

NEWS AND COMMENTARY

Conservation and population genetics

Wolf of a different colour

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Wolf individuals and populations have a striking variety of colours, ranging from white in arctic regions to black in some forested areas of western Canada and Alaska. The white colour in regions with ice and snow has long been suggested to be adaptive, concealing coloration to aid successful survival and predation. Similarly, some researchers have suggested that black colour is adaptive in forested areas with low light.

In most mammals, black colour is the result of pigment type-switching to the black pigment eumelanin from the yellow pigment pheomelanin and is controlled by two genes, *Melanocortin 1 receptor* (*Mc1r*) and *Agouti*. However, in dogs the dominant black variant is determined by a three base pair deletion in the β -defensin gene *CBD103* (also called the *K* locus). β -Defensin competitively binds to the melanocortin receptor, inhibits agouti expression, and results in the production of black eumelanin (Candille *et al.*, 2007). Anderson *et al.* (2009) have now examined wolves from North America and Italy and found that the dark coloured wolves have the same dominant variant at the *K* locus (K^B) as do dogs.

Anderson *et al.* (2009) proposed the following scenario for the introgression of this variant into North American wolves from dogs. First, they suggested that the K^B mutation arose in the ancestor of wolves and dogs or in early domesticated dogs. They estimated the time to most recent common ancestor of this variant as 47 000 years before present (ybp) (95% confidence interval 13 000–121 000 ybp), which overlaps with the estimated date of dog domestication (15 000–40 000 ybp) (Vila *et al.*, 1997; Savolainen *et al.*, 2002). Humans presumably then selected for the black colour in early dogs, making the K^B allele relatively common in dogs. Today this allele is found in at least 38 diverse breeds, such as the Dalmatian, Great Dane, Poodle and Portuguese Water Dog (Candille *et al.*, 2007). Subsequently, dogs with this variant accompanied Amerindians to North America 12 000–14 000 ybp, where they interbred with

wolves and introduced the variant into the wolf population around 500–14 000 ybp. Because of the selective advantage of this mutation in forested areas, it then became established in the wolf population. Both human selection for the dark colour in dogs and natural selection for dark colour in forest wolves could have contributed to the selective sweep signal at K^B alleles documented by Anderson *et al.* (2009). However, if a limited amount of the dog introgression occurred only into the forest population, then there could be a selective sweep signal, as well as more dark animals in the forest population, without selection actually favouring black wolves in the forest.

The events in this scenario have been dated using the amount and pattern of variation at single-nucleotide polymorphisms linked to *CBD103*. For example, there is very little variation at single-nucleotide polymorphisms closely linked to the K^B allele in wolves, resulting in a very recent estimate of time to most recent common ancestor and indicating a recent selective sweep. Conversely, there is more variation and evidence of recombination, among the same single-nucleotide polymorphisms on the K^B haplotypes from dogs, suggesting a somewhat older time to most recent common ancestor.

One particularly surprising finding is that Anderson *et al.* (2009) suggested that the very same K^B variant has entered wild canid populations at least three times, that is, into North American wolves, Italian wolves (these appear to be very recent wolf-dog hybrids; Randi and Luccini, 2002) and coyotes. It will be interesting to see if the same mutant determines black colour in wolves from the Himalayas (LD Mech, personal communication). This suggests that the mutation rate for this deletion is very low and also that it may have a strong selective advantage in particular situations. From these data it does not seem that this mutation has ever occurred in wild canids, in addition, other mutants producing dark colour—of which there are many in other mammals (Searle, 1968)—have also not occurred in wild

canids. A contrast is in rock pocket mice where melanism in Arizona is caused by a variant at the *Mc1r* locus and melanism in a New Mexico population is caused by a variant at another unidentified gene (Nachman *et al.*, 2003).

Anderson *et al.* (2009) suggested that there is a strong correlation between wolf colour and habitat (see also Gipson *et al.*, 2002; Musiani *et al.*, 2007); however, there are a number of counter-examples, that is, wolves of light colour in forest populations and black wolves on tundra (Mech *et al.*, 1998). Given the large number of generations in the selective scenario suggested by Anderson *et al.* (2009), one might have predicted a much higher frequency, maybe near fixation, of the dominant K^B allele in forest populations. From theoretical models, the conditions for a balanced polymorphism of a dominant allele based on selection in different environments are generally quite restrictive in a random-mating population (Hedrick, 1986). However, if there is a limited gene flow between habitats—as there may be between forest and tundra habitat—or if there is habitat selection where animals select the habitat in which they have the highest fitness, maintenance of polymorphism becomes more likely (Hedrick, 1986). Direct estimates of the selective differences in both environments for the colour phenotypes would be important support for the scenario proposed by Anderson *et al.* (2009). Perhaps the Yellowstone wolves, which segregate for the K^B allele and for which there is good pack and pedigree information (vonHoldt *et al.*, 2008), would provide a good population in which to estimate the putative selective advantage of black wolves.

Anderson *et al.* (2009) claimed that there is no other dog ancestry in wolves with the dark variant because they found no association with colour phenotype of 28 739 single-nucleotide polymorphisms in 20 wolves, 10 with genotype K^B/k^y and 10 with k^y/k^y . However, if dog ancestry was from a number of generations in the past it is unlikely—unless it was very tightly linked—that such an association would still remain today. If the K^B variant came from dogs, one might expect that there would still be a signal of dog ancestry at some other loci. Comparison of populations with the K^B allele to populations without the allele for levels of dog ancestry potentially might provide such a signal. Lack of dog ancestry at other loci either suggests that the level of introgression was quite low and that

there was subsequently a strong selection favouring the dark colour or that the variant did not initially come from dogs. Importantly, these findings also seem to imply that there are few other dog variants that are adaptive in wolves, in other words, this colour variant may be quite atypical of other dog genes. In Italy, the presence of a dewclaw (vestigial first toes or polydactyly) on the hind legs indicates hybridization with dogs (Ciucci *et al.*, 2003). This trait, which is selected for in a number of large dog breeds, has never been found in wild wolves without dog ancestry (although many wolf biologists do not look for it), potentially indicating that it is selected against in nature.

Several related questions also come to mind. First, the case for the dog origin of the K^b allele would be greatly strengthened if there were some evidence from ancient DNA that the variant was in North American dogs thousands of years ago or if there were historical data indicating dark coloured dogs among early Amerindians. Second, as wolves are not nocturnal ambush-hunters, similar to jaguars which have melanistic individuals (Eizirk *et al.*, 2003), black concealing colour may not be an important factor in their survival or hunting success. On the other hand, the heavy human hunting pressure throughout much of the twentieth century in many populations of wolves in Alaska and Canada may provide selection pressure in some environments against wolves that do not have black concealing colour. However, most aerial hunting is done with snow as background, and even in forested or open areas black wolves are generally more obvious from the air than grey wolves (LD Mech, personal communication). Third, the *CBD103* gene codes for a β -defensin. These molecules are highly polymorphic in both sequence and copy number and have been extensively investigated because of their importance in immunity. Are there any negative pleiotropic effects of agouti suppression from the K^b allele? Or are there positive pleiotropic effects of having agouti expression? Finally, a similar study on the genetic basis of white colour in wolves would be interesting. As white colour is also found in arctic foxes, which do not breed successfully with wolves or dogs, one might predict that the white allele in wolves did not come from dogs.

The conclusion at the end of the Anderson *et al.* (2009) study states that 'Our results imply that variants that appear under domestication can be viable in the wild and enrich the genetic legacy of natural populations' has broad implications. Efforts to conserve the gene pool of organisms that have had

hybridization with domesticated animals, such as bison, wild cats, wolves and so on, have emphasized the reduction of hybridization or exotic ancestry because it has always been thought that variants from domesticated species would be detrimental, or neutral at best, in wild populations. For example, there are estimates that about 1% of autosomal North American bison ancestry in some conservation herds is from domestic cattle (Halbert and Derr, 2007; Hedrick, 2009). Some bison researchers have advocated that even this small amount of cattle ancestry should be eliminated from bison. On the other hand, if this cattle ancestry were truly detrimental, one would predict that it should have been eliminated, or greatly reduced, by natural selection over the past century. If this cattle ancestry were neutral, it does not appear to significantly influence phenotype, although this has not been examined in detail.

On the other hand, the example of a domesticated species providing adaptive genetic variation for a wild species is sensational. One of the chief concerns in endangered species is that their small population size is expected to result in a loss of genetic variation necessary for future adaptation. Mutation generally can only slowly regenerate variation and only if the population size is not small. The scenario of Anderson *et al.* (2009) suggests that another alternative is possible; that is, hybridization with another species or a domesticated relative can provide genetic variation that has important adaptive consequences. Most adaptive polymorphisms appear to be present in only one species (Hedrick, 2006) and the main examples of adaptation over species are at the few genes that show strong selection and *trans*-species polymorphism, such as those in the major histocompatibility complex in vertebrates, self-incompatibility loci in plants and the sex determination gene *csd* in hymenoptera. Castric *et al.* (2008) calls the incorporation of advantageous genetic variation from related species as adaptive introgression, and gives a number of potential examples. If Anderson *et al.* (2009) are correct then other adaptive variants may be expected to be present in related species and because of gene flow may be important for adaptation in endangered species.

This scenario is extremely intriguing; it is based on some very convincing genomic data and has far-reaching implications for evolution and conservation. However, data confirming the selective advantage of the black K^b allele in forest populations, evidence of the K^b allele in early Amerindian dog samples and data from wolves showing ancestry from other

dog loci would increase the believability of the details of this evolutionary story.

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