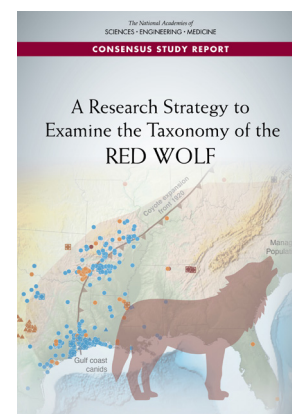




October 2020

A Research Strategy to Examine the Taxonomy of the RED WOLF



Dramatic reductions in the population of modern red wolf, *Canis rufus* in the eastern United States led to their re-introduction by the U.S. Fish and Wildlife Service (USFWS) into North Carolina in the early 1980s. Since then, USFWS has managed a population of red wolves that was established from re-introduced individuals. However, subsequent genetic studies of the managed population indicated that these wolves displayed evidence of significant coyote ancestry. This discovery raised the issue of whether the extant managed¹ population in North Carolina was a valid species that was eligible for conservation and recovery.

In March 2019, at the request of USFWS, the National Academies of Sciences, Engineering, and Medicine issued a report that provided evidence that retained the existing taxonomic designation of the red wolf and reinforced the validity of conserving and restoring red wolves (see Box 1). While the 2019 report was being prepared, genetic research on canid populations in Louisiana and Texas provided evidence that certain genes characteristic of historical red wolves might have been missing from the population introduced in North Carolina.

Given this new information, the USFWS requested the National Academies to carry out a second study with two tasks. The first task was to solicit and review applications to carry out research to determine the taxonomy of the canid population in southern Louisiana, which was completed in January 2020. The second task was to develop this report which lays out a research strategy to examine the evolutionary relationships between ancient red wolves, the extant managed red wolf population, and the unidentified canid populations in southern Louisiana.

WHY DO MORE RESEARCH ON THE RED WOLF?

There are three possible explanations for the significant amount of coyote ancestry in modern red wolves. First, the extant red wolves may be hybrids between coyotes and either gray wolves or what were once red wolves. Hybridization may have occurred long ago or in more recent years. Evidence suggests that some hybridization certainly occurred in the 20th century as red wolf populations dwindled in size while coyotes expanded their range eastward. Second, the extant red wolves may have diverged from coyotes recently enough that they continue to share alleles, even though each species has its own forward evolutionary trajectory—a situation referred to as incomplete lineage sorting. Third, hybridization and introgression of coyote genes into red wolves could reflect historical phenomena associated with the ancestral origin of the red wolf. In this case, the presence of coyote alleles would be the result of hybridization between coyotes and gray wolves (or another ancient canid) that gave rise to the red wolf, in which some level of reproductive isolation exists, but is incomplete.

Another reason why the controversy over the status of red wolves persists is the paucity of data from ancient specimens (specimens dated prior to 1800) that could serve as a standard for the red wolf before hybridization with coyotes in the 20th century. There are only data for mitochondrial DNA (mtDNA) sequences from just four ancient specimens. Those data indicate a close relationship between coyotes and red wolves but not the nature of that relationship. The mtDNA from historical specimens (specimens collected between 1800 and the start of the eastward expansion of coyotes in 1920) cannot

¹ Also known as nonessential experimental population (NEP) and is composed of 20 individuals that roam their native habitats in eastern North Carolina.

BOX 1

Conclusions from the 2019 National Academies Report *Evaluating the Taxonomic Status of the Mexican Gray Wolf and the Red Wolf*

Evaluating the Taxonomic Status of the Mexican Gray Wolf and the Red Wolf, published in March 2019, addresses the taxonomic status (*Canis rufus*) and evolutionary history of the red wolf. The report concluded that available evidence at present supports species status for the extant red wolf for three reasons: (1) there is evidence that the historical population of red wolves was a lineage distinct from other canids, (2) there is evidence that the extant (captive and managed^a) populations of red wolves in the United States represent a lineage of canids distinct from modern gray wolves (*Canis lupus*) and coyotes (*Canis latrans*) (Figure 1), and (3) there is evidence that the extant populations traced some of their ancestry to the historical red wolf. However, the authors acknowledged that additional genomic evidence from historical specimens could change this assessment.



FIGURE 1. From left to right: red wolf (*Canis rufus*), coyote (*C. latrans*), and gray wolf (*C. lupus*). SOURCE: B. Bartel, U.S. Fish and Wildlife Service (left) and Melba Coleman (middle and right).

^a Captive red wolves refer to the approximately 245 red wolves maintained in 43 breeding facilities all throughout the United States. Managed red wolves refer to the individuals comprising the nonessential experimental population (NEP) in eastern North Carolina (or the extant managed population in North Carolina).

be resolved without an analysis of nuclear DNA from the same specimens, so they do not resolve questions about the ancestry of red wolves. Finally, genetic and genomic data of extant putative red wolves come from populations at the southwestern edge of the historical range and the managed population (Figure 2), and it is unclear how well those populations represent what was once a numerically large, geographically widespread population of canids.

EXAMINING THE EVOLUTIONARY RELATIONSHIPS AMONG ANCIENT RED WOLVES, THE EXTANT MANAGED RED WOLF POPULATION, AND THE UNIDENTIFIED CANID POPULATIONS IN SOUTHERN LOUISIANA

The guiding principle for the research strategy described in this report is that it must address the relationships among ancient red wolves, historical red wolves, the extant managed population of red wolves, and the unidentified canids along the Gulf Coast. Resolving those relationships requires answering five research questions:

1. Are the ancient red wolves (i.e., specimens dated prior to 1800) a distinct lineage from the lineages of gray wolves and coyotes?
2. Are the historical red wolves (i.e., those in the 19th century, before the eastward expansion of coyotes) a

continuous lineage to ancient specimens in the eastern United States?

3. Are the red wolves from the North Carolina managed population a continuous lineage to those in the eastern United States before the coyote expansion in the 20th Century?
4. Are the Gulf Coast canids part of the distinct lineage of canids that lived in the eastern United States before the coyote expansion?
5. Is the genetic diversity of the North Carolina managed population a subset of the diversity of the Gulf Coast canids?

To clarify the relationships among these canids, new research should employ three types of data: morphological data from ancient specimens and extant animals, ancient DNA, and genomic data from the unidentified canids and the extant managed population. Figure 3 outlines the five questions, the canids to which each question pertains, and where this report describes the necessary data. The next sections summarize the committee's conclusions.

Morphological, Behavioral, and Ecological Data

Morphological data from ancient and historical red wolves are somewhat limited, but even more limited are behavioral and ecological data. Nevertheless, an understanding of these types of data collected from ancient

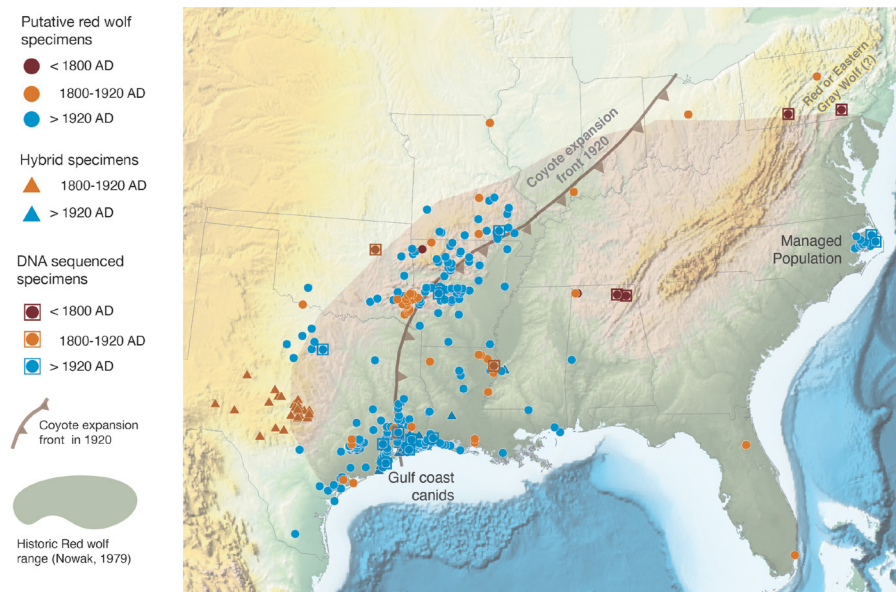


FIGURE 2. Map showing the reconstructed historical range of the red wolf and the locations where natural history specimens were collected that are thought to be non-admixed (circles) or hybrid (triangles). Locations from which DNA samples were taken are indicated with squares.

and historical red wolves, extant North Carolina managed population, and the Gulf Coast canids could be helpful in understanding their evolutionary relationships and the consequences of those relationships.

Conclusions

2-1: To distinguish among the origin hypotheses for the red wolf using morphology, additional kinds of data and analysis are required. The existing studies based on skulls can be supplemented by similar analyses of teeth, which are likely to better capture genetic relationships among taxa, and of postcrania, which are likely to supplement the understanding of ecological differentiation of red wolves from coyotes and gray wolves. Geometric morphometric data will enable finer level discrimination based on details of shape that were not captured in the original analyses.

2-2: New analytical techniques are required to assess the congruence between morphological and genetic evidence for the distinctiveness of the red wolf. Prior analyses relied solely on discriminant function analysis, which is a powerful tool for determining what is different between known groups, but it is less powerful for determining which groups are more similar, how variation at the subspecific level compares with differentiation between species, or whether differences are due to hybridization. The same hierarchical analyses of variance, cluster analyses, and pairwise distance analyses that are commonly used on genetic data can be applied to morphological data to facilitate these comparisons.

2-3: The behavioral data that exist appear to be consistent with the hypothesis that hybridization happened late in the red wolf's history, not at its inception, but behavioral data for historical red wolves are sparse. The behavioral data that exist deserve to be more comprehensively analyzed with regard to hypotheses about hybridization in canids, and morphological indicators of behavior are needed to supplement direct observations of behavior.

Recommendation: The committee recommends that existing morphological datasets be submitted to new analytical techniques that are directly comparable to genetic results, that ancient DNA sequencing and morphological analyses be performed on the same individuals, and that new morphological data be collected that is relevant to assessing the ecological and behavioral distinctiveness of historical red wolves in their core range.

Genomic Data from Historical and Ancient Canids

Genomic data from ancient and historical canids would enable the investigation of the ancestry and population history of red wolves. To be useful, genomic data must be sufficiently fine-grained to make it possible to discern whether the red wolf lineage is distinct from other wolf lineages in North America and to detect the occurrence and timing of historical hybridization events among lineages. For such analyses, a well-planned selection of samples and careful data collection and filtering will be essential.

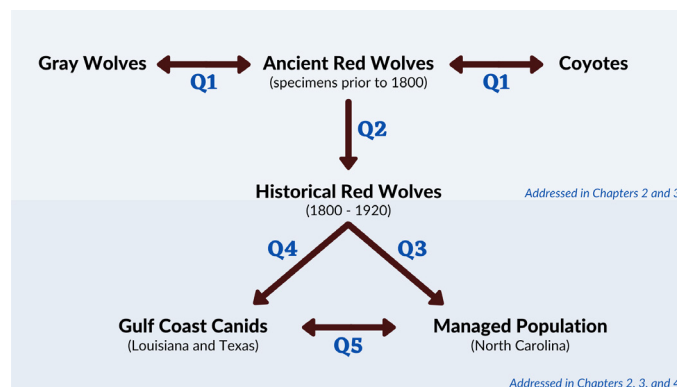


FIGURE 3. Five research questions addressing various relationships among six canid lineages. Single-pointed arrows indicate relationships via direct descent. Double-pointed arrows indicate relationships through a common ancestor.

Conclusions

3-1: Biases in collection and analysis of genetic data can be mitigated through care in selecting samples, application of appropriate methods to isolate DNA, and analysis of data from the entire genome rather than from small subsets of the genome.

3-2: Genomic data from ancient and historical canids would make it possible to investigate the ancestry and population history of red wolves. In particular, high-resolution whole-genome data will be needed to determine with high confidence whether the historical red wolf lineage is distinct from other wolf lineages in North America and to screen for the occurrence and timing of possible hybridization among lineages.

3-3: To discern the taxonomic identity and relationships of contemporary and late 20th century red wolf populations with each other and with earlier canids, it will be necessary to have genomic data from individuals that lived prior to the coyote expansion to the eastern United States. In addition, genomic data from other canids that lived along the proposed range boundaries and from further afield, across a range of time periods, will be needed to provide a data set for comparison.

Genomic Analysis of Extant Canids

The examination of the evolutionary relationships among ancient red wolves, the extant managed red wolf population in North Carolina, and the unidentified canid populations in southern Louisiana will require placing these populations in the context of genomic data from a broader continuum of modern canid populations. Different types of genomic data differ in their power to address questions about lineage continuity among contemporary and historical red wolf populations.

Conclusions

4-1: In tracing the genetic history of the managed red wolf population in North Carolina and the Gulf Coast canid population, the use of whole-genome sequencing methods on modern fresh tissue samples will provide the most informative data for assessing both genetic continuity between populations over space and time and historical admixture with other North American canids.

4-2: Although expensive to produce and computationally intensive to analyze, whole-genome data will provide the best chance to identify introgressed genomic fragments (i.e., ancestry haplotypes) and to estimate the time scale at which gene flow occurred.

Recommendation: The committee recommends that new whole-genome sequence data be built upon the existing data with a carefully constructed set of contemporary samples including all North American canid genetic lineages and the full geographic range of all North American canids (red wolf, gray wolf, coyote, and domestic dog).

OVERARCHING RECOMMENDATIONS

Beyond the specific findings, conclusions, and recommendations about the types of data and analyses best suited to address the five research questions, this report recommends that the USFWS adopt the research strategy and support it via funding. The USFWS should be open-minded about refining the strategy as initial results emerge and should work with the research community, including professional societies, to create opportunities for larger community involvement in the synthesis and dissemination of research results.

COMMITTEE ON A RESEARCH STRATEGY TO EXAMINE THE TAXONOMY OF THE RED WOLF

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For More Information . . . This Consensus Study Report Highlights was prepared by the National Academies of Sciences, Engineering, and Medicine based on the Consensus Study Report *A Research Strategy to Examine the Taxonomy of the Red Wolf* (2020). The study was supported by the National Academy of Sciences and U.S. Fish and Wildlife Service. Any opinions, findings, conclusions, or recommendations expressed in this publication do not necessarily reflect the views of any organization or agency that provided support for the project. Copies of the Consensus Study Report are available from the National Academies Press, (800) 624-6242; <http://www.nap.edu> or via the Board on Life Sciences web page at <http://www.nationalacademies.org/bls>.

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