a distance. Such dogs typically have black nose leather however. On closer examination, the coat of such dogs is composed of intermingled black and reddish hairs. Some of these hairs may be banded. Is this another expression of the K^{br} mutation or is this caused by another allele or another gene?

Ramifications of selection for coat colour

In some breeds only a single coat colour or a very limited range of coat colours are allowed within the standard. Pollinger *et al.* (2005) used an example of two breeds which separated relatively recently on the basis of brown (German Longhair) vs. black (Large Munsterlander) coat colour. Using a set of microsatellite markers on CFA11, they showed that there was a region spanning approximately 20 cM that was affected by this selection. Hence, any other genes in this region would also be under inadvertent selection pressure because of the selection for coat colour.

In recent years, there seems to be an interest in breeding dogs to have colours uncommon in a particular breed. Is this simply selection of rare mutations or is it intentional introgression? At the present time, this is impossible to differentiate. The former is generally acceptable provided that the colour in question is allowed by the breed's standard, the latter is almost universally rejected as it does not conform to the regulations of the major registries.

DNA testing is currently used by many breeders to plan matings to obtain specific coloured pups. DNA testing is also

used to exclude certain mates in order to avoid pups of undesirable coat colour.

Diseases associated with pigmentation

There is a condition called Grey Collie Syndrome, more formally known as Cyclic Neutropenia. This disorder seems to occur only in Collies and is caused by a mutation in *AP3* (Benson *et al.* 2003). The *AP3* mutation is responsible for defects in specific protein sorting processes that also cause a pleiotropic effect on coat colour causing the pups to be a pale colour with a grey tinge.

Some ($n = 30$) of the 119 grey/blue dogs we studied (unpubl. data) showed evidence of hair loss and much more rarely skin problems, symptoms typical of colour dilution alopecia (CDA) and black hair follicular dysplasia (Schmutz *et al.* 1998; von Bomhard *et al.* 2006). This was not true of all adult blue dogs however. The symptoms also appeared to vary by breed with the Large Munsterlanders displaying complete hair loss in all grey areas by 12 weeks whereas most dogs of other breeds were a few years old before this degree of hair loss occurred. Several dogs 5 years of age or older were reported to have no hair loss or skin problems. Several of the dogs were pups or <2 years of age or had large areas of white fur and therefore we could not determine whether these dogs had or would develop CDA. Dogs that were blue or blue fawn, male or female, long or short-haired and with or without white spots were affected. There may be a slight tendency for earlier symptoms in dogs with longer hair. CDA was reported in 26 of the 100 dilute dogs with a *T*/*T* genotype and four of the 19 dogs that showed a dilute phenotype but did not have a *T*/*T* genotype at the synonymous c.106C>T polymorphism in exon 2 of the *MLPH* gene (unpubl. data).

Not all 'blue' or genotypically *d*/*d* dogs (Fig. 1i,j), have problems associated with CDA and not all dogs that have symptoms develop them at a similar age of onset or with similar severity. For example although all Weimaraners are dilute and all of the eight dogs of this breed we studied had a *T*/*T* genotype, not all had CDA. Laffort-Dassot *et al.* (2002) likewise described variable symptoms in five Weimaraners. Miller (1990) suggested that there were possibly multiple recessive alleles of the dilution gene. Although this may be true, it does not appear that dogs with and without CDA necessarily have different mutations in *MLPH*. Since MLPH binds to RAB27A in the region (Strom *et al.* 2002) where we have identified some mutations in *MLPH*, we wanted to determine if a mutation in *RAB27A* might interact and cause some dogs to experience symptoms of CDA whereas others did not. We sequenced the entire coding region of *RAB27A* (GenBank DQ494380) in a Large Munsterlander that had severe symptoms of black hair follicular dysplasia, an Italian Greyhound with CDA symptoms, and a black-and-white Large Munsterlander and a chocolate Labrador Retriever which were not dilute and had no symptoms. No polymorphisms in the *RAB27A* sequence were detected.

Deafness, whether unilateral or bilateral, has been associated with some forms of white spotting. Dalmatians, in particular, have been studied extensively in this regard (Cargill *et al.* 2004). Now that polymorphisms in *MITF* have recently been shown to co-segregate with some forms of white spotting (Rothschild *et al.* 2006), this may open new avenues of research.

Albinism was thought to be very rare in dogs by Little (1957). Several individuals have reported dogs that fit the phenotype expected of oculocutaneous albinism to our lab. Such dogs are born white with no pigmentation in their nose leather, pads or irises. However, we do not have clinical examinations of these dogs and cannot comment on any pleiotropic effects of these dogs. A strain of Doberman Pinschers exists with this colouration. The complete coding sequence of *tyrosinase* in such 'albino' Doberman Pinschers was compared to the sequence of a cream poodle (GenBank AY336053) but showed no polymorphisms that would explain albinism in these dogs, inherited as an autosomal recessive (Schmutz & Berryere 2007). Genomic sequence was also obtained from an albino Lhasa Apso and an albino Pug. *Tyrosinase* was mapped to CFA21 using a SNP in exon 1, I159V (Schmidtz & Schmutz 2002). In addition to this polymorphism, two others cause amino acid changes: R408Q and L438F, both in exon 4. Both alleles of all these SNPs occur in dogs that are cream/white, white/albino, and coloured. Hence, none appear to be associated with either albinism or white in dogs. Our studies are not complete enough to exclude mutations in *tyrosinase* promoter sequence however.

Conclusion

For several of the genes found to be involved in coat colour (*MC1R*, *TYRP1*, *ASIP* and *K*; Table 1), there are at least three alleles. In some cases, several alleles cause the same phenotype such as brown. The alleles detected to date also illustrate the wide variety of types of mutations found including point mutations, premature stop codons, deletions, duplications, SINE insertions, etc. Interactions among several of these loci are necessary to cause a coat colour pattern, such as a black masked fawn dog. Epistasis by some genotypes also overrides some coat colour patterns. The genetics of dog coat colour offers us a wonderful example of the complexity of inheritance and illustrates the whole is much more than the simple sum of its parts.

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