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# Response to Wayne, Nowak, and Phillips and Henry: Use of Molecular Characters in Conservation Biology

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In our recent letter (Dowling et al. 1992), we stated our concerns over two interrelated issues: (1) the use of genetic (i.e., molecular) characters in identifying species and their hybrids, and (2) the role of hybridization in evolution. The letter was stimulated by Wayne and Jenks' (1991) analysis of mitochondrial DNA (mtDNA) variation in the red wolf, *Canis rufus*, and subsequent interpretations presented in both the professional (e.g., Gittleman & Pimm 1991) and popular literature (e.g., Rennie 1991). Although consequences for conservation of the red wolf were addressed in those papers, our letter was focused more on the broader implications for conservation of endangered taxa.

In this issue, Nowak (1992) and Phillips and Henry (1992) have presented views generally in accord with ours, providing additional behavioral, ecological, morphological, and paleontological evidence supporting distinctiveness of the red wolf and its continued protection under the United States' Endangered Species Act. Nowak concludes that the red wolf is (1) neither the

product of ongoing or recent hybridization between coyote (*C. latrans*) and gray wolf (*C. lupus*) nor, (2) a taxon of hybrid origin, but (3) represents a descendant from an intermediate stage in wolf evolution. We concur with his first conclusion but await further data to evaluate the second and third. The only current evidence inconsistent with the last alternative is the lack of distinctiveness among mtDNA sequences from red and gray wolves. Wayne and Jenks (1991) presumably expected differences as predicted from the "standard" rate of mtDNA evolution based on the calibration from primates (Hillis & Moritz 1990). Explanation of such a discordance requires only a reduction in rate of mtDNA evolution in wolves relative to primates, and such rate variation has been documented for a variety of organisms (e.g., Avise et al. 1992; Martin et al. 1992). The most appropriate test of an hybrid origin will be provided by analysis of codominant, single locus, nuclear gene markers alluded to by Phillips and Henry (1992).

Also in this issue, Wayne (1992) takes exception to

our views and presents further interpretation of the red wolf situation, misconstruing some of our ideas and attributing to us viewpoints we neither presented nor endorse. His discussions and criticisms focus on three aspects of our letter: (1) limitations of available molecular genetic data in evaluating hybrid origin and specific status of the red wolf, (2) protection of hybrids under the U.S. Endangered Species Act, and (3) failure to consider the debate over species concepts. Given the apparent misinterpretation and the importance of these issues to conservation biology, we are compelled to respond and discuss our thoughts in further detail.

Firstly, Wayne indicates that we believe molecular data cannot be used to refute the specific status of a taxon. This is not the case. In our original letter, we simply expressed concern over a general perception that molecular characters are "better" (i.e., Gittleman & Pimm 1991) than other types of data for identifying species, and argued that all available information (rather than a subset) should be applied to the question at hand (e.g., Buth 1984; Hillis 1987; Lande 1991). To our knowledge, this analytical approach has not yet been applied in taxonomic evaluation of the red wolf.

Consider just two sets of data for the red wolf discussed by Wayne (1992): (1) cranial measurements, and, (2) mtDNA restriction site/sequence data. Multivariate analysis of cranial measures identified the red wolf as an entity distinct from both coyote and gray wolf (Figure 1 in Nowak 1992 and Wayne 1992). Wayne chooses to ignore this morphological information (from Nowak 1979) because it has not been analyzed cladistically, and instead bases his evaluation solely on information from mtDNA. MtDNAs from a sample of red wolves were virtually indistinguishable from those in samples of coyotes and gray wolves. Coyote mtDNA is believed to have been introduced by recent hybridization (reviewed in Nowak 1992 and Phillips & Henry 1992). Again (see above), the point of dispute is the limited divergence between red and gray wolf mtDNAs.

Following a perplexing treatment of concepts relative to species, subspecies, and populations, Wayne then seems to conclude that the red wolf does not deserve taxonomic recognition: "Although other canid species we have examined can be defined under the phylogenetic species concept by mtDNA analysis, the red wolf as a separate species cannot because we find no unique mtDNA restriction site or nucleotide substitutions that unite all red wolves in a single clade (Wayne & Jenks 1991)" and later "Furthermore, recent discussions of subspecies require that they be defined by uniquely shared traits (Avice & Ball 1990; Dizon et al. 1992). The mix of gray wolf genotypes found in red wolves indicates they are not a gray wolf subspecies under this definition."

This treatment is confusing and deeply flawed. Firstly, the phylogeny of a single gene (or linked group of genes

such as found in mtDNA) may differ from that of the species in which the gene occurs due to random sorting of inherited polymorphisms among evolving lineages (Pamilo & Nei 1988). This phenomenon is known to confound phylogenetic analysis of mtDNA data (Neigel & Avice 1986). By ignoring the morphological data, Wayne thus bases his decision solely on characters representing a small fraction of the genome. Given the lack of diagnostic mtDNA characters, it is essential to evaluate variation in additional characters to resolve the issue. Secondly, many morphologically distinctive taxa exhibit little or no allozymic or mtDNA divergence (e.g., pupfishes [genus *Cyprinodon*], Turner 1974; Echelle & Dowling 1992), while others are morphologically indistinguishable but highly divergent for molecular characters (e.g., plethodontid salamanders, reviewed in Larson 1989). Pupfish species are just as valid as those of plethodontids, but the two groups have diverged at different sets of loci.

We neither rejected nor accepted the hypothesis of hybrid origin for red wolf, but submitted, and do so again, that the available data are insufficient to distinguish between competing hypotheses. Morphological intermediacy of a taxon is neither necessary nor sufficient to demonstrate hybrid origin. In multivariate analyses of more than two taxa, some will, by necessity, be morphologically intermediate to others (Wilson 1992). In addition, hybrid taxa are not always morphologically intermediate to their progenitors (e.g., DeMarais et al. 1992), and even  $F_1$  hybrids are not always intermediate to their parental forms (e.g., Neff & Smith 1978; Dowling et al. 1989; Meagher & Dowling 1991). Inference of hybrid origin from mtDNA data alone is suspect. Neigel and Avice (1986) specifically cautioned against using mtDNA for inferring hybridization among closely related taxa because random extinction of mtDNA lineages can cause discordance between organismal and mtDNA phylogenies. A true test of hybrid origin for red wolf requires joint consideration of additional characters, a view also presented by O'Brien and Mayr (1991a).

Secondly, Wayne (1992) disagrees with our views on treatment of hybrids under the U.S. Endangered Species Act. He is understandably vague and indecisive in his discussion of the origin of red wolves (as were we, since more data are required for an objective decision). Unfortunately, he has similar difficulty discussing our views of the potential evolutionary significance of hybridization and the advisability for protection of imperiled hybrid taxa. His hesitation appears to stem from confusion over or apprehension to address the difference between hybrid taxa and hybrids (see also Wayne & Jenks 1991).

Taxa of hybrid origin are self-perpetuating groups of individuals of mixed ancestry following an evolutionary trajectory independent from that of their progenitors. The U.S. Endangered Species Act applies to recognized

entities or populations deemed important enough in some way to preserve, and does not address their origin (Dowling et al. 1992). Genetically distinct taxa of hybrid origin must not be denied protection due to mixed ancestry. If the red wolf proves to represent an historically stable entity generated by long past (maybe even ancient) hybridization between gray wolf and coyote, then it is a taxon of hybrid origin that clearly should be protected.

We define a hybrid as an individual of mixed ancestry tracing its origin to the mating of a single male and a single female of different taxa. Hybrid individuals may or may not give rise to a hybrid taxon, and the point at which such occurs is unknown. Thus, if a red wolf phenotype originated and was perpetuated through hybridization between gray wolf and coyote following colonization by Europeans, it would not be a hybrid taxon nor merit protection until it became self-perpetuating and achieved an independent evolutionary track.

Wayne's discussion further includes the disturbing inference that we might advocate preservation of domestic dog-wild canid hybrid individuals at the "expense" of endangered species. We do not do so now, nor did we originally suggest such action. We stated: "This is not to say that hybridization is always an important evolutionary force or that it is never detrimental, but does indicate that all circumstances must be carefully considered when evaluating the nature and impact of introgression." Like many others (e.g., Anderson 1949; Lewontin & Birch 1966; Short 1972; Grant 1981), we recognize that hybridization can play an important role in evolution of plants and animals. Our approach is conservative. Each instance of hybridization *must* be evaluated separately.

Furthermore, when endangered taxa (including those of hybrid origin) have hybridized with related taxa due to human activity, conservation strategies must take into account the consequences of management actions as well as introgression (Allendorf & Leary 1988, Dowling & Childs 1992). When sufficient numbers persist to preserve genetic integrity, hybrids and conditions promoting hybridization may be eliminated to prevent further introgression. If, however, a taxon is rare, it may be necessary to protect all individuals, even if introgressed, to preserve remaining genetic diversity (Echelle 1991). Whether or not the red wolf is a taxon originating through hybridization or descended from a coyote-like ancestor (Nowak 1992), we will in fact argue strongly for preservation of hybrids of any kind if no other means exists to prevent extinction of a lineage. For another example, introduction of South American cougars to Florida has resulted in introgression of their mtDNA into the endangered Florida panther (*Felis concolor coryi*). We concur with O'Brien and Mayr (1991b) that the rarity and distinctiveness of Florida panther requires

continued protection be afforded these individuals, despite introgression.

Lastly, despite Wayne's assertions to the contrary, we addressed the debate over species concepts in our first letter, which applies equally well here (brackets ours): "One must realize that the legislation [referring to the U.S. Endangered Species Act] was designed to protect distinctive forms (not just species) in danger of extinction, irrespective of mode of origin, making the species concept one chooses to apply irrelevant." Wayne indicates that systematists would be irresponsible to allow the ESA to influence taxonomic decisions. We agree, but submit the converse as also valid. Conservation biologists must not allow long-standing disagreements over species definitions to interfere with decisions to conserve evolutionary entities. Conservation biologists should indeed be concerned with species concepts, but arguments over their application should not confound or otherwise deter conservation efforts. A goal of conservation biology is to preserve the diversity of life, requiring consideration of evolutionary potential as well as taxonomy. The red wolf is a taxon morphologically distinct from coyote and gray wolf, requiring recognition. We leave it to mammalian systematists to decide its taxonomic rank. As conservation biologists, however, we must continue to protect entities like the red wolf to preserve the evolutionary potential of each species.

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