

Canine coloration: dark domesticated dogs and the bright side for wild wolves

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The skin, feathers, and fur of most wild animals are pigmented with melanin, and these black, brown, gray, rufous, and buff colors serve many evolutionary functions. Melanic patterns – alone or in combination with other traits – can act as bold signals of fighting ability, can amplify other signaling characteristics, or, as is most commonly recognized, can create a camouflaged appearance. In many species where natural selection favors cryptic coloration, melanin pigment and pattern variation across individuals is notably low; however, in other instances (most memorably including the peppered moth), remarkable variation remains, and these present exciting opportunities to study both the causes and consequences of melanization in nature.

Melanin coloration, with little inter-individual variation within species, is generally the rule among mammals, with noteworthy exceptions among domesticated varieties and a few wild species. Gray wolves (*Canis lupus*), for example, vary widely among and within western North American habitats in their coat color (e.g. white, gray, and black). Technological advances in recent decades have made it possible to study the molecular genetic bases to such melanin variability. From zebra fish to chickens to rodents, numerous loci have been uncovered that control the melanin synthesis pathway and pigment variations in domesticated species and humans, with special attention paid to the melanocortin-1 receptor (*MC1R*;

Harding et al., 2000; Kerje et al., 2003). Only recently have some of these historical genetic underpinnings of melanization been confirmed in free-ranging animals in an evolutionary context (Mundy, 2005).

Anderson et al. (2009) recently undertook a molecular genetic investigation of melanin coat color variation in gray wolf populations from the Canadian Arctic and Yellowstone National Park in the northwestern United States. An emergent pattern in the interpopulational variation of gray wolves, whose historical distributions rival any terrestrial mammal, is the rarity of individuals with dark coats in the northern tundra (7%) compared to the southern forests (62%; Musiani et al., 2007). Although earlier evidence pointed to *MC1R* control of dark coat color variants in a canid relative, the red fox (*Vulpes vulpes*; Våge et al., 1997), these authors found that, despite the presence of several polymorphisms, typical gain-of-function mutations to *MC1R* failed to associate with black coat color. Instead, a 3-base-pair deletion (CBD103^{AG23}) at an unlinked locus on a different chromosome – the *K* locus – corresponded nearly perfectly (in all but 2 of 255 individuals tested) with the presence of a black coat.

Intriguingly, many of these same authors had recently detected that the same *K* locus mutation is responsible for black coat color expression in domesticated dogs (*Canis domesticus*; Candille et al., 2007) and in coyotes (this study). Given the interbreeding capabilities of these canid taxa, this observation raised three key questions when considering the evolution and maintenance of this genetic variation: (i) did domestic dogs interbreed with and thus introduce CBD103^{AG23} to wild wolves?, (ii) was this mutation present naturally in ancient canid ancestors and/or the product of artificial selection on domestic dogs?, and (iii) is this

mutation currently under directional natural selection in wild wolves?

To answer the first question, Anderson et al. (2009) sequenced a core 4 kb region of *CBD103* in wild wolves from several habitats and from several domesticated dog breeds (e.g., akita, boxer, bulldog, poodle, great dane) and used phylogenetic reconstruction of haplotypes from 142 canid chromosomes to show that the CBD103^{AG23} mutation did not cluster by recent species, but rather is ancient in the lineage and was later spread via interspecific hybridization. In other words, haplotypes from coyotes were no more similar to one another than they were to haplotypes from wolves or dogs. They further investigated *K* locus variation by estimating chromosome recombination history and discovered that wolf chromosomal varieties have occurred much more recently than those of dogs, consistent with the notion that domesticated dogs directly introgressed black coat color genes into natural wolf populations. Data from other *C. lupus* populations in the world also point to this domesticated origin of CBD103^{AG23}, as the only other occurrence of wolf melanism is in Italy, where there is good evidence of wolf-dog hybridization in nature.

Prior studies of interbreeding populations of wild and domesticated mammal (e.g. *Felis* cats; Daniels et al., 1998) had attempted to disentangle geographic and taxonomic contributions to trait expression, but the work of Anderson et al. (2009) provides an unrivaled historical look at the interplay between phenotype, genotype, and environment in this melanic system. Anderson and colleagues went on to estimate the age of the CBD103^{AG23} mutation, in the hopes of pinpointing an origin before or after canine domestication (which occurred between 15 000 and 40 000 years ago), but confidence limits overlapped with this

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date range, leaving an inability to target the ultimate source of this variation. Parsimony suggests that it was an ancestral trait introduced once to wolves by dark domesticated dogs, but it is also possible that this mutation was lost by ancestral canids and then regained in North America as humans spread with their canine companions across the Bering Strait.

The final and unique piece of this puzzle lies in the fitness function of this melanin mutation in nature. Introgression of domesticated genes into wild populations of organisms has been documented elsewhere (e.g. several plant species, European cats, domesticated cattle to North American bison), but assessments of natural selection on those introduced genes are rarely made. While the behavioral consequences of this variation were not tested directly by Anderson et al. (2009) (e.g. by tracking survival/reproduction of individuals carrying the mutations or the hunting success of individuals with presumably background-matching coloration in the differing habitats), the authors attacked this problem at the genetic level. They detected low haplotype diversity for CBD103^{AG23} compared to the ancestral allele, consistent with strong positive selection on it. A second molecular signature of selection was also present in the marked haplotype homozygosity difference between the ancestral and black-coat mutation in

wild wolves, compared to the lack of difference in homozygosity between these two mutations in domesticated dogs.

In summary, yes, 'man's best friend' also appears to be the best friend of its brother, the gray wolf. Given the rapid human-induced alterations to natural landscapes, from tundra melts to forest clearing, we see today, it appears as if human processes of canine domestication by accident equipped natural populations of these threatened animals with the genetic machinery to cope with our anthropogenic disturbances. These fortuitous findings in wolves – that variable melanin genes are the spice of life – provide a special window into the value of selective breeding programs for maintaining broad and adaptive genetic variation, whether they be in organisms disappearing in nature or those especially valuable to us in industry, medicine, or agriculture. Historically, humans have directionally selected against variation, for the most profitable and yielding domesticated crop or breed, but this study is a timely reminder of the delicate balance and complementarity of artificial and natural selection in the modern, ever-changing natural but always-human-influenced world. Studies of melanism have no doubt been central in modern medicine, but perhaps they will come full circle, since the peppered moth, and have just-as-large impacts on human ecology.

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