

Beyond species boundaries: What North American *Canis* can teach us about conservation in a genomics era

Bridgett M. vonHoldt
Department of Ecology and Evolutionary Biology
Princeton University
Princeton, New Jersey USA 08544

North American *Canis* species are an unusually powerful natural system for understanding gene flow, hybridization, admixture, population connectivity, and the conservation value of genetic diversity. Of the species found on the continent, each are compelling in their own ways for their impact on shaping conservation discussions that consider their tremendously diverse demographic history. First and foremost, North American canids remind us that conservation does not always fit neatly into species boxes. Across wolves, coyotes, and Red wolves, genomic data continue to underscore their intertwined, complex history of divergence, connectivity, admixture, and adaptation. Rather than viewing gene flow simply as a threat to species integrity, conservation genomics offers a way to ask a more nuanced question: *which genetic variation is important to preserve, where did it come from, and how can we maintain it into the future?*

A species concept names the criteria needed to consider an entity a distinct species, with more than 20 concepts described and each ultimately focused on valuing a specific data type(s) over others. There are, however, a subset of concepts that remain most popular over the years. The Typological Species Concept (TSC) and the Biological Species Concept (BSC) both declare that members of the same species must share certain characteristics that distinguish them from other, with the latter carrying only a single criterion: reproduction can only occur between individuals that belong to the same species. In time, additional concepts were introduced. The Recognition Species Concept (RSC) and the Cohesion Species Concept (CSC) both have more esoteric description that a species is united by some emergent property. Although these appear to be quite flexible, with the CSC possibly the most inclusive concept to date. To note, there are a few concepts that rely upon evolutionary theory. The Phylogenetic Species Concept (PSC) is focused on evolutionary relationships while the Evolutionary Species Concept (ESC) adds verbiage focused on sharing a common ancestor with the species being the single lineage that has a single evolutionary origin.

Gray wolves (*Canis lupus* and the endangered subspecies *baileyi*), eastern wolves (*C. lycaon*), Red wolves (*C. rufus*), coyotes (*C. latrans*), and other North American canids have complex histories of divergence and gene flow, often violating much of the overly simplistic species concepts. Further, nearly all the North American wolf species have been listed as endangered, threatened, or of concern at some recent time point or another. Thus, North American canids provide a remarkable case study for the tension between evolutionary history and conservation categories. Of which, the Red wolf is perhaps the most compelling example of how genomic information can complicate and optimistically improve conservation decisions.

One possible explanation for these canids breaking most species concepts is their recent origin and behavioral ecology. Their evolutionary origin in North America was roughly 1 million years ago (approximately 250,000-500,000 generations assuming 2-4 years per generation), combined with often an effective population size substantially less than census size, should dramatically shape our expectations about reproductive isolation and permeable species boundaries. North American *Canis* species have a rich history of gene flow, admixture, and

hybridization that produces physiologically and genetically fertile offspring. Behaviorally, their early social life experience is expected to shape how their own ability to integrate, or not, in a socially structured pack. Further, body size plays a significant role in restricting reproductive events, often successfully with size-matched individuals, such as a smaller female gray wolf could be comparably sized to that of a larger male coyote. To back up even more, inter-species breeding requires some degree of synchronous reproductive cycles. Finally, set these events in a landscape that has been significantly disturbed and disrupted by human activity. There is a general set of observations that disturbed ecosystems can promote hybridization either through pure drive to reproduce and lowering preferences or by simply breaking their social group through targeted elimination and recreating hunting programs. Hence, evolutionary theory is no longer simply that divergence and intrinsic reproductive isolation lead to the formation of a new species. Rather, divergence is compounded by subsequent shifts in ecology and behavior, which may work to restrict gene flow despite retained reproductive compatibilities.

Where gene flow has occurred, mixed genetic ancestry is likely detectable. Possibly also remnant signatures are found in morphology. Although there are clearly many more complicating factors that need to be considered, several North American *Canis* species have measurable amount of mixed ancestry. And in the era of genomic data, we need to change the questions we are asking regarding species concepts and permeable boundaries. It is fact that scientists and regulatory communities need to move beyond simply asking “Is this animal pure?” toward ancestry, demographic history, adaptive variation, and genetic diversity. And in the effort of species conservation, what exactly do those efforts aim to conserve (e.g., species name, evolutionary lineage, ecological role)? And such conservation decisions have genetic consequences.

One thing we do know is that inter-breeding between North American *Canis* appears to be more of the norm rather than the exception. When species conservation decisions are made that influence management practices such as translocations, hunting, or initiating a new *ex situ* breeding population, the source populations of individuals for these events matter with respect to their evolutionary history and genomic variation, which can alter evolutionary trajectories. Again, the critically endangered Red wolf is current under a strict federal species membership definition that requires an animal to carry at least 87.5% Red wolf ancestry to be eligible for protections under the United States federal Endangered Species Act of 1973. Yet recent research has published empirical research documenting coyotes in the American southeast that carry just about 70% Red wolf genetic ancestry. If more than half the genome is composed of critically endangered genetic material, what new conservation decisions need to be considered? If these coyotes carry morphology that is distinct from lower Red wolf ancestry individuals, if these coyotes have a social ecology similar to that of a group living wolf, if they fill the ecological role of a wolf (when indeed wolves do not exist in the region), should these admixed coyotes carry an elevated value for Red wolf conservation?

Which leads me to a call for the North American canid research and regulatory community. If we are to present a united front against species extinction, researchers, conservation practitioners, managers, state and federal biologists, and policy makers need to be in the discussion together. We need to share genomic datasets, samples, ecological observations, and expertise, and we need conservation decisions to be informed by the broadest possible picture of canid diversity. Whole-genome sequencing, demographic modeling, ancestry inference, non-invasive genetics, bioacoustic monitoring, and other emerging approaches increasingly allow us to understand not only what a canid is called, but also its evolutionary

history, ecological function, and potential conservation value. But better tools alone will not solve the problem. We also need to be willing to ask different questions.

For North American *Canis*, reproductive compatibility does not erase evolutionary divergence, just as genomic admixture should not erase evolutionary or conservation value. These lineages have persisted through divergence, secondary contact, gene flow, demographic contraction, range expansion, and profound human alteration of their landscapes. Their genomes record that history; our conservation frameworks should be capable of recognizing it. Rather than asking only whether an animal is sufficiently “pure” to conserve, we should ask: *what evolutionary history does it carry and what genetic diversity would disappear if we lost it?* This is particularly urgent when endangered genetic variation persists outside the boundaries in which we traditionally expect to find it. If historically important or potentially adaptive variation survives through introgression into another population, dismissing that variation because it occurs in an admixed genome may mean choosing to lose a component of the evolutionary legacy we are trying to conserve.

The challenge for the next era of canid conservation is therefore to build conservation strategies sophisticated enough to accommodate the evolutionary processes that created the diversity we seek to protect. For North American *Canis*, permeable species boundaries are not an evolutionary anomaly; they are part of the evolutionary history of these lineages.