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Genetic Interactions Among Three Pigmentation Loci in Domestic Dogs

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ABSTRACT: This paper focuses on the genetic interactions among three genes in the pigmentation pathway: the Agouti signal protein (*ASIP*), Melanocortin receptor 1 (*MC1R*) and Beta-defensin 103 (*DEFB103*). There are currently four *ASIP* or A alleles ($a^y > a^w > a^t > a$), four *MC1R* or E alleles ($E^M > E^G > E > e$) and three *DEFB103* or K alleles, only two of which ($K^B > k^y$) are discussed here. However, because of epistasis and gene interactions we suggest are either multiple modification since multiple genotypes are affected, or specific modification since only a single genotype is affected, there are 10 coat color phenotypes produced. The mutations responsible for these alleles are reviewed and their mechanisms within the pigmentation pathway are discussed. The mutations include nonsynonymous substitutions, premature termination, and deletions with the coding sequences of these genes, as well as promoter mutations.
Keywords: *ASIP*; *MC1R; DEFB103;* coat color; epistasis; modifier

Introduction

There is no domestic animal with as much variation in coat color, as the dog. There are 343 breeds of dogs recognized by the Fédération Cynologique Internationale based on 2010 figures (http://www.fci.be/stats.aspx). The breed standard in over half of these breeds includes specific coat colors or patterns that are permissible within the registered dogs of that breed. The other breed standards often make allowance for "any hound colour", or "any colour but white", etc. Hence dog breeders have tried to select for specific colors or patterns for a very long time. Many depended on two classic works by C.C. Little (1957) or O. Winge (1950), or various dog association's or private individual's websites to understand the inheritance of color and pattern in their breed of interest.

DNA studies have shed additional light on coat color in dogs (Schmutz and Berryere (2007), as well as coat length and texture (Housley and Venta (2006); Cadieu et al. (2009); Dierks et al. (2013)). Coat color is too broad a topic to review in entirety here (see http://homepage.usask.ca/~schmutz/dogcolors.html).

This paper will focus on interactions of the Agouti signal protein (*ASIP*), with two other genes: Melanocortin receptor 1 (*MC1R*) and Beta-defensin 103 (*DEFB103*), as examples of non-additive, polygenic inheritance. We view coat color and pattern as a complex trait, rather than several individual single gene effects. There are complex traits that are controlled by many genes, with non-additive gene action (Roth et al. (2009); Dowell et al. (2010). Although the infinitesimal model based on additive gene action has served quantitative geneticists studying several production traits well.

Non-additive genetic interactions are classically described as either dominance deviation or epistasis. Recent discoveries in regard to the complex trait of dog coat color have suggested additional forms of non-additive genetic interactions that we suggest would benefit from further definition. These fall into the classical use of the term "modifier". We will expand upon this and suggest possible classes of modifier and names for each.

Results and Discussion

ASIP. ASIP is a 131 amino acid paracrine signaling molecule (Bultman et al. (1992)), that includes a 22 amino acid signal peptide (Jackson et al. (2006)). Its only function appears to be on coat color (Jackson et al. (2006)). It binds alternately, with melanocyte stimulating hormone (MSH), on the melanocortin receptor 1 (MC1R). When ASIP is bound, phaeomelanin pigments are produced, causing colors such as cream, yellow and red. When MSH is bound to MC1R, then eumelanin pigments such as black, brown and gray are produced.

The wild type allele of *ASIP* is a^w, and the phenotype associated with it is usually called wolf sable. The dorsal area of a Timber Wolf has some black hairs intermingled among some cream to tawny colored hairs, along with several hairs that have black at the base, cream to tawny in the middle, and black tips. The ventral area of a wolf is typically cream to tawny. These various differences in the coat color illustrate that different promoters are active in different parts of the body. This same coat color pattern occurs in several dog breeds, such as the Keeshond and Norwegian Elkhounds, as well as the Swedish Vallhund shown in Figure 1.

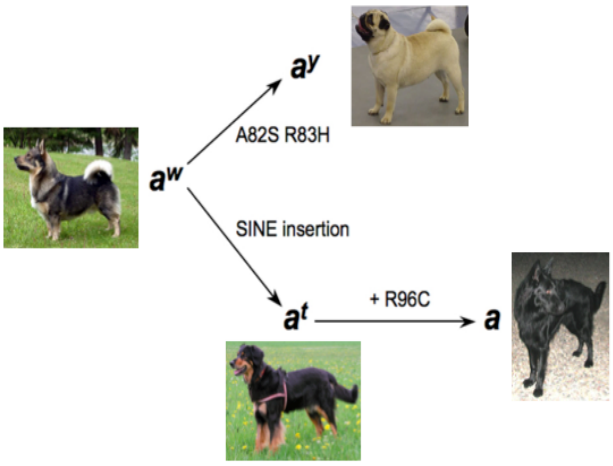

Figure 1. Proposed *ASIP* allele evolution, with a wolf-sable Swedish Vallhund illustrating the phenotype of an a^w genotype, a fawn Pug with melanistic mask illustrating the homozygous a^y genotype, a black-and-tan Hovawart illustrating the homozygous a^t genotype, and a black German Shepherd Dog illustrating the homozygous *a* genotype.

There is another phenotype in dogs called black-and-tan wherein the dorsal surface of the dog has eumelanin pigmentation, such as black, brown or gray and the muzzle, chest and legs as well as "eyebrows" have phaeomelanin pigmentation. This pattern is evident on the Hovawart in Figure 1, but is fixed in some breeds such as Doberman Pinschers and Bernese Mountain dogs. This *ASIP* allele, a^t, has a short interspersed nuclear element (SINE) insertion in intron 1 in the reverse orientation (Dreger and Schmutz (2011)(Fig. 2).

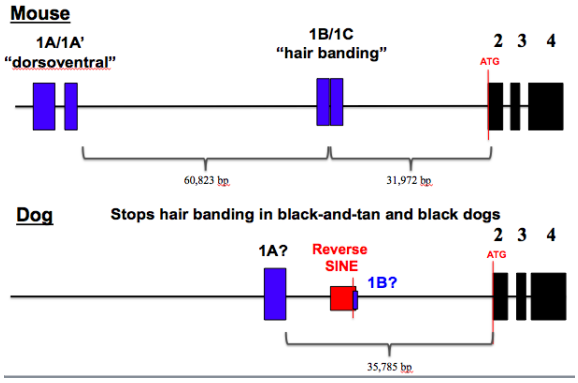

Figure 2: Diagram comparing the *ASIP* promoter region in mice (adapted from Vrieling et al. 1994), and the predicted region in dogs.

This SINE insertion is 215 bp 5' of a constant SINE, found in all dogs in the forward direction. In the reverse orientation, this canine SINE introduces a 3' splice acceptor site (Wang and Kirkness (2005)). We predict that this additional SINE halts the hair banding typical in wolf-sable and fawn dogs by altering the binding of ASIP to MC1R in a body-area specific manner.

In addition to this SINE insertion, there is another mutation in exon 4, R96C (Kerns et al. (2004)). This mutation, only occurs in conjunction with this SINE insertion, which suggests that it is a more recent mutation (Fig. 1). Another cysteine is added to an already cysteine rich carboxy-terminal domain (Yu and Millhauser (2007). This group of cysteines is responsible for the "cysteine knot" structure common to this gene family. This additional cysteine may alter cysteine bridges in the part of the peptide that binds to MC1R, changing the three dimensional structure so that it no longer is capable of binding. In dogs that are homozygous for the 96C allele, or *a/a*, all ASIP binding is halted and only eumelanin is produced. An example of this is the solid black German Shepherd dog shown in Figure 1. This allele is the major cause of black coat color in herding breeds, but not in other breeds (Candille et al. (2007)).

DEFB103. In the majority of dog breeds, eumelanin pigmentation is caused by a deletion of the glycine at amino acid 23 in the *beta-defensin 103* gene (Candille et al. (2007)). This K^B mutation is dominant in the *DEFB103* or *K* locus series. It is also epistatic to all of the *ASIP* genotypes. For example, many hunting breeds such as black or brown Labrador Retrievers and brown Chesapeake Bay Retrievers have various genotypes at *ASIP* that are masked by a K^B allele (Dreger and Schmutz (2011)). It has been postulated that the deletion in the K^B allele makes that form of the peptide have increased binding affinity to MC1R, and it is more easily secreted from cells (Candille et al. (2007); Dorin and Jackson (2007)). Combined, these differences seem to prevent ASIP binding and hence phaeomelanin production.

In order for the wolf-sable, fawn or black-and-tan phenotypes to be expressed in dogs, the *DEFB103* alleles must both be wild type or k^y/k^y (Table 1).

Table 1. Phenotypes produced by the genotype interactions of *ASIP*, *DEFB103*, and *MC1R* in dogs.

Genotype			Phenotype
ASIP	*DEFB103*	*MC1R*	
$a^y/\_$	K^B/K^B or k^y	not *e/e*	black
a^w/a^w	K^B/K^B or k^y	not *e/e*	black
a^w/a^t	K^B/K^B or k^y	not *e/e*	black
a^w/a	K^B/K^B or k^y	not *e/e*	black
a^t/a^t	K^B/K^B or k^y	not *e/e*	black
a^t/a	K^B/K^B or k^y	not *e/e*	black
a/a	K^B/K^B or k^y	not *e/e*	black
$a^y/\_$	k^y/k^y	*E/E or e*	fawn
a^w/a^w	k^y/k^y	*E/E or e*	wolf sable
a^w/a^t	k^y/k^y	*E/E or e*	wolf sable
a^w/a	k^y/k^y	*E/E or e*	wolf sable
a^t/a^t	k^y/k^y	*E/E or e*	black-and-tan
a^t/a	k^y/k^y	*E/E or e*	black-and-tan
a/a	k^y/k^y	*E/E or e*	black
$a^y/\_$	k^y/k^y	$E^M/\_$	fawn, masked
a^w/a^w	k^y/k^y	$E^M/\_$	wolf sable, masked

a^w/a^t	k^y/k^y	$E^M/\_$	wolf sable, masked
a^w/a	k^y/k^y	$E^M/\_$	wolf sable, masked
a^t/a^t	k^y/k^y	$E^M/\_$	black-and-tan, masked
a^t/a	k^y/k^y	$E^M/\_$	black-and-tan, masked
a/a	k^y/k^y	$E^M/\_$	black
a^y/a^y	k^y/k^y	E^G/E^G, E, e	fawn
a^y/a^y	k^y/k^y	E^G/E^G, E, e	fawn
a^t/a^t	k^y/k^y	E^G/E^G, E, e	grizzle
$a^y/\_$	$K^B/\_$ or k^y/k^y	e/e	red
a^w/a^w	$K^B/\_$ or k^y/k^y	e/e	red
a^w/a^t	$K^B/\_$ or k^y/k^y	e/e	red
a^w/a	$K^B/\_$ or k^y/k^y	e/e	red
a^t/a^t	$K^B/\_$ or k^y/k^y	e/e	red
a^t/a	$K^B/\_$ or k^y/k^y	e/e	red
a/a	$K^B/\_$ or k^y/k^y	e/e	white

*All possible genotype combinations have not been observed, so only those that have been observed are shown.

MC1R. Fawn dogs (a^y) that have a M264V *MC1R* mutation have a melanistic mask (Schmutz et al. (2003)), such as the one on the Pug in Figure 1. This mutation often causes eumelanin pigment to occur on the ears as well, and often on the dorsal surface of the tail. Therefore, this phenotype requires a gene interaction that causes eumelanin to be produced on specific parts of the body, instead of the typical fawn phaeomelanin, when *MC1R* has this valine substitution or E^M allele. Although the melanistic mask is not as readily visible on a dog with a wolf-sable phenotype (a^w/a^w, a^w/a^t or a^w/a), these mask markings are still produced. Likewise black-and-tan dogs (a^t/a^t or a^t/a) show their melanistic mask only on their muzzle since the other areas typically affected are already eumelanin pigmented.

Black-and-tan dogs (a^t/a^t) of two ancient breeds, the Saluki and Afghan Hound, sometimes have a mutation in *MC1R* that causes them to have a grizzle or domino phenotype. This mutation alters the glycine at amino acid 78 to a valine (G78V). A single copy of this E^G allele will cause dogs of a^t/a^t genotype to have this altered phenotype in which the black area is reduced and somewhat "grizzled" around the edges. The face of these dogs also has a distinctive widow's peak. The E^G allele is recessive to the E^M allele, and because the E^M allele is very common in Afghan Hounds, the domino phenotype is relatively rare.

A third allele at *MC1R*, R306ter, was reported almost simultaneously by Newton et al. (2000) and Everts and Rothuizen (2000). When homozygous, or *e/e*, the dog has only phaeomelanin pigmentation (Table 1). This is true even if there is a K^B allele. Thus this premature stop codon that truncates the terminal intracellular region of MC1R prevents the binding of MSH completely. This *e/e* genotype is epistatic to both the *DEFB103* genotypes and the *ASIP* genotypes.

A final interaction between *MC1R* and *ASIP* genotypes occurs in the Samoyed, which is always white (Schmutz and Berryere (2007)). The *e/e MC1R* genotype typically causes only production of phaeomelanin or red pigment. The *a/a* genotype of *ASIP* typically causes only eumelan or black pigment to be produced. The Samoyed appears to be fixed at both *a/a* and *e/e*, does not produce either pigment, and is therefore white, or lacking in all hair pigment (Table 1).

Genotype Interaction Effects

An additional allele, k^{Br}, occurs at the DEFB103 locu (Candille et al. (2007)). It has not been discussed relative to either *ASIP* or *MC1R* in this paper intentionally. *DEFB103* has multiple copy numbers in the dog (Leonard et al. (2012)) and several other species (Hollox et al. (2003); Dreger and Schmutz (2009)), and a discussion of the function and interaction of the k^{Br} allele goes beyond the limitations of this presentation.

The line drawing dogs in Figure 3 are an attempt to illustrate the interactions of *ASIP* and *MC1R* genotypes, in dogs that are all k^y/k^y at *DEFB103*. Reinsch et al. (1999), attempted to convert the amount of white on Holstein cattle into a quantitative trait and mapped it near *KIT* on BTA6. In a similar manner, these line drawings try to depict the amount of phaeomelanin versus eumelanin pigmentation. However, in contrast to Holstein cattle where the melanin, or lack thereof (white), is quite random, the phaeomelanin and eumelanin pigmentation patterning is not random.

The interaction of the E^M allele only replaces small and specific areas of phaeomelanin with eumelanin. This change seems to occur equally in dogs of all *ASIP* genotypes (Fig. 3), although one cannot prove this is happening in the *a/a* dog that is already solid black. I suggest that the type of interaction of the E^M allele on *ASIP* genotypes is a "multiple modification".

Conversely, the E^G allele has been shown to affect only dogs with an a^t/a^t genotype (Dreger and Schmutz 2010) (Fig. 3). Therefore this type of interaction is better described as a "specific modification".

However, since the E^G allele has been found only in Afghan Hounds and Salukis thus far, and only the a^y and a^t alleles of *ASIP* occur in these breeds, it is not possible to comment on how this allele might affect a crossbred dog with an a^w allele. One would assume that a dog that is *a/a* would remain solid black. Hence the phenotype is shown in parentheses for such dogs, in Table 1. The phenotypic change results in less eumelanin and more phaneomelanin in dogs that have an E^G allele and no E^M allele.

Such a scenario with an interaction that affects only a single genotype at another locus, a "specific modification", can lead to atypical or misleading results on a genome scan. This was demonstrated in dogs that had the

saddle tan phenotype (Dreger et al. (2013)), a phenotype not discussed here previously, that causes the black area in a black-and-tan dog to be reduced in size to a "saddle" area on the torso of the dog. The GWAS showed *ASIP* as the highest peak when saddle tan and black-and-tan dogs were compared to all others, as both phenotypes require the same a^t variant at the *ASIP* locus. Only when saddle tan dogs were compared to black-and-tan dogs did a separate association peak appear that helped to identify the allele causing the saddle tan phenotype.

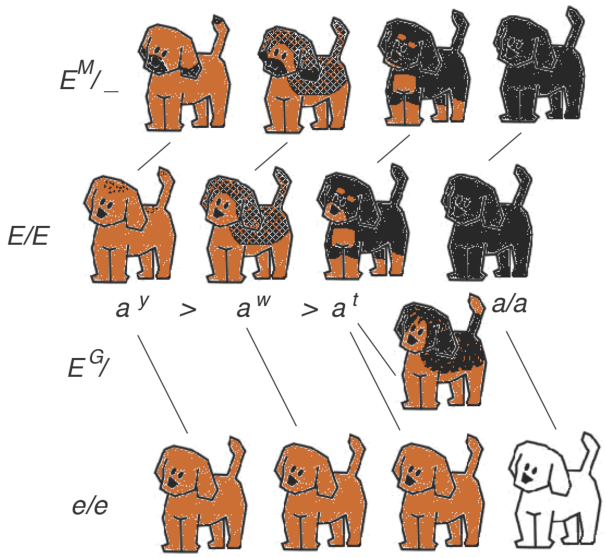

Figure 3: Drawings of dogs with a k^y/k^y wild type genotype at *DEFB103* to illustrate the *MC1R* (*E* locus) and *ASIP* (*A* locus) genotype interactions on pigmentation.

The *e/e* genotype shows complete epistasis to all *ASIP* alleles (Fig. 3) and to the K^B allele at *DEFB103 (Table 1)*. However, dogs that are *e/e* and *a/a* do deviate from the typical red color and are white.

Conclusion

Although genetic interactions are broadly acknowledged to exist, the two main classifications are epistasis and dominance deviation. We suggest that other types of genetic interaction are demonstrated among the three genes *MC1R*, *ASIP* and *DEFB103*. These include multiple modification and specific modification.

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