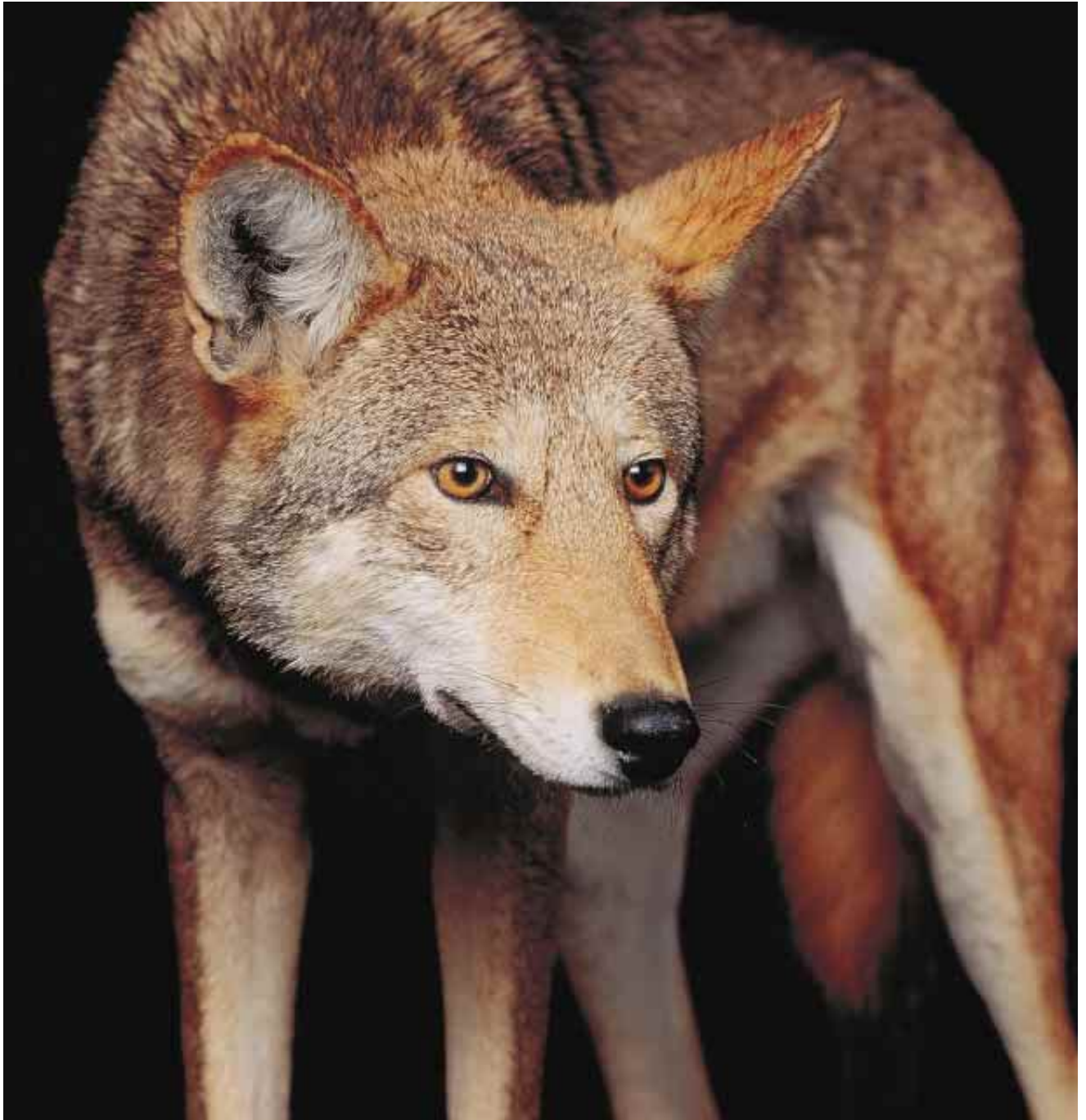


The Problematic Red Wolf

Is the red wolf a species or a long-established hybrid of the gray wolf and the coyote? Such distinctions may affect ongoing efforts to save a variety of endangered species

by Robert K. Wayne and John L. Gittleman



© DAVID LIITTSCHWAGER AND SUSAN MIDDLETON

In the 18th and 19th centuries, residents of the American Southeast often described encounters with creatures they called red wolves: long-legged, cinnamon-colored animals that came to be named *Canis rufus*. By the early 1970s, however, hunting of animals that preyed on livestock and the conversion of woodlands to crop fields had caused the once widespread red wolf population to shrink to a single group in eastern Texas and southwestern Louisiana. By the late 1970s even that cluster had disappeared.

Fortunately, quick action by the U.S. Fish and Wildlife Service—proceeding under the authority of the Endangered Species Act—established a captive population before the wolves vanished entirely. The task was not easy: many of the remaining animals had mated with an abundant close relative, the coyote (*C. latrans*), producing hybrid offspring. But scientists were able to find 14 red wolves apparently having no trace of coyote ancestry, and they succeeded in establishing a breeding program with those animals. As a result, several hundred red wolves live in captivity today, providing a source for ongoing reintroduction of wolves into such places as the Great Smoky Mountains National Park in Tennessee. The preservation effort has been successful beyond expectation and serves as a model for how to rescue an endangered species from the brink of extinction.

Nevertheless, saving a species through captive breeding and reintroduction is expensive. The projected budget of the Fish and Wildlife Service for field study and maintaining captive breeding facilities for red wolves over the next five years is about \$4.5 million. The cost of preserving the red wolf and other endangered species has recently focused new interest on the criteria biologists should use to decide which animals should receive the greatest attention.

The issue remains open because the Endangered Species Act extends to all manner of endangered groups equally, regardless of whether they are species, subspecies (populations displaying minor differences from one another) or, under certain circumstances, hybrids, which result from the cross of two species. Yet in the face of growing numbers of threatened populations and declining resources, conservation biologists are forced to practice a form of triage:

RED WOLF, protected by the Endangered Species Act, may not be a distinct species after all. New genetic evidence indicates the red wolf could be a hybrid of the coyote and the gray wolf.

they must determine which groups among the many endangered ones should be saved and how much effort to devote to each organism.

Some decisions are straightforward. The giant panda, a symbol of the conservation movement, is nearly extinct and is the only living representative of an entire subfamily of bears. Few would doubt that it is more deserving of preservation than a minor subspecies of an otherwise well-populated species. But many cases are less clear-cut. In those instances, biologists generally concentrate more on the last representatives of a species than on a dwindling subspecies or ephemeral hybrid population. Yet, as our research into the proper taxonomic classification of the red wolf demonstrates, determining whether a population constitutes a separate species can often be problematic.

What Is a Species?

Deciding whether a given population constitutes a species can be difficult in part because there is no single accepted definition of the term. Years ago evolutionary biologist Ernst W. Mayr, propounding what is called the biological species concept, proposed that the definition be based on reproductive compatibility. Specifically, he considered a species to be a group of animals that can mate with one another to produce fertile offspring but cannot mate successfully with members of a different group.

Yet this idea can be too restrictive. First, mating between species (hybridization), as often occurs in the canine family, is quite common in nature. Second, in some instances, the differences between two populations might not prevent them from interbreeding, even though they are rather dissimilar in traits unrelated to reproduction; one might question whether such disparate groups should be considered a single species. A third problem with the biological species concept is that investigators cannot always determine whether two groups that live in different places are capable of interbreeding.

When the biological species concept is difficult to apply, some investigators use phenotype, an organism's observable characteristics, as a surrogate. Two groups that have evolved separately are likely to display measurable differences in many of their traits, such as the size of the skull or the width of the teeth. If the distribution of measurements from one group does not overlap those of the other group, the two groups might be considered distinct species. Another widely discussed idea designates a spe-

cies based on the presence of some unique characteristic not found in any other closely related organism—for example, the upright posture of humans—or a distinguishing sequence of nucleotides (DNA building blocks) in a gene.

Proving that the red wolf fits any of these descriptions has been extremely challenging. For instance, the red wolf is not a species by Mayr's definition, because it can breed extensively with the coyote and the gray wolf (*C. lupus*). And efforts to classify the red wolf based on its phenotypic traits have yielded ambiguous results. John James Audubon and John Bachman, who described the red wolf in their classic 1851 book, *Viviparous Quadrupeds of North America*, had difficulty distinguishing the red wolf from the physically similar coyote and gray wolf. Modern researchers looking at phenotypic traits have variously concluded that the red wolf is a subspecies of the gray wolf, a hybrid of the coyote and the gray wolf, and a full-fledged species.

The strongest evidence that red wolves constitute a separate species comes from Ronald M. Nowak of the Fish and Wildlife Service. In 1979 he studied 15 cranial and dental characteristics of domestic dogs, red wolves, coyotes and gray wolves. He found that in size and structure, skulls of red wolves collected before 1930 fell roughly midway between those of the coyote and the gray wolf and could indeed be distinguished from skulls of those two species. Additional analysis of the fossil record in North America led him to conclude that the red wolf first appeared about one million years ago, early in the Pleistocene period and thus before the emergence of the modern coyote and the gray wolf. Because of the red wolf's apparent ancient origin, he surmised it was the ancestor of the other two species and had a long and distinct evolutionary history.

Nowak also determined that red wolf skulls collected after 1930 more closely resembled coyote skulls. He reasoned that beginning in the 1930s red wolves became rare as a consequence of human activities, including destruction of the animals' habitat and establishment of predator-control programs. The remaining red wolves then began to breed with coyotes, so that a red wolf-coyote hybrid population emerged.

Our study has led us to conclude, however, that measurements of skulls are of dubious help in determining the species status and evolutionary history of the red wolf. We now maintain that, in fact, Nowak's cranial data are consistent with the possibility that the red wolf is a hybrid of the gray wolf and the

coyote. If those two species crossbred, one would expect to find precisely the intermediate phenotype Nowak detected. Indeed, coyotes and gray wolves have been observed to mate in certain parts of Canada, and the resulting animals had skull dimensions resembling those Nowak found for the red wolf.

Diagnostic Genes

We came to our conclusion after performing extensive genetic analyses that we originally anticipated would prove red wolves constitute their own species. Dissatisfied with the ambiguity of the existing data, in 1989 we sought evidence that the third definition of species would apply: the red wolf would

DNA—a property that is useful for tracing an organism's genetic history.

Certain species, particularly those that have appeared relatively recently on an evolutionary time scale, cannot easily be distinguished solely by differences in their nuclear DNA. But analyses of selected sections of mitochondrial DNA often reveal changes that can discriminate one species from another. For example, the sequences of nucleotides in the mitochondrial DNA of coyotes and gray wolves differ by about 4 percent, whereas the sequence of African black-backed jackals (*C. mesomelas*) differs from those two species by about 8 percent. This latter difference is comparable to that separating humans and apes, indicating that the

characteristic of coyotes and gray wolves but no unique red wolf pattern.

The discovery of coyote and gray wolf sequences in DNA from red wolves was unexpected and is one of the findings that makes us suspect the red wolf is a hybrid. We had an idea of how to explain this odd result because we had previously encountered a similar phenomenon. When analyzing segments of mitochondrial DNA from gray wolves in Minnesota and eastern Canada, we noted that many of the gray wolves carried coyote mitochondrial DNA.

We speculated that this situation arose because coyotes had entered Minnesota and eastern Canada during the previous 90 years, expanding their range as the number of gray wolves declined in response to predator-control programs and altered habitats. The thinly dispersed gray wolves then mated with the numerous and widespread coyotes, producing hybrid offspring. When male



JOEL SARTORE National Geographic Image Collection

RED WOLF



RED WOLF, COYOTE AND GRAY WOLF can be hard to tell apart by appearance alone. But the red wolf (left) is generally larger than the coyote (top, center),

possess some unique trait—perhaps an unusual stretch of DNA—that marked it as an entity distinct from the coyote and the gray wolf. We therefore examined segments of DNA from both the nucleus and mitochondria of cells obtained from the three putative species.

Nuclear DNA differs from mitochondrial DNA in a few ways. Nuclear DNA contains the multitude of genes that give rise to the physical and behavioral traits of an organism; it is inherited from both parents, so that each individual receives one set of genes from the mother and a matching set from the father. Mitochondrial DNA, in contrast, includes relatively few genes, which primarily code for proteins the mitochondria need in order to produce energy for cells. Mitochondrial DNA is inherited only through the mother. Further, its nucleotide sequence changes, or evolves, faster than that in nuclear

jackals are distant cousins of the more closely related gray wolves and coyotes, but all three should be considered distinct species.

We began our genetic studies by comparing segments of mitochondrial DNA from red wolves currently in the breeding program with the corresponding segments in coyotes and gray wolves. We found nothing to distinguish the red wolf from the other two species. For example, one region of mitochondrial DNA from red wolves that we examined was virtually identical to the corresponding region from coyotes living in Louisiana. We subsequently examined mitochondrial samples saved from red wolves, coyotes and their hybrids captured in east Texas between 1974 and 1976, during the establishment of the captive breeding program. (Gray wolves had disappeared from Texas by then.) We found gene sequences char-

gray wolves mated with female coyotes, their hybrid offspring inherited only coyote mitochondrial DNA, which, as noted, is transmitted solely along maternal lines. But these early hybrids inherited half of their nuclear DNA from their coyote mothers and half from their gray wolf fathers. In consequence, they looked like an intermediate between a coyote and a gray wolf.

When these hybrids mated with pure gray wolves, in a phenomenon called backcrossing, the resulting offspring appeared slightly more wolflike than their parents. As subsequent generations of hybrids continued to mate with gray wolves, traces of the original coyote nuclear DNA disappeared, and the progeny increasingly came to resemble gray wolves. But all hybrids that could trace their heritage back to the original gray wolf-coyote cross through female ancestors retained the coyote mitochondrial DNA from the original coyote mother.



E. R. DEGGINGER, Bruce Coleman Inc.

Recalling this scenario, we began to wonder whether the original red wolves in the captive breeding program were actually recent descendants of red wolf-coyote hybrids and possibly even descendants of gray wolf-coyote hybrids. But perhaps early generations—born before frequent crossbreeding began to occur—were pure red wolves. We examined this possibility by going back further in time, to before the 1930s, the era when, according to Nowak, red wolves supposedly had not yet begun to crossbreed to any significant extent. If the red wolf was once a distinct species that began to crossbreed only in the 1930s, historical samples should reveal a genetic sequence unique to the red wolf. Alternatively, if the red wolf resulted from breeding between gray wolves and coyotes, older specimens should also show a mix of coyote and gray wolf DNA, just as the modern samples did.

Thanks to the recently developed

polymerase chain reaction, which can produce large numbers of copies of selected bits of DNA [see “The Unusual Origin of the Polymerase Chain Reaction,” by Kary B. Mullis; *SCIENTIFIC AMERICAN*, April 1990] and to the Smithsonian Institution’s fur vault, we were able to examine sections of mitochondrial DNA from the skins of six red wolves that died before 1930. To our surprise, we once more failed to find diagnosable red wolf DNA sequences different from those of the coyote or the gray wolf. On the basis of such findings, we deduced that the red wolf may not be a unique species.

Our views were not well accepted by the Fish and Wildlife Service, whose researchers argued that their evidence still supported the theory that the red wolf was a species and indeed an ancestor of the gray wolf. Although some of the resistance to our hypothesis may have been motivated by politics—the

COYOTE

GRAY WOLF



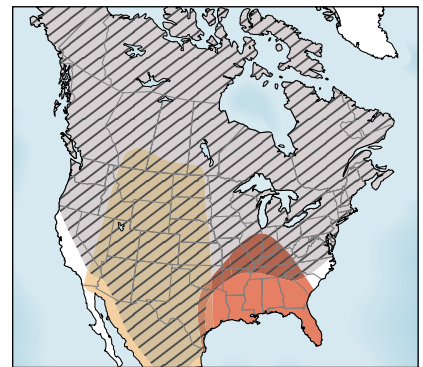
TOMIO NARASHIMA

and its fur can be tawnier. The gray wolf (*below*) is typically the largest; its coloring varies a great deal. Measurements of skulls have revealed that the dimensions of the red wolf’s skull fall in between those of the coyote’s and the gray wolf’s skulls.

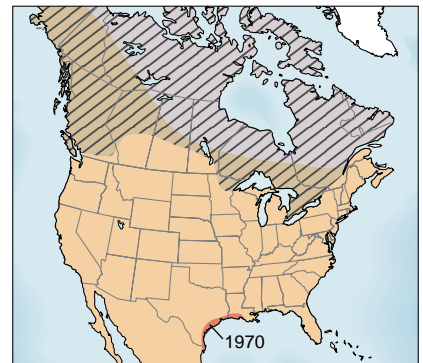


KENNETH W. FINK, Bruce Coleman Inc.

HISTORICAL



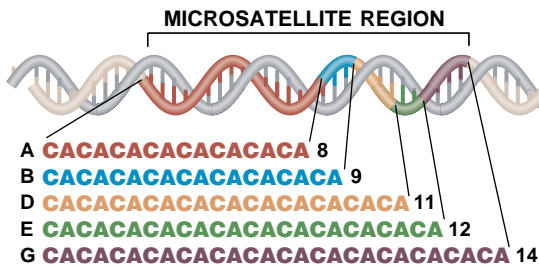
MODERN



MICHAEL GOODMAN

▨ GRAY WOLF ■ COYOTE ■ RED WOLF

OVERLAPPING RANGES of the red wolf, coyote and gray wolf, deduced from historical records (*top*), may have led to considerable crossbreeding among the groups in the 18th and 19th centuries. Human changes to the environment have significantly diminished modern ranges (*bottom*) of the red wolf (shown shortly before protection efforts began) and the gray wolf. But the coyote’s range has expanded.



Clues from Nuclear DNA

Distinctive sections of nuclear DNA, known as microsatellites, have provided strong evidence that red wolves do not constitute a unique species. Microsatellites consist of short, repeating sequences of nucleotides; a single microsatellite may consist of, say, eight repeats of a unit composed of two nucleotides—cytidylate (C) and adenylate (A). If at some given DNA site, or locus, a population possesses a microsatellite that is shorter or longer than the microsatellites found at the same locus in other animals,

the divergence can be a sign that the population in question belongs to a separate species.

In a study involving several hundred red wolves, coyotes and gray wolves, the authors catalogued the number of repeating CA units that occurred in 10 microsatellite loci. For example, at one selected site, they identified five microsatellite variants, labeled A, B, D, E and G (above). Microsatellites in this region from red wolves and coyotes were always of the A or B types (below), but those from gray wolves included additional, unique versions. Similar analyses at the nine other loci demonstrated that only coyotes and gray wolves possess unique microsatellites; all microsatellites carried by red wolves can be found in either coyotes or gray wolves. This finding indicates red wolves have not diverged enough genetically from gray wolves and coyotes to be considered a separate species.

RED WOLF	COYOTE	GRAY WOLF
A CACACACACACACA	A CACACACACACACA	A CACACACACACACACA
B CACACACACACACACA	B CACACACACACACACA	B CACACACACACACACACA
		D CACACACACACACACACACA
		E CACACACACACACACACACACA
		G CACACACACACACACACACACACA

MICHAEL GOODMAN

red wolf project was a flagship program for endangered species preservation—complaints that our data seemed inconclusive were fair. After all, we had been able to examine one type of DNA from only six wolves that lived before 1930; perhaps we had simply missed the distinct genetic trait.

To expand our studies, we turned to nuclear DNA, comparing selected segments in red wolves, coyotes and gray wolves. We made use of a discovery by Diethard Tautz, now at the University of Munich, who found that some short, simple and common sequences of nucleotides repeat themselves at particular sites, known as microsatellites, in nuclear DNA and that the number of repeats in these microsatellites can vary from species to species. The length of the final unit can thus serve as an indicator, or marker, that an animal belongs to a particular species.

Along with several colleagues, we studied microsatellite DNA from several hundred contemporary red wolves, coyotes and gray wolves. Again we found no evidence that red wolves form a unique species. Of course, the similarity between red wolves, coyotes and gray wolves may have been the result of recent crossbreeding, and so we sought a historical perspective once more.

Using pre-1930s skins stored at the Smithsonian's fur vault, our colleagues Michael S. Roy of the Institute of Zoology in London and Deborah Smith of the University of California at Los Angeles

examined 10 different microsatellite regions from 16 skins identified by others, including Nowak, as deriving from red wolves. Still, we detected no sign that the DNA of red wolves was clearly distinct from that of either gray wolves or coyotes living in the same period.

Hybrid Hypothesis

The collected findings from both modern and historical wolves and coyotes has led us to the following hypothesis, which is accepted by many investigators but is still disputed by some. Sometime in the recent past, crossbreeding between the gray wolf and the coyote began to occur. Our data do not allow us to pinpoint exactly when the crossbreeding began, but we speculate it might have been during the early years of European migration. As European settlements expanded in the 1700s, the conversion of woodlands for agriculture and the killing of gray wolves produced conditions similar to those seen recently in Minnesota and eastern Canada. The gray wolf population dwindled, leading the survivors to mate more frequently with coyotes. The resulting hybrid animals, gray wolf-coyote crossbreeds, were of intermediate size and had characteristics that were later classified as attributes of the red wolf. In subsequent years, as gray wolves disappeared in the southern U.S., gray wolf-coyote hybrids mated with coyotes more frequently and became more coy-

otelike, a trend we believe is reflected in Nowak's observation that the skulls of red wolves became more coyotelike after 1930.

Furthermore, our study suggests crossbreeding among the three supposed species was well advanced by the turn of the 20th century. Red wolves captured before 1930 are very similar genetically to those captured in the 1970s and used to start the captive breeding program. This result is good news for the breeding program in that it means reestablishment of a wild population of red wolves with individuals from captivity has indeed preserved the genetic makeup possessed by the wild red wolf. News of the genetic similarities among red wolves, coyotes and gray wolves, however, is disappointing to those who believed the program was protecting a long distinct species. Because crossbreeding among the three groups continued during various periods in their history and throughout their range, we feel the red wolf never truly developed into a separate species.

If biologists focused solely on species status as the guide for determining whether an endangered group should be protected, such findings could be the death knell for the red wolf. Yet there are compelling reasons protection should continue. Captive breeding of the red wolf may have preserved unique physical characteristics or behaviors not revealed in the studies done so far. More important, such qualities may not

be easily regenerated through the mating of modern gray wolves and coyotes. Some investigators have suggested that the red wolf arose from hybridization between the coyote and a southern subspecies of gray wolf that is now extinct. Consequently, a cross of the modern eastern gray wolf with the coyote would produce an animal similar in many ways but potentially different in behavior, fur coloring or other characteristics. Thus, the red wolf may be the last, albeit impure, repository of genes from a now extinct gray wolf subspecies and as such should certainly be preserved. Additionally, ecological concerns need to be considered. Red wolves, even if they are hybrids of coyotes and gray wolves, are once again important predators of many wild animals, including rodents, rabbits and deer, in the south central U.S. The red wolf may also play a role in some habitats that its smaller kin, the coyote, cannot entirely fill.

Protecting the Red Wolf

To protect the genetic makeup of today's red wolves, conservation programs must strive to keep red wolves from breeding with coyotes, which are abundant throughout the planned reintroduction sites. Hybridization might be reduced if several red wolf packs were placed in an area simultaneously; large groups of red wolves stand a better chance of excluding coyotes from their habitat than do small groups. Because coyotes are ubiquitous in the American Southeast, it is hard to find regions where they are sparse. Removal or extermination of coyotes might alleviate this problem, but that solution implies a measure of red wolf chauvinism.

The case of the red wolf suggests to us that in deciding which animals to protect most assiduously, biologists must look beyond the taxonomic classification of an endangered hybrid or



FUR VAULT at the Smithsonian Institution provided skins of red wolves (at right in inset), gray wolves (at left in inset) and coyotes (above) for genetic analyses of animals that died before 1930.

ALEX WEBB Magnum

subspecies; they should also take into account its unique function in an ecosystem or possession of special traits that cannot be reproduced by crossbreeding of contemporary representatives from the parental species.

The fact that the red wolf may be a repository of genes from an extinct subspecies of gray wolf is just one example of this principle. In another example, a hybrid species may result from a single initial crossing of two species and the subsequent evolution of the offspring in isolation from their ancestral species; consequently, those descendants will have unique traits and, in our opinion, deserve protection as a separate species. Many plant species, for instance, arise in this way.

Such situations must be distinguished from those in which crossbreeding occurs frequently over a wide geographic area and may reflect human changes to the surroundings. In these cases, the hybrids may not possess unique traits. Even these hybrid groups, however, should not automatically be excluded from conservation efforts; they should

be examined on an individual basis.

Human changes to the environment can sometimes bring together two populations that proceed to crossbreed; we believe the resultant hybrids from such unnatural matches generally should not be protected if their numbers start to fall. But hybrids that have arisen from populations that overlap naturally deserve special consideration as integral parts of their ecosystems.

Molecular analysis of DNA can provide insight into the history of endangered or rare species. Moreover, such an approach can provide a yardstick with which one can measure the differences between populations. For the red wolf, we feel the problem of classification was largely resolved by DNA testing. But the issues raised by identifying the red wolf as a hybrid species highlight the difficulties of determining how to rank endangered species, subspecies and hybrids in protection efforts. Those challenges need closer attention if conservation biologists are to make the best possible choices for the preservation of our natural heritage.

The Authors

ROBERT K. WAYNE and JOHN L. GITTLEMAN are both carnivore biologists who share a concern for problems associated with the red wolf reintroduction program. Wayne, a professor at the University of California, Los Angeles, specializes in molecular population genetics, systematics and conservation. He is particularly interested in the consequences of hybridization between reintroduced red wolves and resident coyote populations. Gittleman is a professor at the University of Tennessee, where he focuses on carnivore ecology and evolution. He is investigating the ecological and evolutionary effects of carnivore reintroduction programs.

Further Reading

- CRYING WOLF IN NORTH AMERICA. John L. Gittleman and Stuart L. Pimm in *Nature*, Vol. 351, pages 524-525; June 13, 1991.
- MITOCHONDRIAL DNA ANALYSIS IMPLYING EXTENSIVE HYBRIDIZATION OF THE ENDANGERED RED WOLF *CANIS RUFUS*. Robert K. Wayne and Susan M. Jenks in *Nature*, Vol. 351, pages 565-568; June 13, 1991.
- CONSERVATION BIOLOGY. Various articles on the red wolf hybrid controversy. Vol. 6, No. 4, pages 590-603; December 1992.
- MOLECULAR EVOLUTION OF THE DOG FAMILY. Robert K. Wayne in *Trends in Genetics*, Vol. 9, No. 6, pages 218-224; June 1993.
- PATTERNS OF DIFFERENTIATION AND HYBRIDIZATION IN NORTH AMERICAN WOLF-LIKE CANIDS REVEALED BY ANALYSIS OF MICROSATELLITE LOCI. Michael S. Roy, Eli Geffen, Deborah Smith, Elaine A. Ostrander and Robert K. Wayne in *Molecular Biology and Evolution*, Vol. 11, No. 4, pages 553-570; July 1994.