Genomic analysis of McCleery Buffalo gray wolves:
Final report for Wolf Haven

NORTH AMERICAN
CANINE
ANCESTRY

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Introduction. A distinct species originally described (Goldman 1944; Hall 1981), *C. nubilus* Say 1823 was observed in eastern Nebraska and later documented to be the gray wolf subspecies (*C. l. nubilus*) widespread and common to the interior of the contiguous United States and eastern Canada (Chambers et al. 2012). Their vernacular name of the "Great Plains" wolf is a misnomer as this subspecies occurs from the west coast rain forests to the Arctic landscape in eastern Canada. The difficulty in describing and defining *C. l. nubilus* is due to the ongoing contact with several other lineages of *C. lupus* (*occidentalis, mackenzii, arctos*), *C. lycaon* (eastern wolf), and *C. rufus* (red wolf) (Chambers et al. 2012). Yet research has investigated the genetic structure across *C. l. nubilus* gray wolf range and found that habitat and prey specializations contribute towards genetic distinctions populations (Carmichael et al. 2001, 2007, 2008; Musiani et a. 2007). As these wolves track the migratory movements of buffalo herds throughout the expansive Rocky Mountains, they have been also referred to as the "Buffalo" gray wolves. Similar drivers of genetic structure have been described in *C. lupus* populations, predominantly due to unstable prey base, natal dispersal events, ephemeral land bridge opportunities, or climate change (Carmicheal et al. 2001, 2007, 2008; Musiani et al. 2007). Specifically, Carmichael et al. (2008) evidenced limited genetic connectivity between the Buffalo gray wolves and the northern timber gray wolves (*C. l. occidentalis*). During the early 1900s, the US Biological Survey began an initiative to eradicate all gray wolves (*Canis lupus*) from the Great Plains alongside other predator species. The Animal Damage Control Act of March 2, 1931 (7 USCA § 8351-8356 Ch. 109A) was a directorate for the US Biological Survey to control "...mountain lions, wolves, coyotes, bobcats, prairie dogs, gophers, ground squirrels, jack rabbits, and other animals injurious to agriculture, horticulture, forestry, animal husbandry, wild game animals, fur-bearing animals, and birds, and for the protection of stock and other domestic animals through the suppression of rabies and tularemia in predatory or other wild animals; and to conduct campaigns for the destruction or control of such animals….". Between 1921 and 1930, Edward Heber McCleery (1867–1962), a student at Princeton University and then a physician in McKean County in Pennsylvania, purchased several gray wolf pups to preserve Buffalo gray wolves from extinction. These pups were captured from dens in the Highwood Mountains of Montana. Today, the lineage of these original wolves survives in the Wolf Haven International, a 501(c)(3) nonprofit sanctuary. Their genomic uniqueness remains unknown. With the recent Endangered Species Act status changes for many gray wolf populations of the United States, we quantified the genomic uniqueness of the Buffalo gray wolves to determine if ghost genomic variation persists in the captive lineage.

Results. We genotyped 72,792 loci in 226 canids from North America. We found that the Buffalo gray wolves form a distinct genetic group (**Fig. 1A**). In a similar fashion, the domestic dogs and eastern wolf individuals, both lineages of known genetic isolation and inbreeding, also formed distinct and tight clusters. The gray wolves split into their two representative geographic regions (Rocky Mountains and the Western Great Lakes). Coyotes displayed a wide range of spatial occupation, reflecting their known genetic subgroups. Buffalo gray wolves formed the most spatially restrictive cluster, suggestive of isolation or distinctiveness.

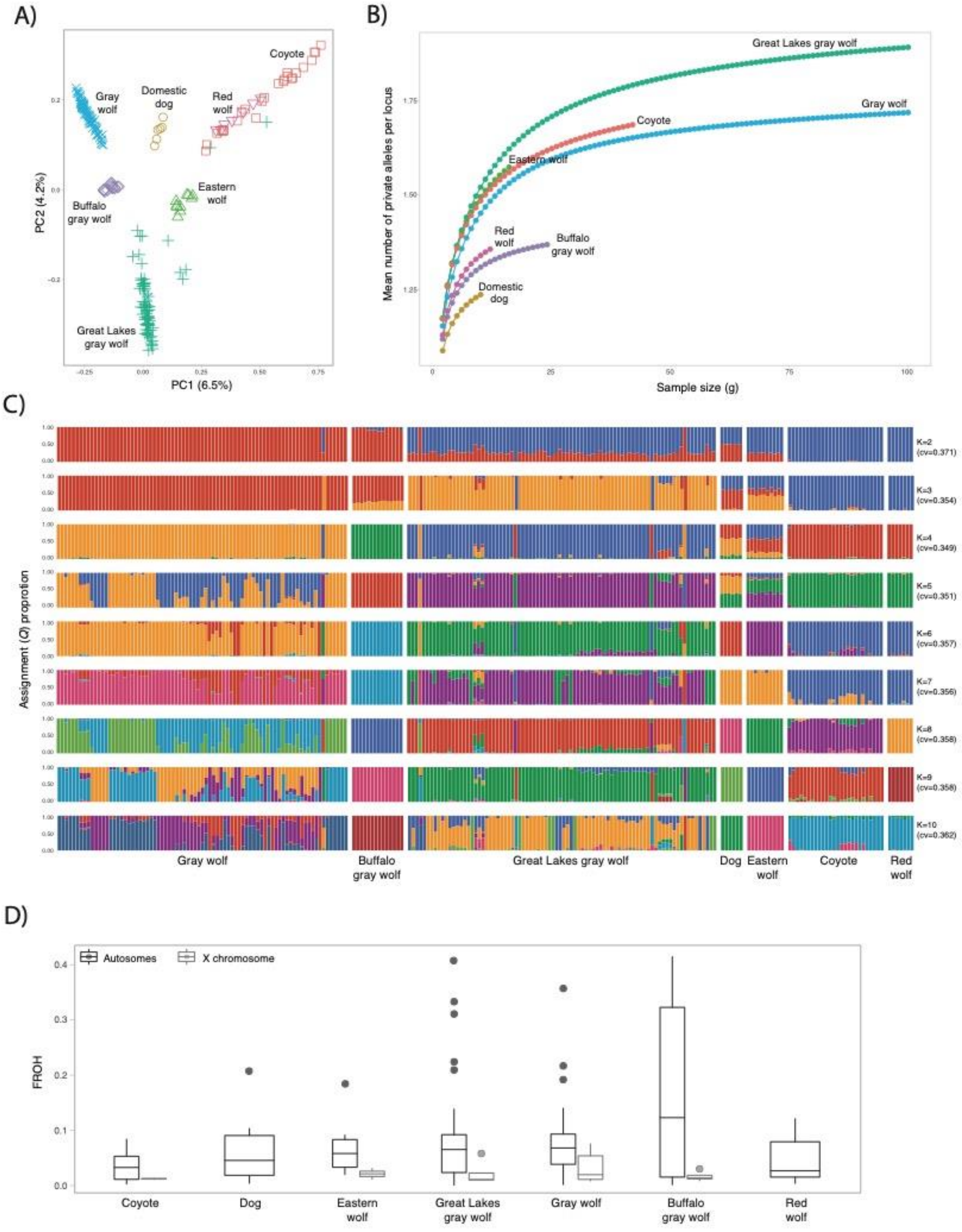

Figure 1. A) *Principal components analysis and the percent of variance explained (in parentheses) for 226 North American canids genotyped at 72,972 statistically neutral and unlinked loci.* ***B)*** *Private allele rarefaction for each cluster identified in panel A.* ***C)*** *Maximum likelihood analysis of 2 to 10 genetic partitions (K) with their respective assignment proportion (Q) and each partition's cross-validation (cv) value in parentheses.* ***D)*** *Inbreeding estimates (referred to as FROH) for autosomes separately from the X chromosome.*

For each of the PCA clusters found in Figure 1A, we found that Buffalo gray wolves had the second lowest nucleotide diversity of the canids analyzed (pi=0.120) with only 54 alleles private to them (**Fig. 1B**). This is in comparison to the genetically diverse and unique Rocky Mountain gray wolf (1,412 private alleles and pi=0.148), the Western Great Lakes gray wolf (1,718 private alleles and pi=0.171), and coyotes (156 private alleles and pi=0.199). Similar to the Buffalo gray wolves, we found that eastern and red wolves also showed very few alleles private to their lineages (0 private alleles for eastern wolves and only 5 alleles private to red wolves). The model-based clustering analysis also found this distinction of Buffalo gray wolves (**Fig. 1C**). However, when this pattern is considered in light of inbreeding estimates, we find that Buffalo gray wolves had the highest average estimates of inbreeding, which were significantly higher than estimates found for gray wolves, eastern wolves, coyotes, and red wolves (**Fig. 1D**). We found that the genetic ancestry of these Buffalo gray wolves composed of two identities, with a collective average of 74.7% gray wolf (range=71-79%) and 25.3% Western Great Lakes gray wolf.

Conclusion. Despite the near ubiquitous distribution of the North American Buffalo gray wolf (subspecies *C. l. nubilus*), they have had several taxonomic revisions and modifications due to their wide geographic representation and contact with several other gray wolf lineages. Here, we had the unique opportunity to collect genome-level variation of Buffalo gray wolves that have populated McCleery's sanctuary for the past century. With foresight, McCleery anticipated the decimation of canids across the continent as federal predator control programs sought to eliminate their presence. The McCleery Buffalo gray wolves have been successfully maintained as a captive population, yet their role in gray wolf conservation has been uncertain to date. We found that the Buffalo gray wolves represent a distinct genomic group among several North American wild canid lineage and domestic dogs. However, this unique grouping is due to their exceedingly elevated level of inbreeding, thus significantly removing all signatures of genomic uniqueness among the other wild North American canids. Buffalo gray wolves are genomic allies of Rocky Mountain and Western Great Lakes region gray wolves. Their genomic ancestry is predominantly of western gray wolf (75%) yet carry a fraction (25%) that aligns with their Western Great Lakes gray wolf neighbors. There is still uncertainty about the historic levels of genomic variation that was lost due to population declines in the ancestor. Similar to other captive populations established with a few individuals, genetics that are unique to the species or lineage were quickly lost and were not retained in the modern-day wolves.

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