
Adapting to a New Diet During Dog Domestication: Implications for the Domestication Process and Dog Health

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Abstract

Genome-wide comparisons of dogs and wolves are offering new perspectives on the origin of dogs. We recently used this technique to map selection during dog domestication and identified several genes involved in digestion and fat metabolism. In particular, selection at three genes responsible for the digestion of dietary starch indicates that dog domestication was accompanied by a change from a mostly carnivorous to a relatively starch-rich diet. Through surveys of genetic diversity in these loci in dog populations from across the globe, we may gain insights into how and where this adaptation took place as well as how this dietary change may affect dog health.

Introduction to Dog Domestication

Dogs were likely the first animal to be domesticated, yet they still are important today to modern human society. It is not clear when and where this domestication process started. Fossil remains of canids in Europe and Russia dating 12,000 to 33,000 years before present (YBP)^{1,2} suggest that it may have started among Palaeolithic hunter-gatherers. It is, however, uncertain whether these remains represent ancestors to modern dogs,³ failed domestication attempts or natural morphological variation among wolves.⁴ Canine remains from Israel that are 12,000 years old could instead represent the earliest verified dog remains,^{5,6} potentially associating dog domestication with the human transition from a nomadic to sedentary lifestyle.

Large-scale genetic analyses indicate that dog domestication began between 18,000 to 11,000 YBP,^{7,8} but as all wolves appear to be equally closely related to dogs,⁸ these analyses are less informative with regard to the location of the domestication process. On the other hand, worldwide surveys of mitochondrial and Y-chromosome haplotype variation in dogs show that Chinese village dogs harbor unrivaled levels of genetic diversity, suggesting that most modern dogs have a Chinese origin.^{9,10} Adding to these uncertainties about the origin of dogs, it also is not clear why and how dogs were domesticated. Humans may have begun

Glossary of Abbreviations

AMY2B: Pancreatic amylase in the small intestine of dogs that initiates starch digestion

GO: Gene Ontology

MGAM: Maltase-Glucoamylase responsible for the hydrolysis of maltose to glucose

PCR: Polymerase Chain Reaction

SGLT1: Sodium-Glucose Co-Transporter 1

YBP: Years Before Present

this process by capturing wolf pups for use in guarding or hunting. Alternatively, as humans changed from a nomadic to sedentary lifestyle, wolves themselves may have approached waste dumps near human settlements to scavenge for food.⁶ Selection for characteristics that allowed an efficient use of this alternative food resource may have resulted in the evolution of

a type of scavenger wolf that constituted the proto dog.

Genomic Differences Between Dogs and Wolves

Genome-wide comparisons of domestic and wild relatives at a population level recently have started to shed light on several domestication processes. Identifying genomic regions where domestic varieties share a haplotype that is typically absent or rare in wild relatives can be used to map selection during initial domestication. Functional characterization of genes in these regions may then provide insight into traits characterizing domestic animals. Such knowledge can, in turn, be used to explore the genetic basis for these traits and to extrapolate on the environmental changes that accompanied the transition from wild to domestic animal, hence, offering a new perspective on the domestication process.

Following this reasoning, we recently mapped selection during early dog domestication by comparing pooled whole-genome sequence data from 12 widely distributed wolves and 60 dogs¹¹ and identified 36 genomic regions that differ significantly between dogs and wolves. By functionally characterizing genes within these regions using gene ontology (GO) analysis, we identified two major themes of functional change during dog domestication.

The first theme relates to the GO term *nervous system development*, which supports the hypothesis that selection for altered behavior was important during dog domestication and that mutations affecting developmental genes may underlie these changes.^{12,13} The second theme relates to *digestion* and includes genes involved in both starch and fat metabolism. In particular, we found evidence for selection affecting three consecutive steps in the pathway responsible for starch digestion and subsequent glucose absorp-

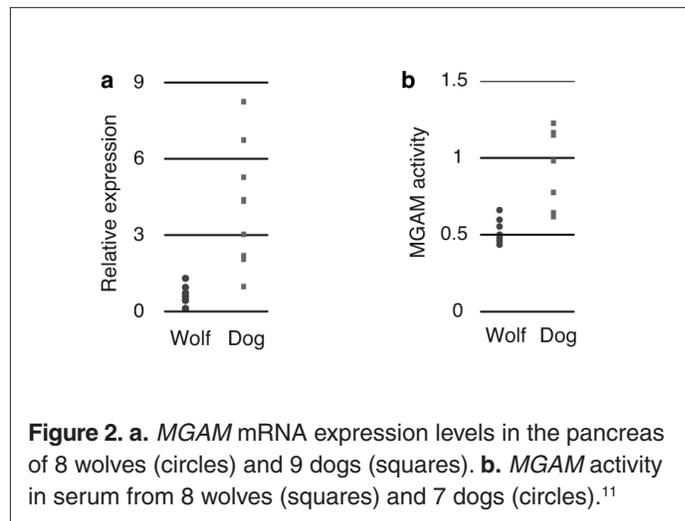
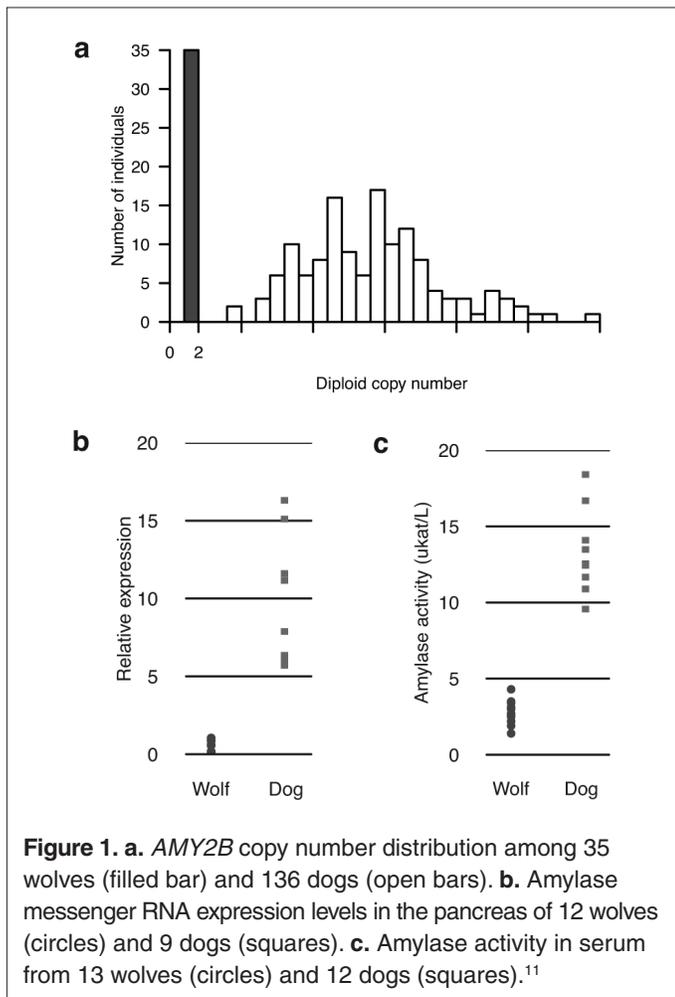
tion, indicating that dogs may have adapted to a change from a mainly carnivorous to a more starch-rich diet.

AMY2B

In dogs, starch digestion is initiated in the small intestine by pancreatic amylase (*AMY2B*) catalyzing the breakdown of starch to oligosaccharides maltose and maltotriose.¹³ Following initial observations of biased sequence coverage near *AMY2B* in dog, we used quantitative polymerase chain reaction (PCR) to show that repeated gene duplications have resulted in an average sevenfold *AMY2B* copy number increase in dogs relative to wolves (Figure 1).¹¹ We subsequently determined that this difference is associated with higher pancreatic *AMY2B* expression (28-fold higher average expression in dog, [Figure 1] as well as higher serum amylase activity in dog compared to wolf [4.7-fold higher activity in dog, Figure 1]).¹¹ Our observations argue that *AMY2B* duplications likely resulted in a selective advantage to early dogs by increasing the amylase activity.

MGAM

After the initial breakdown of starch to oligosaccharides, maltase-glucoamylase (*MGAM*) is responsible for the hydrolysis of maltose to glucose.¹⁴ *MGAM* is located in a region on chromo-



some 16 that is highly divergent between dogs and wolves. We identified several candidate mutations within this locus including two conservative amino acid substitutions, a 2 bp deletion that disrupts the *MGAM* stop codon in dogs and a mutation in intron 37 that affects a predicted transcription factor-binding site.

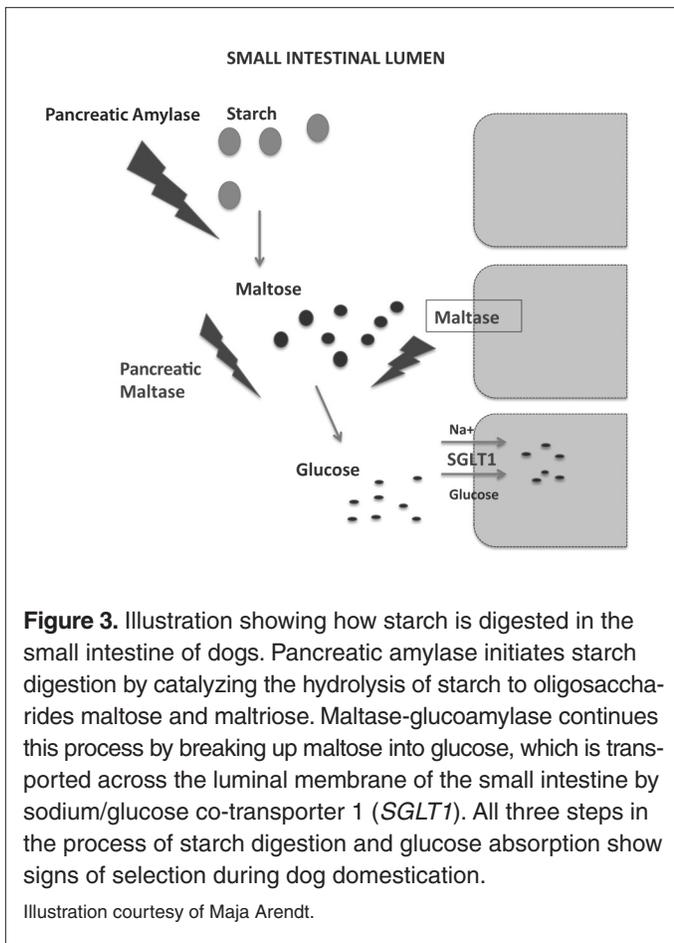
Although we have not been able to determine which of these mutations were targeted by selection during dog domestication, our functional assays concluded that both *MGAM* expression and *MGAM* protein activity have changed during the domestication process: *MGAM* is, on average, expressed at ~12-fold higher levels in dogs compared to wolves, and maltose is converted to glucose at twice the rate in dog compared to wolf serum (Figure 2).¹¹

SGLT1

After the hydrolysis of starch has been completed, the resultant glucose is absorbed through the wall of the small intestine by the sodium-glucose co-transporter 1 (*SGLT1*).¹⁵ *SGLT1* resides in a region on chromosome 26 that also differs markedly in haplotype frequencies between dog and wolf. All dogs tested were carriers of a particular haplotype that was completely absent in 18 of 19 wolves.¹¹ This region includes a conservative amino acid substitution in *SGLT1*, though so far we have not been able to determine that this mutation, indeed, represents the target of selection in this region.

AMY2B Copy Number and Amylase Activity Varies Among Dogs

Although our observations argue that dogs, in general, digest starch more efficiently than wolves (Figure 3), strong variability in *AMY2B* copy number (diploid *AMY2B* copy number range in dogs $2N=4-30$) and widely varying serum amylase activity values among dogs¹⁶ indicate that the ability to handle starch may vary in a genetically determined manner within the dog population. By comparing *AMY2B* copy number and amylase activity in a diverse set of dogs, we have confirmed that amylase activity is associated with *AMY2B* copy number (beta 0.69 per



copy; $p=0.028$, linear regression), arguing that individual dogs, indeed, may be unequally fitted to digesting starch.¹⁶

Due to founder effects during breed creation and possible differences in selection pressures among breeds from diverse geographical locations, it is furthermore likely that this ability may vary in a breed-dependent manner. In support of this idea, we found that *AMY2B* copy numbers vary considerably in a large set of dogs and that nearly 70% of the individual variation can be attributed to breed.¹⁶ Copy numbers are, for instance, low in some breeds that traditionally have relied on a diet that was relatively rich in meat.¹⁶ Dogs' ability to handle starch, thus, seems to vary both at the individual and breed level.

Conclusion

In conclusion, our genome-wide comparison of dogs and wolves argues that selection affected three starch digestion genes during dog domestication: *AMY2B*, *MGAM* and *SGLT1*. This indicates that adaptations that allowed the ancestors of modern dogs to thrive on a diet that was relatively rich in starch constituted an important step during the domestication process. Although it is likely that most dogs, thus, digest starch more efficiently than wolves, this ability seems to vary significantly within the dog population, with some breeds being better adapted to starch than others. Ongoing research is now addressing whether this variability may be linked

to traditional feeding habits and whether it could be associated with susceptibility to metabolic disorders.

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