



29 June 2011

Public Comments Processing
Attention: FWS-R3-ES-2011-0029
Division of Policy and Directives Management
US Fish and Wildlife Service
4401 N. Fairfax Drive
MS 2042-PDM
Arlington, VA 22203

Re: Proposed Rule to Revise the List of Endangered and Threatened Wildlife for the Gray Wolf (*Canis lupus*) in the Eastern United States, Initiation of Status Reviews for the Gray Wolf and Eastern Wolf (*Canis lycaon*)

Dear Sir or Madam:

In reply to the USFWS proposed rule I am writing in support of the delisting of the Western Great Lakes Wolves and for the need to review the conservation status of wolves in the eastern USA. However, it is my professional opinion that it would be a serious mistake to recognize *Canis lycaon* as a distinct species. This taxa does not have strong support and a 'mitochondrial capture' hypotheses explains the genetic pattern found in eastern wolves much better than the 'unique species' hypothesis.

1. Genetic data shows WGL wolves are a distinct population of *Canis lupus*.

I agree with the assessment that Western Great Lakes (WGL) wolves are a Distinct Population Segment (DPS) of the gray wolf (*Canis lupus*). My conclusion is supported by a new study presenting the most detailed genetic study of the region's canids (vonHoldt et al. 2011). This paper used over 48,000 genetic markers (Single Nucleotide Polymorphisms, SNP's) to characterize the genetic makeup of 18 WGL wolves, and compared this with a global sample of *Canis* diversity.

In this study, the WGL wolf samples formed a contiguous population in overall genetic similarity, analogous to other established wolf populations around the world (vonHoldt et al 2011, figure 3). The WGL wolf population was less unique than many other subspecies of wolves, emerging as the 10th most distinct genetic group in this global comparison (vonHoldt et al 2011, figure 4). This is consistent with them being recognized as a DPS, and possibly a subspecies, but certainly not a unique species, as some have argued based on more limited sampling and more limited genetic data (Wilson et al. 2000). By relating the genetic composition of WGL wolves to other *Canis* populations we showed relatively low levels of hybridization:

two separate analyses of the data (Structure analysis, figure 4; Saber analysis, figure 6; vonHoldt et al 2011) indicated a low level of coyote genetic introgression into the WGL wolf gene pool. The saber analysis shows that the WGL wolf population genetic makeup is, on average, 85.1% *C. lupus* and 14.9% *C. latrans* (vonHoldt et al 2011, figure 6). This genetic makeup was relatively consistent across the 18 GLW samples included in this study (variation of $\pm 0.3-0.4\%$, vonHoldt et al 2011, table S5), suggesting the introgression is well mixed in the modern population. Finally, vonHoldt et al (2011) found no genetic signature of a unique species of eastern in the samples from the WGL wolves (see below), or from other eastern wolf populations.

Hybrid populations of animals may have unique genetic signatures in some analyses because they have unique combinations of genes from two different species, not because they have long independent evolutionary origins. This confusing genetic structure requires detailed genetic data to resolve. Given that some low level of hybridization has long been recognized in the WGL area (Lehman et al. 1991), we view earlier conclusions that the population is a unique species with caution, given the limited genetic data it was based upon (8 microsatellites and 238 base pairs of mtDNA with Wilson et al 2000). Subsequent papers with slightly more genetic resolution (e.g. 12-26 microsatellites) have since recognized some unique aspects of the WGL population, but also recognized gene flow with other wolves the east and west (Koblmuller et al. 2009, Wheeldon et al. 2010). The newest, and most detailed, study has more than two orders of magnitude more resolution (>48,000 markers) than previous studies, giving it unparalleled resolution of the genetic patterns of the region (vonHoldt et al 2011). This shows how the hybridization between wolves and coyotes has resulted in complex genetic patterns that led previous lower-resolution genetic studies to reach contradictory conclusions.

Based on this genetic evidence I support the recognition of the WGL wolves as DPS of *Canis lupus*. Given that the population size of this wolf is healthy, and that the states have well written management plans, I support the delisting of this DPS.

2. Disagree with reclassification of eastern wolves from *Canis lupus lycaon* to *Canis lycaon*.

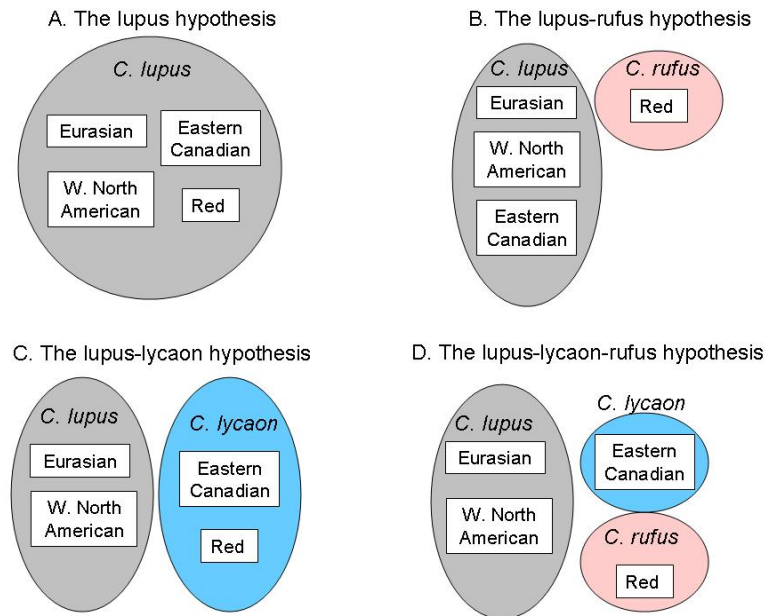
I disagree with the proposal to reclassify *Canis lupus lycaon* to *Canis lycaon* because this is not supported by recent high-resolution genetic studies, and because there are alternative hypotheses to explain the genetic patterns found in previous studies. Here I will first outline the established hypotheses for wolf taxonomy and review the traditional data available to test them, and the limitations of these traditional approach to resolve complex genetic patterns from hybridization. I will then and summarize the results of a new high-resolution genetic study and its support for recognizing eastern wolves as *C. lupus*. Finally, I will introduce an evolutionary hypothesis that may explain inconsistencies in previous findings, and supports the continued recognition of eastern wolves as *C. lupus*.

Taxonomic hypotheses for eastern wolves-

There are four published hypotheses to explain the evolutionary origin of wolves in the eastern USA and Canada (Figure 1). The ‘lupus’ hypothesis posits that all eastern wolves were versions of *Canis lupus* that should be recognized as subspecies or ‘ecotypes’ (Musiani et al. 2007, Koblmuller et al. 2009, vonHoldt et al. 2011). The ‘lupus-rufus’ hypothesis suggests that *C. rufus* evolved independently from *C. lupus* in the Southeastern USA (Nowak 2002). The ‘lupus-lycaon’ hypothesis suggests that all eastern wolves, including *C. rufus*, were actually a large sister-species to *Canis latrans*, evolving independently for 150-300K years (Wilson et al.

2000). In this case, the species name *lycaon* would have priority over *rufus* because it was described first (Schreber 1775). Finally, the fourth, and most complex, hypothesis, which is recommended by the new USFWS proposal, is the ‘lupus-lycaon-rufus’ hypothesis, which recognizes two independently evolving wolves in eastern North America as separate species from the wolves in the rest of the world.

Figure 1. Four hypotheses for the placement of different wolf populations within taxonomic groupings.



Holes in the case for the species status of *Canis lycaon*-

The strongest line of evidence for a *C. lycaon* to be recognized as a distinct species are the two unique mtDNA haplotypes (C1, C13) reported from some eastern wolves but not found in coyotes, and one similar haplotype (C3) found in eastern wolves and one wolf from Manitoba (Wilson et al. 2000, Wheeldon et al. 2010, Rutledge et al. 2010a). These three haplotypes are very similar (2.1% divergence) to a mtDNA haplotype (C2) found in many red wolves, and more similar to coyote mtDNA (3.2% divergence) than to that of *C. lupus* (8% divergence). This result is consistent with the lupus-lycaon or the lupus-lycaon-rufus hypothesis. However, many other haplotypes are also found in eastern wolves, including those of *C. latrans*, *C. lupus*, and others that are similar to these three putative *lycaon* makers (Leonard and Wayne 2008, Koblmuller et al. 2009, Wheeldon et al. 2010, Rutledge et al. 2010a). This shows the hybrid nature of the region’s canids, not only in modern animals, but also historic specimens. Clearly, in the face of this recent and historic hybridization, mtDNA alone can not be used to define *C. lycaon* as a unique species-level taxa.

There have been at least seven studies using microsatellites to evaluate the population genetics of northeastern wolves with nuclear markers (Wilson et al. 2000, Wilson et al. 2009, Koblmuller et al. 2009, Wheeldon and White 2009, Fain et al. 2010, Wheeldon et al. 2010, Rutledge et al. 2010b). All of these have found significant differences between various populations of eastern and western canids and also found evidence for widespread hybridization. The key question is – have any of these studies shown convincing evidence for the existence of a unique species in the east? Showing that a modern population is genetically distinct from others

is not a basis for designating it a species, especially when hybridization is known to have occurred. Unique gene frequencies of a population could be the result of unique combinations of genes from the hybridization of two species, rather than the independent evolution of a unique taxa.

If *C. lycaon* is a valid taxa resulting from 150-300K years of isolated evolution (Wilson et al 2000) then it would have many unique nuclear genetic markers not found in *C. latrans* or *C. lupus*. No one has identified a pure *C. lycaon*, instead finding only hybrids, leaving it a statistical challenge to find the signature of this species introgressed with other species. However, even with hybridization, an introgression analysis such as Structure should also be able to identify those alleles that came from the each of these parental species involved in the hybridization, including the unique aspects of the now introgressed *C. lycaon*. However, none of these microsatellite studies has found such a pattern in eastern wolf samples. Indeed, the strongest of these studies (with 26 microsatellites, Koblmuller et al. 2009, figure 3) showed that Great Lakes Wolves are a unique population of *C. lupus*, and that animals from Ontario include hybrids between western *C. lupus*, Great Lakes *C. lupus*, and eastern coyotes. Fain et al (2010) did not include any Ontario samples, and had confusing results from their Structure analysis of 8 microsatellites, which incorrectly grouped coyotes and dogs together (figure 6, K=2&3), and then arbitrarily identified two separate *C. lupus* components (figure 6, K=5). Wilson et al. (2009) recognized eastern wolves as a hybrid in the text, yet in their Structure analysis (figure 3) they categorized eastern wolves as an unmixed genotype, probably reflecting the inability of their small sample (8 microsatellites) to resolve hybridization.

Thus, despite concluding that modern eastern wolves are hybrids between *C. lycaon*, *C. lupus*, and *C. latrans*, none of the proponents of the *C. lycaon* hypothesis have been able to actually partial out the genetic components from their proposed parental species in a structure analysis of microsatellites. Indeed, their statistical power has not appropriate for this question as their use of 8 microsatellites falls well below the 25 recommended for distinguishing five different populations (Haasl and Payseur 2011). Furthermore, Haasl and Payseur (2011) point out that microsatellites are much worse than SNP's at detecting population structure in the face of hybridization (e.g. the statistical power of a panel of 10 microsatellites to detect 2 populations with F1 hybrids is zero), while others have shown that microsatellites give poor representation of true genomic diversity (Vali et al. 2008). The resolution of microsatellites is made worse when considering species-level (rather than population level) differences due to homoplasy caused by their rapid mutation rate (Haasl and Payseur 2011). Finally, it should be pointed out that the geographic sampling was limited in each of these seven studies that analyzed microsatellites for eastern wolves, with most comparing only within a limited region, and none including the full variety of *Canis* species and subspecies for proper comparison.

A new global genomic evaluation of *Canis* variation-

A new study by vonHoldt et al (2011) addresses all of the shortcomings of the various microsatellite studies that evaluated eastern wolves described above. This study used over 48,000 nuclear SNP markers to analyze 259 animals, making it the largest genetic study of any vertebrate group. We analyzed a global sample, with all major Eurasian and North American populations of wolves and coyotes represented. This study provides unparalleled genetic resolution as well as a global framework with which to compare populations. Thus, if any study was suited to detect the signature of a unique eastern wolf species that has since hybridized with coyotes and wolves, this was it. If *C. lycaon* did originate as an independent offshoot of coyotes,

the Structure analysis of these data should group the populations of coyotes and eastern wolves at lower K, and show a unique coyote-like *C. lycaon* component within eastern wolves at higher K, even if modern animals have since hybridized with other species (and thus also show genetic components of other species in the Structure diagram).

However, the Structure analysis of this paper found no support for a unique eastern wolf species (vonHoldt et al 2011, figure 4). Indeed, the population of Great Lakes Wolves is less distinct than many other *C. lupus* subspecies, and both the WGL and Algonquin wolves were better characterized as hybrids between *C. lupus* and *C. latrans*, as was the red wolf. A more detailed analysis of this hybridization (Saber analysis, figure 6 in vonHoldt et al 2011) was able to show exactly which genes on which chromosome of an individual animal originated from *C. latrans* and *C. lupus*, producing estimates of the overall genetic composition (e.g. Algonquin animals 58.1% *C. lupus* and 41.9% *C. latrans*), as well as the age of hybridization (e.g. 546 to 963 years ago in the WGL). Although this study only included 2 animals from Algonquin park, this is sufficient to test for a species-level taxon, for which the standard is only one voucher specimen. Furthermore, unpublished sensitivity tests show this Structure result was not biased by low sample size.

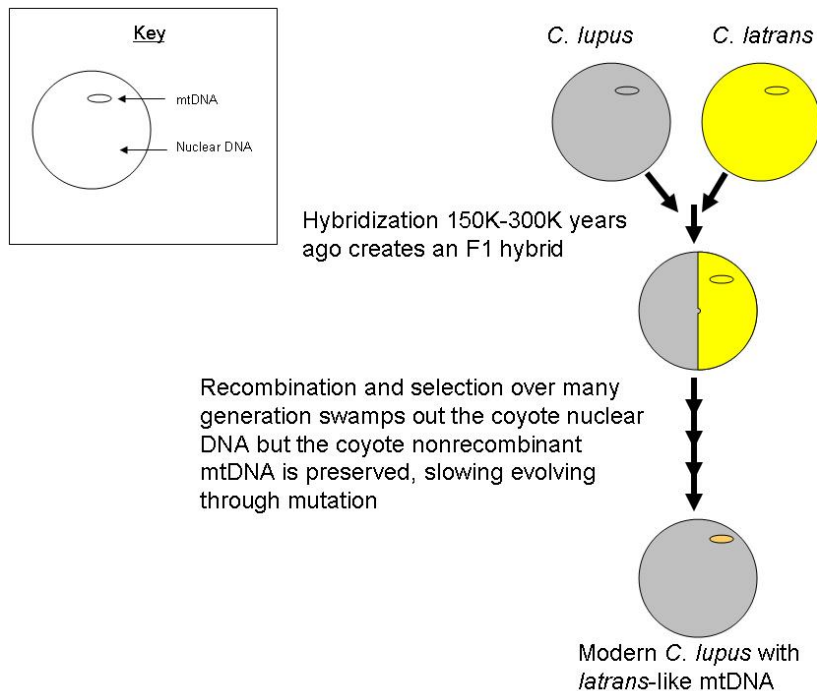
Using the results of this new study, which presents unmatched genetic resolution and taxon sampling, to evaluate the wolf taxonomy hypotheses (Figure 1) gives strong support to the *C. lupus* hypothesis and no support for the recognition of *C. lycaon* or *C. rufus* as unique species resulting from long, isolated, evolution.

Resolving differences in the evolution of nuclear and mitochondrial DNA-

Inconsistencies between the evolutionary pattern shown by nuclear and mtDNA are commonplace in the scientific literature, and a number of mechanisms have been proposed to explain these differences (Zink and Barrowclough 2008). Of these, mitochondrial capture, offers a potential explanation for the differences in the genetic patterns of mtDNA and nuclear DNA in eastern wolves (Figure 2). In the mitochondrial capture hypothesis, a prehistoric hybridization between an eastern population of *C. lupus* and coyote would have transferred a coyote-like mtDNA haplotype into the eastern wolf lineage. In this scenario, the nuclear DNA of the eastern *C. lupus* would have continued to evolve, swamping out most of the coyote DNA through recombination and selection. However, these wolves would retain the coyote nonrecombinant mtDNA, which would continue to evolve through mutation. Based on the estimates of Wilson et al. (2000) this would have taken place 150k-300K years ago.

This is an example of one hypothesis to explain the patterns of genetic variation seen in Eastern wolves, resolving differences between the mtDNA and nuclear DNA. A similar case was recently published showing the introgression of the mtDNA haplotype of a now extinct species of bat in the Caribbean (Larsen et al. 2010), and mitochondrial capture through ancient hybridization has been used to explain the mismatch of nuclear and mtDNA results in various other mammal taxa (Good et al. 2008, Keller et al. 2010). This hypothesis remains to be tested in a formal way with Eastern wolves, but shows that there are more parsimonious explanations for the pattern of genetic variation seen than the distinction of *C. lycaon* as a unique species.

Figure 2. A schematic describing the hypothesis of mitochondrial capture to explain the mismatch between nuclear and mtDNA evolution in eastern wolves.



3. Agree with need for rangewide review of the conservation status of *C. lupus lycaon* in the eastern USA.

I agree with the proposal to initiate a rangewide review of the conservation status of wolves in the eastern USA. Although I disagree on the taxonomy of eastern wolves, I see the need for a review of their conservation based on the presence of suitable habitat and the evidence that some large wolves are already migrating into the area on their own.

Modeling studies have shown that there is substantial suitable wolf habitat in Northern New York and New England that could support a viable population of Wolves (Harrison and Chapin 1998; Mladenoff and Sickley 1998). The distance between surviving Wolf populations in Canada and this suitable habitat in northeastern states is relatively small when compared with potential Wolf dispersal distances (70-230km, Harrison and Chapin 1998). This suggests that wolves could disperse into the area on their own.

Indeed, there have been at least 8 large wolves shot in the northeastern USA between 1984 and 2001 (Kays and Feranec In Press). Each of these animals was substantially larger than eastern coyotes and classified as a wolf (*C. lupus*) or possible wolf hybrid by state and/or federal wildlife officials. In two cases physical conditions suggested the animal had been in captivity, but no evidence of a captive past was noted for the others (USFWS 1993, USFWS 1997, USFWS 2002, USFWS 2004). Isotopic tests later support the conclusion that three of these animals had not been raised in captivity (Kays and Feranec In Press).

In summary, the northeast USA appears to have suitable wolf habitat and a few natural wolf immigrants that could form the start of a naturally recovered population. However, there remain conservation challenges (e.g. coyote hunters, public perception) that should be evaluated in a rangewide review of their status.

Sincerely,

Roland Kays

Literature Cited

- Fain, S., D. Straughan, and B. Taylor. 2010. Genetic outcomes of wolf recovery in the western Great Lakes states. *Conservation Genetics* : 1-19.
- Good, J., S. Hird, N. Reid, J.R. Demboski, S.J. Stepan, T.R. Martin-Nims, and J. Sullivan. 2008. Ancient hybridization and mitochondrial capture between two species of chipmunks. *Molecular Ecology* 17: 1313–1327.
- Haasl, R.J. and B.A. Payseur. 2011. Multi-locus inference of population structure: a comparison between single nucleotide polymorphisms and microsatellites. *Heredity* 106: 158-171.
- Kays, R. and R.S. Feranec. In Press. Using stable carbon isotopes to distinguish wild from captive wolves. *Northeast Naturalist* .
- Keller, C., C. Roos, L.F. Groeneveld, J. Fischer, and D. Zinner. 2010. Introgressive hybridization in southern African baboons shapes patterns of mtDNA variation. 142: 125-136.
- Koblmuller, S., M. Nord, R.K. Wayne, and J.A. Leonard. 2009. Origin and status of the Great Lakes wolf. *Molec. Ecol.* 11: 2313-2326.
- Larsen, P.A., M.R. Marchan-Rivadeneira, and R.J. Baker. 2010. Natural hybridization generates mammalian lineage with species characteristics. *Proceedings of the National Academy of Sciences* .
- Lehman, N.A., K. Eisenhaver, L. Hansen, D. Mech, R.O. Peterson, P.J.P. Gofan, and Wayne R.K. 1991. Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. *Evolution* 45: 104-119.
- Leonard, J.A. and R.K. Wayne. 2008. Native Great Lakes wolves were not restored. *Biol. Let.* 4: 95-98.
- Musiani, M., J.A. Leonard, H.D. Cluff, C.C. Gates, S. Mariani, P.C. Paquet, C. Vila, and R.K. Wayne. 2007. Differentiation of tundra/taiga and boreal coniferous forest wolves: genetics, coat colour and association with migratory caribou. *Molecular Ecology* 16: 4149-4170.
- Nowak, R.M. 2002. The Original Status of Wolves in Eastern North America. *Southeastern Naturalist* : 1 (2): 95-130.

- Rutledge, L.Y., K.L. Bos, R.J. Pearce, and B.N. White. 2010a. Genetic and morphometric analysis of sixteenth century *Canis* skull fragments: implications for historic eastern and gray wolf distribution in North America. *Conservation Genetics* 11: 1273–1281.
- Rutledge, L.Y., C.J. Garroway, K.M. Loveless, and B.R. Patterson. 2010b. Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. *Heredity* .
- Schreber, J.C.D.v. 1775. Die Saugthiere. Erlangen,
- USFWS. 1993.Serology and Morphology Reports on 1993 wolf from Maine (MCZ62506).
- USFWS. 1997.Morphology and serology report on 1997 Maine wolf (MCZ62507).
- USFWS. 2002.Agency Case No 00FW01519
- USFWS. 2004.Saratoga county canid.
- Vali, U., A. Einarsson, L. Waits, and H. Ellegren. 2008. To what extent do microsatellite markers reflect genome-wide genetic diversity in natural populations? *Molecular Ecology* 17: 3808–3817.
- vonHoldt, B.M., J.P. Pollinger, D.A. Earl, J.C. Knowles, A.R. Boyko, H. Parker, E. Geffen, M. Pilot, W. Jedrzejewski, B. Jedrzejewska, V. Sidorovich, C. Greco, E. Randi, M. Musiani, R. Kays, C.D. Bustamante, E.A. Ostrander, J. Novembre, and R.K. Wayne. 2011. A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. *Genome Research* .
- Wheeldon, T. and B.N. White. 2009. Genetic analysis of historic western Great Lakes region wolf samples reveals early *Canis lupus/lycaon* hybridization. *Biology Letters* 5: 101-109.
- Wheeldon, T.J., B.R. Patterson, and B.N. White. 2010. Sympatric wolf and coyote populations of the western Great Lakes region are reproductively isolated. *Molecular Ecology* 19: 4428-4440.
- Wilson, P.J., S. Grewal, I.D. Lawford, J.N.M. Heal, A.G. Granacki, D. Pennock, J.B. Theberge, M.T. Theberge, D.R. Voigt, W. Waddell, R.E. Chambers, P.C. Paquet, G. Goulet, D. Cluff, and B.N. White. 2000. DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. *Canadian J. Zool.*

78: 2156-2166.

Wilson, P.J., S.K. Grewal, F.F. Mallory, and B.N. White. 2009. Genetic Characterization of Hybrid Wolves across Ontario. *J Hered* 100: S80-89 .

Zink, R.M. and G.F. Barrowclough. 2008. Mitochondrial DNA under siege in avian phylogeography. *Molecular Ecology* (2008) **17**, 2107–2121 17: 2107-2121.