

Review of Proposed Rule Regarding Status of the Wolf Under the Endangered Species Act

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NCEAS

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Statement of Work

Introduction

The National Center for Ecological Analysis and Synthesis (NCEAS) was asked to perform an independent scientific review of the US Fish and Wildlife Service's (USFWS or 'Service') proposed rule regarding Grey Wolves. The full statement of work is appended. The key issues were stated as:

The purpose of this review is to provide an objective, independent, external scientific peer review of the information in the proposed rule. The proposed rule is 246 pages long and synthesizes the existing best available scientific and commercial information regarding the status of various gray wolf populations and subspecies that occur within portions of the lower 48 States where the species is currently listed. Factors to be addressed in the peer review include the scientific merit of the proposed rule's primary analysis components (i.e., gray wolf taxonomy and status) which provide the basis for the proposal. The peer reviewers should confirm that any scientific uncertainties are clearly identified and characterized by the Service, and the potential implications of the uncertainties for the technical conclusions drawn are clear.

Specifically NCEAS was asked to directly address the following four issues

Task 1. Did the service consider the best available scientific information, including the scientific literature, in developing this proposal?

Task 2. Are the assumptions, analyses, and conclusions reflected in the proposed rule reasonable in light of the best information available?

Task 3. Does the proposed rule draw reasonable and scientifically sound conclusions from Chambers et al., 2012?

Task 4. Does the proposed rule utilize the best available scientific information and draw reasonable and scientific sound conclusions concerning the status of wolves?

NCEAS selected Dr. Steven Courtney to lead the review. Dr. Courtney has extensive experience with Endangered Species issues, and with panel-based independent peer review processes. He has also published in ecology, taxonomy and genetics, and served as the fifth reviewer at the end of the peer review process. He has particular expertise with evaluations of wolves (having led two previous review efforts) and with complex genetics analyses under ESA (e.g. with the Preble's Meadow Jumping Mouse)

Dr. Courtney worked with the Director of NCEAS, Prof. Frank Davis to design the overall process, and to select peer reviewers. These two scientists were in sole charge of all aspects of the review, from selection of reviewers, through conduct of the review itself, and preparation of this report. The USFWS provided the initial scoping request, provided extensive documentation, and answered questions as needed. However the Service had no role in the selection of reviewers; nor did the Service direct the conversations or deliberations of the reviewers. They similarly had no role in the drafting of this report.

The overall goal then of this effort was a full and completely independent scientific review of the proposed rule. The impartiality of this review was emphasized by the lack of contact between the reviewers and the Service. NCEAS acted as the sole point of contact, and ensured that reviewers were

themselves committed to a full and completely impartial review.

Panelists were selected (discussed below) and then sent a large volume of scientific material with which to evaluate the proposed Rule. We then invited the reviewers to attend an in-person review meeting in Santa Barbara, at NCEAS. In the event one reviewer withdrew immediately prior to the meeting, while a second was unable to attend in person because of winter travel difficulties.

At the review meeting, a carefully controlled process was followed. The full details are provided in the meeting notes (appended). In essence the panelists were invited to respond in turn to the four questions posed above. There was discussion among the panelists on each question in turn, but the panelists were asked to provide their individual opinions, rather than to come to a consensus. This process ensures that there is a clear understanding of the full range of scientific opinion; if consensus is reached that indicated that there is little diversity of opinion. Conversely, if opinions vary greatly, this may indicate that there is a wide range of opinion in the larger scientific community.

Selection of Panelists

Previous efforts to ensure an impartial scientific review of the proposed Rule had encountered problems with the selection process for identifying peer reviewers. In this effort, only Drs. Courtney and Davis were responsible for reviewer selection. We sought scientists who we believed would be appropriate subject matter experts in ecology, genetics, taxonomy and the ESA. We also sought some scientists with direct engagement in wolf genetics and taxonomy, while seeking others who did not have direct engagement in these issues. While many of these scientists were easily identified through the literature, we sought the advice of other qualified scientists in identifying appropriate reviewers. However we did not seek the advice or input of the USFWS in selecting reviewers. The Service remained ignorant of the identity of reviewers until the date of the meeting at NCEAS.

Our primary goal in selecting reviewers was the individual scientist's ability to perform the task. However it was also important (following OMB and NRC guidelines) to include the full range of opinion on substantive science issues. In the case of wolf taxonomy and genetics there are two predominant opinions, notably regarding the status of eastern wolves. We therefore deliberately included among our reviewers one representative of each point of view. In the event these two scientists did not substantively disagree on most issues.

Another important criterion for selection of reviewers was their willingness to undertake a large task in the public interest, without pay and at short notice. Although one reviewer did eventually turn down our request because of time constraints, most reviewers were commendably willing to undertake this very significant task.

Finally, we were careful to select reviewers who we felt were able to commit themselves to a completely impartial review. Wolf protection under the Endangered Species Act has received a great deal of attention, and is an emotional issue for many (including both those favoring and opposing delisting of the species as a whole). We sought those reviewers who we believed would be able to focus on the specific scientific issues with which we were charged. However we did not avoid selecting reviewers who had previously made known their personal (as opposed to scientific) opinions on the issue. This distinction is important; it is entirely possible for a scientist to have a strong opinion on policy or a proposed action, but also for that scientist to make an impartial assessment on (for instance) the precise genetics or taxonomic techniques and data that were used. Similarly, we did not consider a scientist's professional affiliation to be grounds for exclusion, although we attempted to find a panel unaffiliated with USFWS (to provide independence).

Each reviewer that we selected was interviewed in person by Dr. Courtney who asked in detail whether the scientist would be able to perform an impartial and purely scientific review, limiting their comments solely to technical issues, and avoiding any recommendations or discussions on policy. This process followed the guidelines set out by NAS. All reviewers that we approached committed to this process. As can be seen from this report, and from the individual panelists' comments, these commitments were met – this report is devoid of policy recommendations, and is focused entirely on the science at hand

It is important to emphasize that the key criterion in selecting reviewers was technical capability. One of the selected reviewers, Prof. Wayne, had been apparently excluded from a previous review effort. This played no part in our considerations (either to include him or not). He was included solely on merit. All the other reviewers were similarly included on scientific merit and ability to carry out the task as set.

Seven reviewers were eventually selected and approached for inclusion in the process. One of these (Dr. Dumbacher) although willing to serve, was unable to make the extensive time commitment. A second reviewer (Prof. Patton) also withdrew later, for personal reasons. Dr. Courtney at this point took

on the role of fifth reviewer.

The key qualifications of the reviewers are:

Dr. J. Dumbacher. Curator of Mammals and Birds, California Academy of Sciences.

Dr. Dumbacher is a population geneticist and taxonomist. He has previously evaluated mammal genetics and population status in reviews of ESA proposed actions

Dr. S. Fallon. Senior Scientist, NRDC

Dr. Fallon is a genetics expert, with extensive experience with ESA issues, including wolf genetics.

Dr. W. Murdoch. Professor (retd) UCSB.

A member of the National Academy of Sciences, Prof. Murdoch is an ecologist who has participated in many previous reviews of ESA issues.

Dr. J. Patton. Professor (retd) UC Berkeley

Prof. Patton is an eminent taxonomist of mammals, with extensive experience in the west

Dr. R. Wayne, Professor, UCLA

Prof. Wayne is a geneticist, with a long-standing research program in canid genetics world-wide

Dr. P. Wilson, Canada Research Chair, Trent U

Prof. Wilson is an expert in conservation genetics, particularly of large mammals.

Dr. S. Courtney, NCEAS

Dr. Courtney is an expert in ESA issues, with publications in quantitative genetics and taxonomy. He is also expert in the use of scientific peer review in applied contexts.

Summary of Discussions at Meeting at NCEAS

The individual reviewer reports show the detailed scientific opinions of each reviewer on the four questions posed in the statement of work.

The following key conclusions can be drawn from these reviews

- There was unanimity among the panelists that, although there was much good scientific work in the Proposed Rule, the rule is heavily dependent upon the analysis of Chambers et al.
- Some reviewers also noted a lack of appropriate use of the literature on species level taxonomy
- There was unanimity among the panelists that Chambers et al was not universally accepted and that the issue was 'not settled'. The issues raised by Chambers et al could be definitively answered relatively soon.
- There was unanimity among the panel that the rule does not currently represent the 'best available science'
- Neither the panel as a whole, nor any of its members in their individual reviews, made any management or policy recommendations

Individual Panelists Opinions

The following sections report the individual opinions of the four selected reviewers, and of Dr. Courtney (fifth reviewer)

Gray wolf National Delisting proposal peer review – Sylvia Fallon

As part of the peer review process I was asked to review the proposed rule and evaluate the “quality of any information and analyses used or relied on in the document.” Specifically, I was asked to address the following questions:

(1) Did the Service consider the best available scientific information, including the scientific literature, in developing this proposal? Is there additional biological, commercial, trade, or other information relevant to our analysis of the current *C. lupus* listed entity that we did not consider, but should? (2) Are the assumptions, analyses, and conclusions reflected in the proposed rule reasonable in light of the best available information? (3) The Service determined that the synthesis and conclusions of Chambers *et al.* (2012. *North American Fauna* 77:1-67) reflect the best available scientific information regarding taxonomy of wolves in North America. In doing so, does the proposed rule draw reasonable and scientifically sound conclusions concerning the taxonomy of the eastern wolf, *Canis lycaon*? (We are not requesting information on the status of *C. lycaon* because we are conducting a status review for this species and peer review of that document will occur separately.) (4) Does the proposed rule utilize the best available scientific information and draw reasonable and scientifically sound conclusions concerning the status of the gray wolf in the Pacific Northwest United States; the gray wolf subspecies *Canis lupus nubilus*; the gray wolf subspecies *C. l. occidentalis*; and the gray wolf subspecies *C. l. baileyi*?

Although some of the above questions can be interpreted broadly, I was asked to focus my comments on the taxonomic issues presented in Chambers *et al.* 2012. I was additionally instructed to only comment on issues related to science and not on policy decisions.

Fundamental to each of these questions is the fact that the proposed rule relies entirely on the taxonomic scheme presented in Chambers *et al.* 2012. It is my professional opinion that Chambers *et al.* 2012 does not represent the best available science. Although Chambers *et al.* 2012 does present a thorough review of taxonomic literature related to the *Canis* genus in North America, it ultimately puts forward a taxonomy that is not sufficiently supported by the available science. Because of this, the proposed rule itself is also not based on the best available science.

Many of the issues related to why Chambers *et al.* 2012 is not the best available science, as well as other problematic conclusions from the proposed rule, were discussed verbally by the panel and will be summarized in the synthesized report. Below I discuss a few notable issues.

1. *C. lycaon* should not be recognized

The entire scientific basis for the proposed rule depends on the taxonomic scheme put forward by Chambers et al. 2012. However, there was consensus among the panelists, including two of the leading researchers in this field, that Chambers et al. 2012 does not represent the best available science. Specifically, there is agreement that there is not currently sufficient scientific basis for recognizing *C. lycaon* as a species. Furthermore, this is an area of active scientific research with new scientific studies being published several times a year. In fact, research papers that are in preparation or in review/in press right now could significantly alter the current conclusions drawn by Chambers et al. 2012 and the Service. Because of this, there was consensus among the panelists that it is premature for the Service to be adopting the conclusions of Chambers et al. 2012 as the basis for their proposed rule.

Among other problems, Chambers et al. 2012 justifies the recognition of *C. lycaon* based primarily on two non-recombining markers (a mitochondrial gene segment and some Y chromosome markers). The authors state, "In summary, species-level recognition of *C. lycaon*, the eastern wolf, outside the species limits of *C. lupus*, is supported by the phylogenetic distinctiveness of its mtDNA and Y-chromosome haplotypes." (pg. 25). As the other panelists agreed, this simply is insufficient to determine the existence of a species and specifically is completely insufficient for ruling out the alternative hypothesis that the pattern is explained by ancient (and recent) hybridization between *C. lupus* and *C. latrans*. An examination of the nuclear genome is necessary for this. To date, the most extensive nuclear datasets in relation to this issue in my opinion have indicated a greater likelihood that the observed genetic pattern is explained by introgression rather than the existence of a separate species (Koblmuller et al. 2009, vonHoldt et al. 2011).

In addition to there not being sufficient genetic signal to identify a distinct species, it is worth noting that the entity that is considered *C. lycaon* (by Chambers et al. 2012) hybridizes extensively with both *C. lupus* and *C. latrans* which further complicates recognizing it as a species. Even Chambers et al. 2012 suggests that the entity that they recognize as *C. lycaon* may be undergoing "despeciation" while indicating that they believe "it is premature to conclude that the eastern wolf is no longer an identifiable taxon" (pg. 27). To me, this point is better made in the reverse – that given the lack of distinctiveness, it is premature to conclude that the eastern wolf is an identifiable taxon. Until there is clear and strong support for the existence of a recognizable, independently evolved species (at the exclusion of the hybridization hypothesis), *C. lycaon* should not be recognized.

In short, there is not currently sufficient scientific support for the recognition of *C. lycaon* as a separate species. The uncertainty of the existence of a separate species is reflected in the fact that *C. lycaon* is not recognized by authoritative taxonomic organizations such as the American Society of Mammalogists or the International Commission on Zoological Nomenclature. The fact that the US Fish and Wildlife Service produced the proposed taxonomy (Chambers et al. 2012) and have accepted it as best available science does not, in fact, make it best available science.

2. The assertion that *Canis lupus* did not occur in the eastern US is unfounded

The proposed rule states that "The results of recent molecular genetic analyses....indicate that the gray wolf (*C. lupus*) did not occur in the eastern United States" (pg 35670). However, the papers that are cited to support this assertion do not actually exclude the gray wolf from the range of the eastern US. In fact the authors of the papers that are cited have explicitly made this point to the US

Fish and Wildlife Service previously. This was further supported by the other panelists during the peer review. This assertion is not justified.

3. The Pacific Northwest wolves are likely to be genetically and ecologically distinct

The proposed rule states that even if wolves were to recolonize parts of the PNW west of the NRM DPS that they would not be ecologically or genetically distinct. The rule, however, also acknowledges the differing ecology in this area and the historically distinct wolves that used to occupy it (once considered their own subspecies). Additionally, recent research indicates that wolves just north of the PNW demonstrate ecological and genetic uniqueness typical of a 'coastal ecotype' (Leonard et al. 2005, Munoz et al. 2009, Weckworth et al. 2010, vonHoldt et al. 2011). Therefore, it does not seem to logically follow that wolves establishing west of the NRM DPS in the PNW would not be ecologically and genetically unique.

4. *C. l. baileyi* is ecologically and genetically unique

Chambers et al. 2012 and the proposed rule recognize three subspecies in the lower 48: *C. l. baileyi*, *C. l. occidentalis* and *C. l. nubilus*. Of these *C. l. baileyi* is clearly identifiable and unique in terms of its ecology (desert-adapted) and genetics. If the frame of subspecies or DPS were being used to identify units within *C. lupus*, *C. l. baileyi* would qualify. I think it's important to note, however, that historically Mexican wolves were part of a genetic "southern clade" that extended further north into the southern rockies and greater plains (Leonard et al. 2005) suggesting that Mexican wolves showed evidence of intergradation with other populations of wolves to the north. Therefore although they are geographically and genetically discrete now, they were not so historically and would not necessarily need to be managed as such in the future.

As for *C. l. occidentalis* and *C. l. nubilus*, a significant shortcoming of Chambers et al. 2012 is that it relies heavily on a pre-established taxonomy based on morphology (Nowak 1995) and then tries to superimpose genetic information onto that taxonomy and claim 'concordance.' However, the result of this approach is that the taxonomy is driven largely by the morphological data rather than an examination of newer genetic information. For example, as discussed by the panelists, research indicates that landscape level ecological differences may be important for driving differentiation between groups of wolves (Munoz et al. 2009, Weckworth et al. 2010, e.g.). At the same time, the vagility of wolves and their ability to interbreed likely complicates their associations beyond the morphologically-based, geographically-discrete subspecies structure outlined by Chambers et al. 2012. Therefore, the taxonomy proposed by Nowak 1995 and closely followed by Chambers et al. 2012, including the description of *C. l. occidentalis* and *C. l. nubilus*, is likely not an accurate or meaningful description of the genetic and ecological diversity of wolves in North America.

References:

Chambers, S. et al. 2012. An account of the taxonomy of North American wolves from morphological and genetic analyses. *North American Fauna* 77: 1-67.

Koblmuller, S. et al. 2009. Origin and status of the Great Lakes wolf. *Molecular Ecology* 18: 2313-2326.

Leonard, J.A., C. Vilà and R.K. Wayne. 2005. "Legacy lost: genetic variability and population size of extirpated US gray wolves (*Canis lupus*)." *Molecular Ecology* 14: 9-17.

Muñoz-Fuentes V., et al. 2009. "Ecological factors drive differentiation in wolves from British Columbia." *Journal of Biogeography* 36: 1516-1531.

Nowak, R. 1995. Another look at wolf taxonomy. Pages 375-397 in Carbyn, LN, Fritts, SH, Seip, DR (Eds). *Proceedings of the second North American symposium on wolves*. Edmonton, Alberta: Canadian Circumpolar institute, University of Alberta.

vonHoldt, B., et al. 2011. A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. *Genome Research* 21: 1294-1305.

Weckworth, B. V. et al. 2010. Phylogeography of wolves (*Canis lupus*) in the Pacific Northwest. *Journal of Mammalogy* 91: 363-375.

Peer review comments of Prof. W. W. Murdoch

I cannot comment on the scientific substance underlying the 4 questions, since this is not my field of scientific study. The rule faithfully represents the conclusions of Chambers et al. But other panelists have made what seem to me likely to be reasons for doubting that Chambers et al. represents either the best available scientific information (some key information may have been omitted) or the best available analysis of then-existing information.

I do have a comment on the approach that seems to have been followed by the Service. Chambers et al. is written by four Service scientists and was published in a Service publication. This seems a less than optimal way of establishing the best scientific statement on a controversial issue. A more standard, and I believe preferable, approach would be to convene broadly-acknowledged experts covering different viewpoints, and to produce a paper that would be published in a recognized peer-reviewed journal.

Comments on Proposed Rule Change for delisting the Gray Wolf (FR 78 114)
 Dr. R. Wayne

Task 1. Did the service consider the best available scientific information, including the scientific literature, in developing this proposal?

Summary. No. The information on wolf taxonomy is generally comprehensive. However, the literature on taxonomic concepts is dated and focuses on a single subspecies/species concept first published in 1990 by Avise and Ball. More than 2 decades of research and discussion have passed since this concept was developed and it is not now widely used as originally stated. The justification for the exclusive use of this approach is not well defended. Moreover, it is not clear that the concept is an appropriate one for wolves at the subspecies level as they only recently colonized much of their geographic range since glaciers retreated from Canada and parts of Alaska 10-15 thousand years ago. Diagnostic mtDNA sequence mutations that define phylogroups are unlikely to have occurred and be fixed in this short time period. Further, wolves are highly mobile and ongoing gene flow coupled with recent colonization makes the development of well-supported reciprocally monophyletic subspecies groups unlikely. Finally, the fossil record of canids on the East Coast is not well reviewed or analyzed. There is no convincing evidence that gray wolves were absent historically from the eastern US or Great Lakes region as asserted by the authors. Multiple species, including dire wolves, eastern wolves, coyotes and gray wolves could have occupied and coexisted in the region.

Analysis.

1. Subspecies concepts. The authors have chosen a subspecies concept that defines subspecies based on genealogical concordance in multiple genetic and phenotypic traits. However, the dominant trait used is

Groups	Φ_{SC}	Φ_{ST}	Φ_{CT}	% among groups	P
[Spain, Portugal] [France, Italy] [Romania, Bulg.] [Croatia] [Yugos.] [Poland, Estonia, Sweden, Finland, W Russia] [Greece, Turkey] [Israel] [Iran] [Afghan.] [India] [S Arabia] [China] [Mongol.] [Alaska] [Yukon] [NWT, Alb., Mont., Minn., Labra.] [Mexico]	0.185	0.689	0.619	61.89	< 0.01
[Eurasia] [America]	0.661	0.724	0.186	18.55	< 0.05
[Eurasia] [USA, Canada] [Mexico]	0.650	0.749	0.284	28.38	< 0.01
[Europe] [Asia] [USA, Canada] [Mexico]	0.650	0.689	0.186	8.55	0.05
[Spain, Portugal] [France, Italy] [Romania, Bulg.] [Croatia] [Yugos.] [Greece, Turkey] [Poland, Estonia, Sweden, Finland, W Russia] [Israel] [S Arabia] [Iran] [Afghan.] [India, China] [Mongol.] [Alaska] [Yukon, NWT, Alb., Mont., Minn., Labra.] [Mexico]	0.271	0.689	0.573	57.27	< 0.01
[Spain, Portugal] [France, Italy] [Romania, Bulg., Croatia, Yugos.] [Greece, Turkey] [Poland, Estonia] [Sweden, Finland] [Israel] [W Russia] [S Arabia] [Iran] [Afghan.] [India, China] [Mongol.] [Alaska] [Yukon, NWT, Alb., Mont., Minn., Labra.] [Mexico]	0.344	0.687	0.523	52.34	< 0.01

mtDNA control region sequence information for which time and isolation is needed in order for well supported monophyletic groups to develop that are coincident with geography. In much of North America, the development of reciprocally monophyletic groups of wolves, or even groupings marked by diagnostically fixed mutations, is unlikely because there has not been sufficient time for lineage sorting to have occurred since recolonization of previously glaciated areas after

the last glaciation 10-15 thousand years ago. The expectation of the time to coalescence is on the order of 2-4N generations (Hartl & Clark 1989), and hence given past female effective size continent wide of >10,000, lineage sorting is unlikely to have occurred. Furthermore, ongoing gene flow between populations will obscure past periods of isolation even when differences might have occurred.

Supporting this notion is the observation that only

Table 1. Statistical analysis of molecular variance (AMOVA) approach for hypothesis testing of groupings in North American wolves (Vila et al., 1999).

3% of Nearartic and Palearctic avian subspecies are confirmed by data on genealogical concordance based on mtDNA sequence data (Zink, 2004). In fact, much of the mtDNA data are obtained from Vila et al. (1999) who using a statistically based approach, find few genetically supported partitions in North American wolves or in the Old World (Table 1). Moreover, the units they find are not concordance with those in Chambers et al. 2012, who instead base their conclusions on a non-statistical qualitative approach.

A large body of recent discussion focuses on defining evolutionary units below the species levels based on *statistically supported* differences in allele and haplotype frequencies among

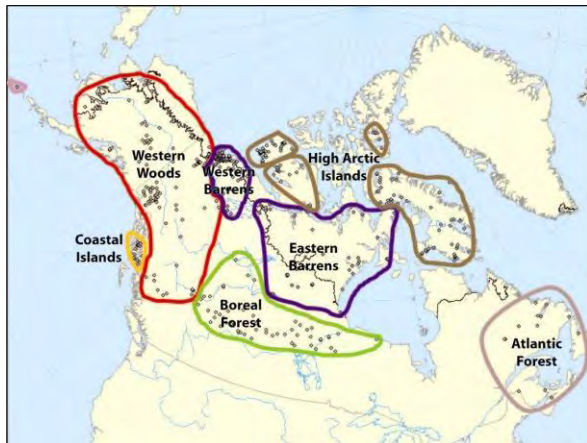


Fig. 1. Genetic clusters in North American wolves and their correspondence to habitats (from Carmichael et al. 2007).

populations as well as data on adaptive divergence (e.g., see reviews in Fraser and Bernatchez, 2001; Allendorf et al., 2001; Pearse and Crandall, 2004; Haig et al., 2006; Palsbøll et al., 2007; Fink et al., 2012). The review by Haig et al. (2006) is especially critical in this regard. This extensive literature is not discussed in this report, and in fact is dismissed as “non taxonomically” based which is not true, as any evidence for differences among population can be used in a taxonomic framework (e.g. see Serrouya et al., 2012). No citations or analysis is provided that support their contention that these newer approaches are “non taxonomic” and do not represent the best and most appropriate scientific

approach. Furthermore, the newest information on gray wolf differentiation beginning in a first report by Carmichael et al. (2001) shows that wolf populations are genetically differentiated along an ecologically axis (Garcia-Moreno et al., 1996; Geffen et al., 2004; Carmichael et al., 2007; Musiani et al., 2007; Pilot et al. 2006, 2010; 2013; Koblmüller et al., 2009; Munoz-Fuentes et al., 2009; vonHoldt et al. 2011), information which can be used in a taxonomic framework (e.g. see Fig. 1 for a possible subspecific scheme and an example from Serrouya et al., 2012). The selective use of mtDNA data in Chamber et al., and in the proposed rule in an *ad hoc* hypothesis framework and the dismissal of other equally meritorious data such as difference in microsatellite allele or SNP frequencies, is not well defended. Considering the history of wolf populations in North America as discussed above, allele frequency differences, rather than reciprocal monophyly is more likely and provides important “taxonomic” information.

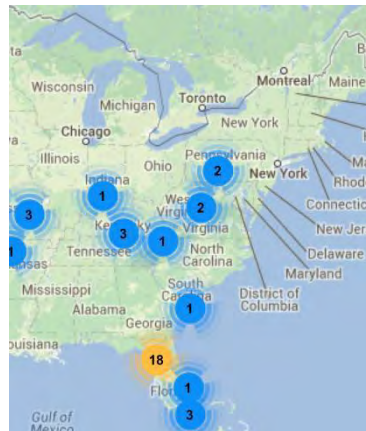
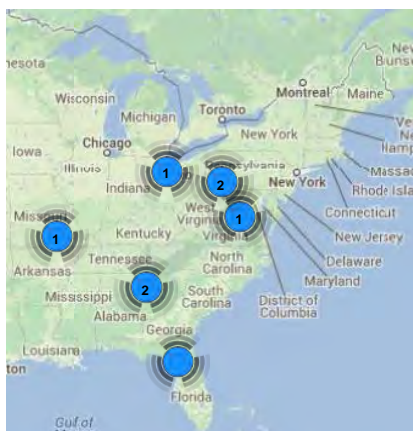


Fig. 2. Number of localities with gray wolves (left) and dire wolf specimens (right) indicated in each circle in the Late Pleistocene record from the east coast. These results indicate the co-existence of multiple wolf species. Both gray wolves and dire wolves are also known from the Rancho LaBrea tar pits in California (see www.ucmp.berkeley.edu)

2. *Evidence for absence of wolves on the East Coast.* In Chambers et al. (2012) and the proposed rule, no direct evidence is provided for the absence of the gray wolf on the East Coast. Rather, the assertion that it was absent derives from the claim that a unique non-protected wolf species, *Canis lycaon*, existed there instead. However, the hypothesis that both species may have existed in the US east coast is not refuted by data. In fact, the fossil data records gray wolves on the east coast, which have been argued by Nowak (2002, 2009) to possibly be red wolves or red wolf-eastern wolf hybrids, but others have considered them gray wolves (see Fig. 2, and references therein). Even if one accepts the opinion of Nowak, then the conclusion is that the eastern wolf was not on the East Coast, alternatively there may have been multiple species there as now exist in the Great Lakes area and existed historically in other parts of the US. Regardless, the fossil literature and data needs to be cited and evaluated, and the possibility that eastern wolf, gray wolf or both species existed in the Northeast supported with evidence.

Task 2. Are the assumptions, analyses, and conclusions reflected in the proposed research reasonable in light of the best information available?

Summary. No. Information contrary to the proposed delisting is discounted whereas that which supports the rule (such as the species status of “lycaon” and lack of distinction of the Pacific coastal wolf) are accepted uncritically. Overall, the subspecies analysis lacks statistical rigor with proposed subspecific designations appearing *ad hoc* (i.e. the data are interpreted within a preconceived subspecies framework rather than analyzed in an unbiased fashion by a statistically based hypothesis-testing approach see Table 1 for an example). Rather than a concordance approach as suggested will be used, important data are often over-looked or discounted, notably data from microsatellite loci and SNP genotyping approaches that identify statistically supported groupings (e.g. Fig. 1) are dismissed in favor of weakly supported phylogroups based loosely on mtDNA patterns that regardless, are not geographically coherent (subspecies share haplotypes from different phylogroups). These groups are interpreted as due to past invasions but how the pattern and timing of these specific invasions might explain geographic patterns in mtDNA are not well explained. In light of previous studies showing flux in the distribution of carnivore phylogroups throughout the Pleistocene (e.g. Barnes et al., 2002; Leonard et al., 2005; Hofrieter et al., 2000), and the glacial history of North America, this invasion hypothesis seems too simplistic and is not consistent with the most recent best scientific evidence.

Analysis.

Diagnosis of *Canis lycaon*. A primary finding of Chambers et al. (2012), and the proposed rule is that parts of the Great Lakes region and the eastern US were inhabited by a distinct wolf species endemic to North America. Both reports further assert that the gray wolf was absent from the East Coast historically, although as discussed above, no direct evidence is provided and there is no reason to suspect that the gray wolf, which is larger than the eastern wolf, was excluded by *Canis lycaon*. The current evidence concerning the taxonomic status of the eastern wolf is controversial, with some scientists maintaining that it is a subspecies of the gray wolf (the traditional view) whereas others maintain it is a distinct species that evolved in the New World, an idea initiated by the report in 2001 by Wilson and colleagues. Since this initial report, a large body of evidence has been published that purports to support either one view or the other. It is widely agreed by both sides that despite this evidence, the theory that the eastern wolf is a distinct species is controversial and therefore, in the absence of dramatic new evidence, the conclusion now that the eastern wolf is well supported as a distinct species in Chambers et al. is surprising. Chambers et al. and the proposed rule arrive at this conclusion through systematically dismissing contrary evidence and accepting uncritically evidence supporting species status. For example, the most extensive genome-wide study to date (vonHoldt et al., 2011) based on 48,000 markers (whereas previous studies generally used less than 25 markers) and published in *Genome Research* (which is the leading journal for such studies) is criticized extensively whereas the original Wilson et al., 2001 paper is accepted uncritically although it is only based on a single marker system (mtDNA). Previous research from both camps using the same markers (mtDNA, microsatellites and Y chromosome data) but with different sample sets, and come to different conclusions regarding species status. The general limitations of these markers for species inference and the bias imposed by sample composition and reference populations are poorly discussed by Chambers et al., and the proposed rule. Specifically, the limitation of using a gene tree of mtDNA sequences to infer a species tree, the poor bootstrap support for branches in the mtDNA tree, homoplasy in microsatellite allele size and control region sequences, lack of resolution in the Y chromosome tree of sequences, alternative networks in this tree, the problem of admixture, and the problem of ancient DNA sequences in canis from NE Canada before the recent coyote invasion are just a few of the issues that could be discussed. Indeed, many of these problems are discussed throughout the literature by the camp supporting the specific status of the *lycaon*. Obviously, the case is not closed, and the lack of attention to unresolved issues and the biased viewpoint of Chambers et al. in this regard is alarming. Nonetheless, even accepting *lycaon* as a separate species, the most fundamental issue concerning the absence of gray wolves in the East Coast is not directly supported by the data and is not a necessary condition of species status for the eastern wolf.

Subspecific designations. As discussed above, subspecific designations ignore the diverse sources of evidence, integral to the concordance approach, that support multiple taxonomic units in North America wolves based on genetics, phenotype and ecology. For example, the subspecific status of the Pacific coast

wolf is supported by genetic, morphologic and life

Criterion	Example	Reference
Morphology	Pelage: shorter and coarser hair than interior conspecifics. Grey morph with conspicuous red tones and brown under-fur common. Brownish red tinge, a feature responsible for the one coastal region's historic sub-specific epithet <i>fuscus</i> .	Young & Goldman (1944); Cowan & Guiguet (1975); Wood (1990)
	Smaller body sizes. Cranial morphology; multivariate analyses identified distinct coastal forms.	Cowan & Guiguet (1975); Friis (1985); Wood (1990) Friis (1985)
Prey specialization	Unique prey-based ecotype: wolf-black-tailed deer system, North America's smallest ungulate. Other regions include black-tailed deer but also other ungulates.	Darimont <i>et al.</i> (2004); Theberge (1991)
	Evidence for insular predator-prey (wolf-deer) dynamics on islands. Deer consumed at frequencies inversely proportional to island isolation.	Darimont <i>et al.</i> (2004, 2009)
	Populations heavily subsidized by marine resources, especially in absence of deer; isotopic data suggest 25–75% of diet is of marine origin (especially spawning salmon and marine mammals). Salmon hunting behaviour; high efficiencies and shared fishing techniques and tissue targets across coastal region.	Darimont & Reimchen (2002); Darimont <i>et al.</i> (2003, 2004, 2008, 2009) Darimont <i>et al.</i> (2003)
Other specific behaviour	Archipelago environment with islands often smaller than home ranges necessitates frequent swimming between landmasses.	Darimont & Paquet (2002); Paquet <i>et al.</i> (2006)

Table 2. Attributes of the pacific coastal wolf used to argue for separate status (from Munoz-Fuentes *et al.*, 2009).

history differences from inland wolves, yet Chambers *et al.*, focusing primarily on mtDNA data suggests it is part of the same hypothesized invasion of North America, and therefore not distinct from wolves of the American West. However, the table provided in the original manuscript outlines many sources of evidence for distinction (Table 2). In fact, an argument for subspecific status for the coastal wolf is similar to that used in Chambers *et al.* for the Mexican wolf, which they accept as a distinct subspecies. For example, the diagnostic haplotype of the Mexican wolf is found in historic wolves of the American west, similar to the coastal wolf, and like the coastal wolf (Table 2), the Mexican wolf is ecologically and morphologically distinct. Such inconsistencies are common in argumentation throughout Chambers *et al.*

Task 3. Does the proposed rule draw reasonable and scientifically sound conclusions from Chambers *et al.*, 2012

Summary. Yes. As discussed above, virtually all taxonomic conclusions from Chambers et al. are accepted uncritically. Such lack of critical analysis is a key problem with the proposed document. Specially, as discussed above none of the taxonomic conclusions of Chambers et al. are supported by a carefully considered modern statistical analysis (e.g. Pease and Crandall, 2004; Palsbøll et al., 2007) and instead rely on a graphical and qualitative approach. Inferences are generally *ad hoc* without formal statistical testing. Finally, the concordance concept is applied unevenly, in some cases concordance is sought between different data, in other cases, additional data are discounted if they do not support the viewpoint of Chambers et al., and the proposed rule.

Task 4. Does the proposed rule utilize the best available scientific information and draw reasonable and scientific sound conclusions concerning the status of wolves.

Summary. No. As the status discussion is predicted on the poorly supported taxonomy at the subspecies level from Chambers et al., it is not reasonable especially with regard to the pacific coastal wolf. The taxonomic conclusions of the proposed rule are poorly supported given the controversial state of wolf taxonomy (see above).

Grey Wolf Peer Review

NCEAS

December 18, 2013

Report by Dr. Paul J. Wilson

January 25, 2014

Assumptions of Report as Articulated on December 18, 2013:

- Scientific review not a policy review
- Use best available science
- Impartial and independent assessment

Comment on the Process:

The membership of the scientific panel maintained independence from the USFWS but less independence in terms of being stakeholders in wolf science and taxonomy. I indicated in pre-meeting e-mails, that my understanding was that non-wolf genetics expertise would be included on the panel. This was acknowledged as a potential gap at the December 18, 2013 meeting with a yet to be determined solution for this expertise to provide an evaluation. This need for additional future consideration demonstrates that the best course of action for next steps include recently published and forthcoming studies into the evaluation.

Review of Chambers et al. (2012):

Consistent with my presentation on December 18, 2013, my summary report is focused on the taxonomy of the eastern wolf and red wolf and an assessment of Chambers et al. (2012) in that context, with little emphasis on wolf sub-species designations.

In terms of evidence for the eastern wolf as a species independent of the grey wolf, a survey of Y-chromosome haplotypes in comparison to mtDNA (Wilson et al. 2012) demonstrates eastern-specific patterns consistent with a divergent eastern wolf more closely related to a North American evolved and coyote lineage than the grey wolf. The use of these non-recombining molecules in assessing the existence of the eastern wolf (*C. lycaon*) was based on divergent DNA markers, originally stated as not occurring in hybrid red wolves or eastern wolves, that are also eastern-specific in terms of their geography. Where they currently exist, i.e. the specific canid type, does not preclude their value in assessing phylogeographic patterns, and the Wilson et al. (2012) study and a subsequent published study (Wheeldon et al. 2013), further shows an increased contemporary complexity as a result of extensive hybridization when the animal type is superimposed, e.g. eastern coyote with eastern wolf Y-chromosomes and potential dog introgression. Based on the communication at the December 18, 2013 NCEAS meeting, it is important to note that emphasis on these genetic markers should be based on their molecular evolution and properties, and not the “newness” of their application, e.g. as compared to genome single nucleotide polymorphisms (SNPs).

The first major issue in the scientific review, is the claim that the evidence for the eastern wolf (*C. lycaon*) and the proposed historic distribution of this wolf species, precluded the presence of the grey wolf in eastern North America. Any publications associated with the model of the eastern wolf do not address this specific question and any interpretation in this regard would be limited given the current state of hybridization that may include eastern wolves (including red wolves), grey wolves, western coyotes, eastern coyotes and dogs. A fundamental example is the interpretation in Chambers et al. (2012) where Y-chromosomes identified in eastern North American canids that are commonly found in dogs were interpreted as being the source of these introgressed haplotypes. The work of myself and others supports a potential dog origin of these Y-chromosomes, but an alternative hypothesis is that these molecules originated from grey wolves inhabiting eastern North America. This is a critical question as the overall “stickiness” of Y-chromosomes in canids may capture the historic distribution of one or more species in eastern North America. Again, the Y-chromosome results (that couple microsatellites and an intron sequence in Wilson et al. (2012)) identifies a divergent eastern-specific Y-chromosome lineage supporting an eastern-evolved wolf, this finding does not reject the presence of the grey wolf. It is not valid to assume *C. lupus* Y-chromosomes are strictly dog in origin. Certainly, historic naturalist records, e.g. DeKay in New York State, describe a stockier wolf, albeit rare, termed the American Wolf and a thinner wolf in larger packs observed hunting deer. This historic account suggests the overlap of two wolves in New York State. I am not arguing that the data supports the presence of the grey wolf in eastern North America, but that this has not been explicitly tested. The ability to survey grey wolf genomic material with emerging next generation sequencing technologies in the current hybrid background of eastern canids does offer the potential to resolve the historic distributions at a course scale.

The second major issue is that the red wolf (*C. rufus*) and the eastern wolf (*C. lycaon*) should be considered as separate species, and this claim is not well-supported. As contentious as the grey wolf and eastern wolf taxonomic debate, the evidence of distinct rufus and lycaon species is not well-established. That is not to say that evidence will not emerge to support this level of taxonomic distinctiveness, but the contemporary characterization of genetic markers in an originally heavily hybridized and then intensively managed population of red wolves makes interpretation of taxonomy that much more challenging. Two statements in particular require some clarification:

Eastern wolf and red wolf do not share mtDNA or Y-chromosome haplotypes and do not together form a single group exclusive of coyote lineages.

Control region C2 in red wolves warrants some consideration of its origin in the context of the eastern wolf given a closer association to C1 and C3 “eastern wolf” haplotypes than to coyotes, and the eastern-specificity. If rejected as eastern wolf in origin, that does not exclude an intra-specific relationship between lycaon and rufus. Again, hybridization and managed breeding have defined the current red wolf, so effort to characterize remnant mtDNA and Y-chromosome markers in natural hybrid canids in the US is recommended. The presence of lycaon (intron-4) Y-chromosome haplotypes in Louisiana (Wilson et al. 2012) supports the lycaon/rufus relationship, although a more comprehensive assessment of more Y-chromosome sequences and full mtDNA genome sequencing should provide insight as to the subspecific or species-level divergence observed among these molecules.

Morphometric, autosomal microsatellite, and canine SNP array data also indicate divergence between red wolf and eastern wolf; although these conclusions must be qualified by acknowledging the gaps in sampling.

F_{ST} estimates are problematic using contemporary samples as a result of the confounding issues of hybridization. Linkage analysis using genome-wide SNP scans (vonHoldt et al. 2011) is recommended, providing the appropriate reference databases, or best available, are applied to testing explicit hypotheses about chromosome segments, divergence times and the amount and timing of admixture events.

My overall recommendation is to incorporate the recently published work of Wilson, Wheeldon, Monzon and upcoming research of Rutledge and Wayne that should more explicitly test for the existence of an eastern wolf evolutionarily independent of the grey wolf and what signatures of grey wolf actually exist in eastern canids. There was some discussion at the December meeting that the panel review this “moment-in-time” and “best available” evidence but the recommendations of myself and Dr. Wayne to incorporate these upcoming findings was not based on a philosophical open-ended view of science, but on the recognition that the controversy has not been resolved (in a sense “agree-to-disagree” in the most formal sense) and that emerging findings will make the position of the USFWS more rooted in the near future. The Chambers et al. (2012) report actually presents a number of limitations within published works but, in my opinion, do not close the loop on these issues and in fact move to full-scale acceptance of the tenants of the report, e.g. the existence of the eastern wolf at the exclusion of the grey wolf in eastern North America, despite these identified limitations.

One significant implementation, to re-iterate, is the design of the analyses to accurately reflect the hypotheses that are being tested. The review of the various studies should not emphasize the “newness” or scale of markers used, e.g. genome-wide SNPs, or implementation of specific types of analyses, but should emphasize the reference databases used to generate the data and consolidated into analytical units. The non-recombining Y-chromosome and mtDNA markers may suit a geographical pattern, specifically eastern North America, that can be tested regardless the types of canids these molecules have introgressed. In this case, interpretation may be confounded by using the canid types as a reference. The linkage analysis in vonHoldt et al. (2011) with the significant number of SNPs, offers strong potential for elucidating the number of taxonomic entities but does require sufficient sample sizes as well as appropriate design of the potential reference databases being applied to the analyses, e.g. the inclusion of eastern wolves representative of the “east”. These explicit tests are predicted to increase the resolution of the ancestry, particularly those segments currently labeled as joint “wolf-coyote” (vonHoldt et al. 2011) that may, or may not, emerge as distinct under a three species model. Emerging terms such as Great Lakes wolf that may be used as a represented eastern wolf may be inappropriate as these wolves likely have a different evolutionary history than the northeastern US wolves, although *C. lycaon* may be predominant in the history of both wolves. Increased sample sizes for representative eastern wolves is a requirement regardless of the number of loci applied. The apparent forthcoming studies from both UCLA and Trent expanding the genome-wide SNP assessment should provide critical input into USFWS recommendations. Also, implementing the geographic characterization of genetic/genomic markers independent of the current wolves or coyotes they inhabit, may further enhance the clarity of the historic distributions and taxonomic relationships, such as the red wolf and eastern wolf, required by the USFWS.

Review comments of Dr. Courtney

Task 1. Did the service consider the best available scientific information, including the scientific literature, in developing this proposal?

It is apparent that the Service has carried out a comprehensive, even exhaustive search of the literature, and this was provided to the panel. Although there were a few noted additions identified by the panel, the majority of information was used by the Service. Other panelists emphasize the lack of discussion of other literature, notably in mammalian taxonomy. I concur with that opinion.

Task 2. Are the assumptions, analyses, and conclusions reflected in the proposed rule reasonable in light of the best information available?

Everything hinges crucially on the validity of the proposition and analysis in Chambers et al 2012. Given that sensitivity, and the unanimity among the panel that that paper is best viewed as preliminary and not fully supported, the Service's proposed rule is NOT easily characterized as reasonable.

Task 3. Does the proposed rule draw reasonable and scientifically sound conclusions from Chambers et al., 2012?

The Service's actions and conclusions flow logically from Chambers et al, and from the other literature considered. However it is clear from the panel's discussions that Chambers et al is yet to receive general acceptance. The Service's strong reliance on this one analysis makes any conclusions heavily contingent upon the analysis being correct.

Task 4. Does the proposed rule utilize the best available scientific information and draw reasonable and scientific sound conclusions concerning the status of wolves?

The proposed rule certainly makes use of a lot of good science, and represents a tremendous synthesis of a lot of information. There are many sound conclusions (e.g. the distinctiveness of the Mexican Wolf taxon). However it is clear that the proposed rule cannot be said to reach uniformly sound conclusions.

Gray Wolf Peer Review

National Center for Ecological Analysis and Synthesis (NCEAS)

December 18, 2013

Participants:

Dr. Steven Courtney – NCEAS Review Leader

LeeAnne French – NCEAS Associate Director

Dr. Sylvia Fallon – Scientist, Natural Resources Defense Council

Dr. William Murdoch – NCEAS, Professor, University of California, Santa Barbara

Dr. Robert Wayne – Professor, University of California, Los Angeles

Dr. Paul Wilson – Professor, Trent University (*participating by video conference due to travel conditions*)

Observers from US Fish and Wildlife Service (USFWS)

(Conference call listening mode, available for questions, not participating in the discussion)

Maricela Constantino, Endangered Species Biologist, Headquarters Office, West Virginia

Hilary Cooley, Wolf Coordinator, Idaho

Shawn Sartorius, Montana field office

Tracy Melbiness, Mexican Wolf Recovery Program, Idaho

John Oakleaf, Mexican Wolf Recovery Program, Arizona

Dr. Courtney Thanks reviewers and USFWS biologists listening

Everything is being recorded

Introductions: (*each participant introduced him/herself*)

Dr. Courtney, NCEAS Associate. He is not a wolf expert, although he did study genetics. He has worked on Endangered Species Act (ESA) issues for many years. He is the chair of the wolf peer review process today.

Dr. Murdoch is a retired professor of ecology at UCSB and has worked in population dynamics primarily as it relates to insects. He doesn't know a lot about wolves but has also worked on several ESA- related peer review processes.

Dr. Wayne from UCLA is a geneticist with a specialty in wolves and carnivores

Dr. Fallon scientist with NRDC, studied ecology and evolution and is a wildlife scientist working specifically on ESA issues.

Dr. Wilson is a geneticist who has done a lot of work on wolf issues and other species at risk and genetics.

Ms. French is the Associate Director of Communication and Outreach at NCEAS and participating in the meeting to provide a public record of the discussion

USFWS participants provided their name, program, and location

Maricela Constantino, Endangered Species Biologist, Headquarters Office, West Virginia

Hilary Cooley, Wolf Coordinator, Idaho

Shawn Sartorius, Montana field office

Tracy Melbihess, Mexican Wolf Recovery Program, Idaho

John Oakleaf, Mexican Wolf Recovery Program, Arizona

Dr. Courtney This is a formal scientific review process to help the USFWS with their determination of the status of the wolf. It is the responsibility of the USFWS to take our input and the input from other efforts and then make a decision.

Rules

This is a scientific review. It is science-based exercise. We are not trying to understand, comment on policy. We are tasked with helping to understand what science says, avoid discussions of policy and not second guessing the decision the Service will make. USFWS

is tasked with the decision about saving species. They will factor in science and other issues such as the adequacy of existing regulations and protections (not a science issue). The Service will weigh many factors, but the decision is their decision.

Scope of the Science

USFWS has asked this panel to address four questions in reference to the proposed ruling on the gray wolf. USFWS has two reviews underway: our scientific peer review and another review looking at the status of the wolves on the ground. We are not limited to discussion on the four questions. After we finish our scientific commentary, it will feed into the USFWS processes and deliberations, and will be available to the public. We understand USFWS may well also be opening the public comment period up again after our review is submitted at the end of January 2014. They will seek other information as well. This is part of the Service's normal commentary process.

Why Are We Here

There are guidelines from OMB, USFWS, and the National Academy about how to carry out a scientific peer review process. Guidelines including things like how to verify that participants don't have a conflict of interest, that participants can focus on the issues of science, and conduct a polite, respectful discourse. Dr. Frank Davis, NCEAS Director and Dr. Courtney went through the selection process for the panelists. The Service was not involved. Drs. Davis and Courtney chose panelists based on their set of skills, yes. In addition, Dr. Courtney interviewed each candidate to evaluate whether the candidate could/would focus on the science, available and willing to dedicate the time, be able to engage in a professional discourse, and be willing to engage in a sensitive, tricky issue.

The Service asked us to bring together experts including ecologists, taxonomists, geneticists, mammalian taxonomist. But we don't have all that expertise in the room with us today. We will talk about what we should do about this.

Why The Panelists were Chosen

Dr. Murdoch – member of the National Academy of Science, eminent ecologist, reasonable, worked on a number of ESA issues, some with Dr. Courtney. He brings a familiarity with the review process, conservation biology,

Dr. Wayne – eminent scholar in canine genetics, well regarded in the specifics of wolf

Dr. Fallon – familiar with ESA and the application of the Act, and has been working on issues of wolf genetics. She works for NRDC which has at times sued the USFWS. Dr. Courtney doesn't see that as a conflict of interest. This is a meeting about science. Dr. Fallon agreed to separate the issues of policy and science. She committed to engaging in a scientific discourse.

Dr. Wilson – eminent Chair in Canada who has worked on many issues in genetics, published in the area of wolves.

While we are well qualified in the area of genetics, we don't have everyone we hoped to have here in the room today. We will need to find a way to bring that side of the conversation in on mammalian taxonomy. We did approach these folks, but had a late cancellation.

USFWS wants, needs, and is committed to a completely impartial assessment. NCEAS agreed to help. Thanks to NCEAS to design a process that could create a good administrative record. Thanks to all panelists for stepping without any recompense to ensure we have a good clear record of discussion on short notice. We are all committed to good science, good conservation, and a good discourse.

We did talk to other people about being reviewers. The only people involved in the process were Dr. Davis and Dr. Courtney. Not the USFWS. Dr. Courtney did seek expert opinion about who might be good to be involved in the panel. The only people who made the decision of who would be invited were Davis and Courtney. One reviewer candidate turned us down based on workload reasons, and another withdrew at late notice. NCEAS solely is responsible for this process, the agenda, the format, which reviewers are at the table.

There is a diversity of opinion amongst us about how to interpret some of the information. Courtney doesn't think this is a problem. With regards to process – our goal is to understand the full range of scientific opinion. It is ok that everyone doesn't agree. Everyone here has committed to professional discourse and to explore any differences the panelists have. It is not our job to come to a uniform decision. We want to understand and help the Service to understand why there is a diversity of opinion.

We don't have all the skills we might prefer, particularly mammalian taxonomy. During the course of the day we should be thinking about this, since it would require expanding this process and that would include the four panelists. One alternative we could consider is to have Dr. Courtney seek out 3-4 mammalian taxonomists to feed into our review. Another possibility is to hold conference call.

I don't want to overload you with additional demands. What should we do now since we may not have all the expertise we need in the room? We will have this discussion later in the day.

Consensus

All the panelists have participated in or watched the peer review process. Often this process concludes in consensus, but not always. What we do is different than academic peer review. For one, it is all out in the open. There is no question of majority rule and there is no right or wrong. Every opinion matters. Everything goes on the record. Everything is being recorded. We are going to have a conversation about understanding the science. Sometimes the minority is correct. Every scientific advance starts with a minority opinion!

We are not trying to get to the definitive statement of what constitutes the best available science. The final product of this process will include everyone's individual opinions. We will not create one single report from all of you, instead you will each write up your own opinions and Dr. Courtney will synthesize reports into an overview. Understanding the scope of diversity of opinion is important and will help the decision makers.

Everything is being recorded. At the end of the day, Dr. Courtney will create an administrative record including every email that has been exchanged and all conversation today will be part of that record. This is important for transparency. There will be breaks today if the panelists need to have a quiet word with Dr. Courtney about process.

Role of the Fish and Wildlife Service

They will be muted once we start. They will have two roles. Firstly, they are our servants for the day. They are available to answer the panelists' questions. Secondly,

they are observers so that they can better understand our final products. The process has been established with USFWS - they are be at arm's length (not in the room) so that there can be no assertion that they have swayed the discussion. They can't speak up directly. If they have something to say, they must text or email Dr. Courtney – but they cannot directly interact with the panelists during the discussion. If the panelists have something they want to ask the Service, we will put them on mute, talk about the question you want to ask. We will then put USFWS on speakerphone live so we can ask the question. The Service is unable to influence what happens today.

How we will proceed

The USFWS has asked us to address four specific questions. For each question, Dr. Courtney will invite the panelists to comment on the question in turn. Then we will have a conversation, some to and fro which Dr. Courtney will moderate. The discussion can range beyond the questions as narrowly written. If it goes seriously off track or to discussions about policy, Dr. Courtney will intervene to keep the group focused on the science. Courtney will be directly in control of the process.

Dr. Wayne How much time do we have for our comments?

Dr. Courtney Take as much time as you need but it is important that we have a discussion about each of the questions. The conversation is important. To that point, Dr. Courtney has indicated a time schedule for each question on the agenda, this is only a guideline and the panelists can take as much time as we need. Dr. Courtney will help guide the discussion to be parsimonious with their time.

Reminder: Ms. French is also taking notes and she will be preparing these and will circulate them swiftly – early January.

Dr. Courtney is hopeful that each panelist can complete the majority of their commentary today. He is mindful that he has asked a lot from the panelists with their participation.

Reiterate: Panelists do have some control over the agenda. Follows established procedure.

Not in the Scope of Work.

How many wolves are there? What is the listing status or what should it be? What's the value of wolves to ecosystem function? What is the meaning of recovery, what are recovery standards? These are all very important to the Service. They are key issues, but NOT *our* key issues, and not the subject of the very specific review. Given our academic strengths, there is limited value for us to weigh in on these issues at this time. Dr. Courtney will focus the discussion on the areas where the panelists can be most helpful to the Service. He will not police the panelists' comments and will not close off anything the panelists chose to discuss.

If panelists elect to include comments that are not a part of our scope of work in their commentary, Dr. Courtney will not redact these statements in the panelist's commentary. Instead he will keep everything on the record, but may italicize the comments that he thinks are off topic, or he might comment on these comments. Courtney will flag the comments and the Service will see them but may or may not view them as part of this review.

The public comment period may be reopened. This would be another opportunity for panelists to submit their views on topics that are not the focus of this discussion.

Questions about process?

Dr. Wayne Are all of our discussions today a part of the public record?

Dr. Courtney Everything is public. This is not unusual. The recording will also be a part of the public record.

Dr. Wilson No questions.

USFWS No questions.

[USFWS goes mute and the proceedings will start after a 5 minute break.]

Question 1 from the USFWS

Did the Service consider the best available scientific information, including the scientific literature, in developing this proposal? Is there additional biological, commercial, trade, or other information relevant to our analysis of the current *C. lupus* listed entity that we did not consider, but should?

Dr. Wilson I need help with the scope of the question, I need clarification on the timeframe – is it the last date of the report?

Dr. Courtney Science is always a moving target. USFWS has to make a decision at a given moment of time. Our task is to ask did they look at the best available at that moment in time. However, they haven't made a decision yet and are collecting more information. Although our comment is retrospective on what the Service has already done, if there is new stuff out there, it behooves us to let them know.

Dr. Wilson I am going to limit my comments to genetics and taxonomies. Given the date of the proposed ruling, the Chambers et al. report was current at that time. I am qualifying my answer to my area of expertise and because I only had time to review in detail the genetics related sections of the report. I did not have time to review all the literature available or cited. Instead I focused on the area that I am most familiar to make sure we have a solid assessment of these.

Dr. Courtney Dr. Wilson, you sent some more current papers to me this morning and alluded to some new work underway?

- Dr. Wilson** Our group has a couple of papers out in Ecology and Evolution that deal with some characterization in the northeaster distribution which I think are relevant. There is also an early online version of a Molecular Ecology paper by Roland Keyes and group also has important information. I am not still xxxx, but there is some work expanding at Trent University on SNIPS in the C. lycaon context and followed up with some new simulations, but these have not been submitted for publication yet
- Dr. Courtney** Best available scientific and commercial information. The Service will look at all available information, but it has to be available. I am sure the USFWS will consider all that is available and hopefully there will be some effort to get that information to them. It is helpful to know what is not yet available that they might want to look at.
- Dr. Wilson** I have given you the contact for the postdoctoral associate who is doing this work. I don't want to speak on her behalf, but she would be the best contact to find out the status on this.
- Dr. Fallon** Besides anything that has been published since the rule was issued, I don't think there is a glaring absence of the science that they looked at. But the rule is highly dependent on the Chambers et al. document. This is part of Question 3 later today. We can talk about whether Chambers is the best available discussion later. However, I want to flag one of my concerns with the proposed rule and decision on taxonomy, which is illustrated by the fact that Dr. Wilson has already identified a number of scientific research studies in press or in prep that would further add to this discussion. This is an area of active research. This indicates to me that it is premature for the Service to come to have come to the particular conclusion they did on the taxonomy.
- Dr. Courtney** We may want a second phase of this review.
- Dr. Murdoch** I just want clarification what Dr. Fallon said. Was your answer to the first part of this question is "yes" the Service did consider the best available literature at the time?
- Dr. Fallon** They considered available science, but I disagree with what the Service concluded was the best available science at the time when coming to their conclusions.

Dr. Courtney I take your point that there is a lot of new stuffing going on all the time, but the USFWS did not ignore anything at the moment they made their decision.

Dr. Wayne I concur with everyone else, that with regards to wolf taxonomy the proposed rule and the Chambers documents are comprehensive. However, with regards to two other things there are glaring insufficiencies in particular, the review on subspecies and species concepts. The whole Chambers document is based on a taxonomy framework suggested by John Avis and Ball in the 1990s. That taxonomic framework while still useful is now two decades old and the field has evolved. A whole new framework for understanding genetic divisions within species that can be put into a sub-specific taxonomy has evolved. Chambers dismissed this research in one caveat toward the end of the document by saying that these new approaches and ideas, which are population genetic-based and evolutionary-based, are “non-taxonomic”. I find this not to be correct. They are taxonomic and can be placed within a taxonomic framework just as easily as the Avis and Ball approach can. They have to address the recent literature. There have been some excellent reviews by Fred Alendorf and Louis Bernache, leaders in the field, about how one defines genetic units or partitions within species.

The Avis and Ball approach is not used very much for mammals and especially not for species like the wolf where there is so much gene flow. The expectation is not reciprocal monophyly. It is what Avis called a Type 4 species where there is a mixture of mitochondrial DNA haplotypes across the species range. I am focusing not on species taxonomy, but the sub-specific taxonomy which is so important because the number of subspecies in wolves have gone from 24 to 3. So clearly there is a problem with defining that taxonomy, which could benefit from the use of modern evolutionary and taxonomic thinking.

Dr. Courtney Clarification. You have given some references. For the record, in your opinion, the work described in the decision is insufficient?

Dr. Wayne The works I cited are reviews in Trends in Ecology. They are reviewing a whole field of thought about how one defines genetic units within species from a population genetics perspective.

Dr. Courtney These reviews should be both incorporated but more important they should be a reanalysis of the overall set of materials in the light of those fundamental reviews.

Dr. Wayne Yes, I think the framework is incorrect. The reason Campbell et al. chose the Avis and Ball approach is not well justified anywhere. It is never well defended especially against the modern thinking about how one might define units within species. Everything else follows from the initial decision about the framework they used.

Dr. Wayne The second concern the fossil record needs a thorough analysis. I don't think they do service to it. Of course, there are taxonomy problems there. Ron Nowak is not the only expert who has worked on the fossil record of canids. A new resource tries to compile the full vertebrate record across the United States. They identify what they call *Canis lupus* in the East Coast – there are several specimens. Whether this is legitimate or not, I don't know, but someone needs to take a serious look at this. It is instrumental in the idea that another kind of wolf was present on the East Coast. They need to solidify the evidence for there being a new kind of wolf in the eastern states. Ron Nowak thought they were red wolves. Their whole case for the revised taxonomy relies on a careful analysis. This also shows dire wolves so you can see in the rancholabrean era more than 10,000 years ago. You can see how abundant they were. So there is a case that there may have been two types of wolves in the area defined by the fossil record, maybe they interacted? The Service has to confront that evidence and they have done a very poor job of it.

Dr. Courtney These are two slightly different commentaries. One is something was missing. The second point is that it was not well put together.

Dr. Wayne No, they are both the same thing. The detailed citations to data on the fossil record were missing, so the scientific literature is not well covered of the paleontological record.

Dr. Murdoch I don't really have anything to add.

Dr. Courtney We will take the discussion in turns. I don't want to get into you are right you are wrong. But there seems to be a general sense that the review work is adequate but not forward. Anyone is free to pick up the conversation at this point.

Dr. Murdoch I do have a question about Dr. Wayne's concerns. The question is, did the Service consider the best available scientific information? Bob, I thought you originally answered the question, "yes" they considered it.

Dr. Wayne With respect to wolf taxonomy, recent work on wolf taxonomy, I think they are comprehensive. With respect to how one uses taxonomy, the recent literature of taxonomic definitions below the species level, I don't think this is considered. It is not just a few citations, but a whole body of research missing. Likewise with the paleontological record, there are missing references, and he will provide reviews of this literature.

Dr. Courtney If we have suggestions for the Service about next steps, it would be helpful. While it is not our job to tell them what to do, any suggestions would be appreciated.

Dr. Wilson I agree with Dr. Wayne's comments. The question is fairly large in scope and only felt like I could comment on my area of expertise. I couldn't comment on the full document and its limitations.

Dr. Fallon I agree with Dr. Wayne discussion on the taxonomic species. I find the rule to be so heavily based on Chambers et al., I am still holding off on other comments until later.

Dr. Courtney Any suggestions for USFWS? We are free to comment on "Maybe you would want to talk to X" or "you should assemble a group on Y".

Dr. Wayne At a subspecies level, the Service needs to read the literature and take on a new taxonomic framework based on modern evolutionary thought. The framework that they based their analysis on doesn't incorporate modern molecular techniques. I think they need to take on a new framework and revisit their analysis and conclusions. I don't think it would be that hard, because all that information is out there.

Dr. Courtney I will make a suggestion to the Service. Some time back I ran a workshop, a “teach-in” for the Federal Government on modern evolutionary biology. We talked about how different types of scientists use different methods species/subspecies, etc. How do you operate in an environment with different taxonomic approaches? There is a need for consistent rules and how to apply modern genetics to these consistent rules. The Service needs to bring these ideas on board.

Dr. Wayne I would even be happy even if they read and discuss this other body of literature but then chose to use Avis and Ball because of the following reasons. They could then defend their decision in the light of the modern evolutionary thinking. Instead, they simply provided a disclaimer at the end, that they are not using these other methods because they are “non-taxonomic”.

Dr. Courtney Is there anyone you would recommend the Service talk to?

Dr. Wayne I could suggest the following and will provide a whole list:

Keith Crandall recently published a paper in Trends in Ecology

Fred Alendorf has published several reviews

Robin Waples – NOAA

Louis Bernache at LeValle

Question 2 from the USFWS

Are the assumptions, analyses, and conclusions reflected in the proposed rule reasonable in light of the best available information?

Dr. Fallon I am most concerned about the Service’s dependence on the Chambers et al. as the best available science. Therefore the conclusions they drew are not correct because they are based solely on the Chambers study.

Dr. Courtney What I think you are saying is that everything hinges on Chambers et al. Everything flows from that.

Dr. Fallon Yes, and it is a big thing because it is so central to the entire rule.

Dr. Wilson I can appreciate the comment that everything stems from Chambers et al.. On some topics there seems to be a comprehensive discussion, but on others there seems to be a bit of a disconnect, particularly around some of the assumptions on the red wolf. Some of the new literature I recommended on the first question might be applicable here. Particularly around the assumptions that were made when the Y chromosome marker was quickly tagged as being from dog. One of the critical steps moving forward, is to recognize that some of the taxonomic labeling of some of these markers, we may not be able to make those assumptions. This is one of the taxonomic questions which we may not be able to resolve given the current status of things on the east coast. Whether you call that Y chromosome from dog or wolf will have a major impact on your interpretation and recommendation. I have concerns with some of the statements about it is common in dog but doesn't close the loop that the alternative may be that it could also from *C. lupus*. Some of the discussions and decisions hinge on the question of whether *C. lupus* inhabited the East. These are pretty fundamental aspects. The assumptions were there but it may have been premature to make those types of taxonomic identifications on those types of molecules. This is a major concern around the specifics related to the red wolf component.

Dr. Wayne The entire proposed rule was based on the acceptance of Chambers et al., I find many problems with the definitive conclusions drawn. Particularly with regards to *Canis lycaon*, I think there is still a controversy out there. There are some groups out there are troubled by the adoption of the Eastern Wolf Species including my own group, Roland Kay, and Jennifer Leonard out of Spain. I am not saying we are right, I am just stating for the record that there is still a controversy out there. I agree with Dr. Fallon, that the controversy requires more data and there is new data being generated. It seems to me that it is a very odd time for the consensus being outlined in Chambers et al.

The second concern is I am surprised that the proposed rule just accepts so uncritically the conclusions of the Chambers report. Of course they can't be correct in everything they say. The USFWS is entitled to have its biologist write a report, and then utilize that

information. But it seems the rule should be far more critical instead of whole scale acceptance verbatim of what Chambers et al. have said.

At the sub-species level, as I have mentioned, I find the taxonomic framework and the analysis in Chambers et al. to be faulted, but it was accepted uncritically. I find the sub-specific analysis in Chambers very qualitative and not statistical at all. There are plenty of statistical analyses available for lupus that the USFWS could have drawn on. This is just one example of where the Service could have been critical of the Chambers report.

Dr. Courtney I know a little bit about process for USFWS. You said three things, but they all sound the same. You feel like the Service analysis insufficient from a normal scientific practice. I want to comment from a regulatory process. The things that we may all aspire to, publications, etc., the regulatory agencies may not have the luxury of. They have to make decisions on a specific timescale with whatever they have on hand, within specific budgets, etc. I am not trying to excuse anything, it is just the context. There is a fine line that we can help the Service negotiate by articulating what else could/should be done from a scientific point of view, versus other things that are really fundamental to making a reasonable and defensible decision. I want to get your sense of what you are articulating. Wayne and Fallon have asked “Why make the decision now?” It is out of our hands. The Service has its own processes that dictate this.

Dr. Wayne I am not questioning their motivation of making a decision now, you are correct, that is out of our hands. I am questioning whether we can make that decision now with the current state of scientific evidence. I feel with regards to species status, not all the data is in, and in that regard, I believe Chambers was too bold. With regards to sub-species, I will talk more about that when we address question 3. I regard their analysis insufficient and the Service should recognize these insufficiencies in their rule, and they have not.

Dr. Courtney (Tells a story about acceptable levels of risk and this is a policy decision.) You are saying it is premature to make a decision. I would like to recast this statement as saying to the Service that their decision is relatively weak and could be made stronger by doing these other things.

- Dr. Wayne** I am saying that there is not a scientific consensus yet to base these decisions. The genetic evidence is not strong enough. I am not talking about probabilities; I am talking about a commonly accepted scientific standard.
- Dr. Courtney** I am trying to get at the subjectivity of what's enough?
- Dr. Wayne** I believe the results are equivocal and not definitive enough to make firm decisions upon. And there is ongoing research on these topics.
- Dr. Murdoch** The answer to this question really relies on whether you agree of whether Chambers is the best available information. The service has made their decisions based on Chambers faithfully.
- Dr. Wilson** I have been nodding in agreement about what is being said. We are not to comment on policy. But, to factor in risk, is to comment on policy. I am in full agreement that Chambers is not the definitive answer to any of these questions. I don't dispute that there is still some a controversy about the species taxonomic question and from sub-species component too. What are you charging us to do from an assessment?
- Dr. Courtney** Thank you. That is exactly why I was pushing Dr. Wayne a bit. Trying to tease apart what is subjective and prescriptive "they should do this" versus a scientific assessment that "this is a decision based on a body of information that is likely to change and may not be stable in the long term". I am trying to help the Service qualify the quality of the information.
- Dr. Wilson** The risk is high. The risk the service is accepting is high. In term, with the reliance on Chambers the risk would be high moving forward
- Dr. Wayne** I would agree with that. I think if the Service said that there is too much controversy to make a decision – I would be happy with that. Rather than making a decision that there is one eastern species that occupied the entire eastern region – this is not well enough supported by the evidence. On the sub-species discussion two of the sub-species designations are not well enough supported by the evidence.

[Break]

Question 3 from the USFWS

The Service determined that the synthesis and conclusions of Chambers et al. (2012. North American Fauna 77:1-67) reflect the best available scientific information regarding taxonomy of wolves in North America. In doing so, does the proposed rule draw reasonable and scientifically sound conclusions concerning the taxonomy of the eastern wolf, *Canis lycaon*? (We are not requesting information on the status of *C. lycaon* because we are conducting a status review for this species and peer review of that document will occur separately.)

Dr. Courtney There are two ways to answer the above question: 1) If Chambers, et al. is the best available scientific information, then do all the USFWS analyses and conclusions flow logically from it? 2) Do you question the validity of Chambers et al. as the best available science?

Dr. Fallon If we are to assume for a moment that Chambers et al is best available, USFWS follows Chambers et al. as it recognizes species and subspecies, therefore all their policy decision flow from that. There are policy decisions that flow from the Chambers but I am trying to respect that we are not here to comment on policy. The ruling follows Chambers closely. USFWS splits *Canis lupus* into differ taxonomic units based on the Chambers paper. The ruling all hinges on the Chambers paper

Dr. Wilson If we first assume that Chambers is the best available, the USFWS decisions did flow from Chambers. However, even within Chambers there are caveats that were identified. When Chambers reviews the literature they state that this or that was not explicitly tested. Chambers et al. did recognize that the science is a moving target In some cases they are explicit about the potential alternatives not being tested or how one might test the question. Whereas, in other instances, such as the red wolf, that same threshold was not applied. To assume that it is the best available doc – they do identify the gaps then default to a recommendation. Chambers has flagged up these outstanding questions or provided criticism for the design or interpretation. Nothing flows from that. So it may in fact signal that maybe the available science that was used for the Chambers paper wasn't as good as they needed.

Dr. Murdoch If you decide that Chambers is correct – the rule did follow it very closely. Chambers may have had doubts, but their conclusions are the conclusions of the rule as far as I can tell.

Dr. Wayne I concur that the ruling follows Chambers et al. very well. I really like with Dr. Wilson's comment. I hadn't thought about that earlier. There are several places where Chambers does try to qualify the conclusions, identifying what's needed or where the data is equivocal, or where there are opposing opinions. And, all that falls away with the ruling.

I have one specific question. In the summary of the ruling, it says [Dr. Wayne quotes from the first paragraph of the summary in the proposed ruling pg 35664]. Paraphrased: The USFWS proposes to delist *Canis lupus* because best available science indicates that it is not a valid species under the Act and that the Mexican wolf (*C. l. baileyi*) is an endangered subspecies. I can not understand how a the parent species *Canis lupus* can be invalid under the Act, while one of its subspecies is valid? The subspecies is subsumed within the species. So if the Species is invalid, then by definition, the subspecies should be invalid?

Dr. Fallon It has to do with how the species is listed nationally in the Lower 48. The USFWS discusses how the worldly species of *Canis lupus* is not endangered, but it is listed in the Lower 48.

Dr. Wayne Is it just a range reduction of that species then?

Dr. Courtney Difference between science and the law. Species is a biological concept but defined differently under the ESA – a “species” is a listable entity that could include species, subspecies, DPS, PSU - all these can be described as a “species” under the Act. This is at the heart of the miscommunication here.

Dr. Wayne I have read through the history of the various revisions where they have changed the taxonomy or the range of one species. This to me seems quite analogous. This is what this seems like - a range reduction of the species – so why does the species need to be considered “not valid”? Analogous changes have been made at the species and subspecies level. This feels like the same thing - a geographic range change from occupying the east coast and Great Lakes regions to having a more restricted range in North America. Maybe our colleagues at the USFWS can answer this question for us.

[USFWS is put on mute]

Dr. Courtney Dr. Wayne raised the issue of asking the USFWS to address the question. Do we want them to weigh in at this time? USFWS may or may not be able to respond in a timely fashion. We need to think about how we would use the answer if it is delayed.

Dr. Wayne I would like to have their opinion.

[USFWS is put back on line]

Dr. Courtney We would like to ask a question of a to help us understand. Please identify yourselves

Maricela Constantino

John Oakleaf

Shawn Sartorius

Tracy Melbihess

Hilary Cooley

Dr. Courtney We are asking you to help us understand something. For the record, we are keeping you at arms length so you can't directly influence what we do.

The listed entity *Canis lupus* is described as not a valid species under the Act, what does that mean exactly? Is it simply a redefinition of the range on the basis of new evidence?

I am not encouraging dialog between you and the panelists.

Dr. Wayne In addition to the change of the range of the species, how can you call *Canis lupus* an invalid taxon, when you call a subspecies *Canis lupus baileyi* and the other named subspecies a valid taxon under the Act? This is not a criticism; we are just trying to understand.

Ms Constantino

I can answer this for you. For reference I am drawing from page 35673 of the rule where we talk about a reevaluating the current listed entity under the Act. We are not saying that *Canis lupus* is an “invalid species”. We go on to analyze the status of that species in the rule making. What we are talking about at the beginning of the rule is the “listed entity”. The listed entity, while called *Canis lupus*, what we are actually referring to is a smaller range of that species. At this time, the range is smaller parts of 42 US States and Mexico. This is a policy issue in my mind. First to consider the status of current listed entity, we first have to describe the species and subspecies, and then analyze the status of that. We determine that the listed entity is not valid because what we describe is the listed entity – not the whole species. It does not represent a whole of a subspecies either - it does not include the range of just one. So under the Act, for it to be listed it must be a DPS. Based on the history of the listing, the listing of *Canis lupus* predates the ability to list as a DPS, so it is incumbent on us to ensure that it satisfies the DPS policy that was determined in 1996. Through this analysis we have come to the conclusion that it does not qualify as a DPS and therefore it is not a valid entity under the Act. That is the first step.

Dr. Courtney For clarification, you are using “species” as synonymous with a geographic range or DPS.

Ms Constantino

Yes, we are analyzing whether the current listed entity is a valid DPS under the Act.

Dr. Wayne If let’s say we found out the range was in error because some historic material was identified as “dog” in Wyoming and gray wolves were not found in Wyoming. Let’s just suppose that the gray wolf has a range throughout the East Coast but we discover an error in the southern limits of the geographic range – that the range designation was less than what we had thought. Would this cause the species to be delisted?

Ms Constantino

There would need to be an analysis to consider if that is the appropriate course of action. I am not clear about your hypothetical. In this case, our analysis was dependent on two things: 1) based on the adoption of the information in Chambers et al. the eastern region was not a part of the historical range for *Canis lupus*. The original listing included large portions of the eastern region that were never part of the historical region for that species. In addition, we are also articulating that DPS, listed entities, are species that are currently in existence. Typically DPSs based on a population on the ground. The current population is not accurately described by a listing that includes all or portions of 42 States and Mexico. This listing is much too broad to describe the population that currently exists in the Southwest, and the few individuals that exists in the Pacific Northwest.

Dr. Courtney I think we all understand, thank you Maricela. Now, you can please go back on mute.

Dr. Wayne Refer to slide deck

I am responding to Question 3. The first part of the question we all agree that the ruling follows Chambers et al. well. The principal problem here is that Chambers is accepted uncritically, but we have made that point already. So the second part of the question is whether Chambers is an accurate document.

The first issue is the subspecies concept. Chambers uses a concept put forward by Avis and Ball in 1990 and further described in his book in 2004. The concept is called genealogical concordance. The notion is that there are a number of characteristics of a population that make it distinct from other populations and that those two populations are generally not sympatric. But they may interbreed when they do become sympatric, then we can consider them subspecies. This is geologic concordance because the notion is that the distinct population characteristics have evolved over time through evolution and genealogic divergence and isolation. This can work well for some subspecies such as the example of the pocket gophers in California where there are hundreds of subspecies. It works well each population is fairly distinct and the individuals are philopatric and don't disperse very far from where they are born. Consequently, they do tend to be isolated and accumulate distinct genealogical differences and even morphological differences. The years of research in large carnivores, particularly wolves, shows that rather than this distinct partitioning you have more of a gradient, a genetic gradient, without the distinct lines between subspecies. To be honest, this is

acknowledge in Chambers et al., but should have lead to different type taxonomic concept. Rather than the genealogical concordance concept that works well for pocket gophers, some concept that fit a species where there is a gene flow on a continental scale. The wolf can travel hundreds of kilometers before they settle in a new territory. Therefore, you wouldn't expect genealogical concordance.

Also, the range of gray wolves, which is all of North America, has been greatly diminished after the last glaciation. During the last glaciation Canada was under ice. Especially on the west coast of the United States, Canada was populated from refugia from the American west. There is historical evidence to support this conclusion. So there has only been a recent reestablishment over the past 10,000 - 15,000 years of wolves in Canada. So we have just that framework to work with to differentiate. Given that framework, you would never expect reciprocal monophyly to appear in those populations. There was just not enough time. On top of that, with all the add mixture and gene flow, there would never be the expectation of this strong genealogical concordance when natural selection is involved.

Slide 11 is from the Vila et al. 1999 paper, that was cited in the Chambers et al., They attempted to reconstruct groupings or subspecies within North America and Europe using a statistical test AMOVA (similar to ANOVA) where the partitioning of genetic variance is divided among groups and significance evaluated. Very few significant groupings expect maybe Alaska and Canada or Mexico. This is a statistical statement of groupings that could be used to support subspecies analysis, although that is not what they did in the paper. Since the time of this paper, a variety of other statistical approaches have emerged.

Chambers et al. puts forth three phyloclades or clades that fit the genealogical concordance concept, but in fact they could equally fit the notion of gene flow and this recolonization that occurred since the last glaciation. [Looking at the slide] These are not geographically partitioned; Vila et al. and subsequent papers conclude that phylogeographic partitions particularly among gray wolves in North America are nonexistent or are weakly supported. This is the basis of the entire subspecies designation. This kind of phylogenetic relationship is not well supported. This is an artifice that has been superimposed on the data. A real problem is the clade on the right – C. l. nubilus clade, the blue color on the slide is historic material, much of that clade is dominated by material from the American west Lyn et al. paper. That is where all the diversity comes from. If it was removed, the remaining green ones would be assigned C. l.

occidentalis. Wolves existed in refugia in the America west, since the glaciation that was the source for colonization.

Example from a paper on Brown bears four distinct clades that have changed over time in the fossil record based on DNA evidence. The genetic pattern we observe today is a veneer over this turmoil that occurred over the pleiocene extinction and recolonization.

What is not discussed in Chambers is the fact that much of the genetic data including the data in Vila et al. is better explained by patterns in ecology. A paper by Eli Geffen in 2004 shows the traditional measure of expectation of differentiation, called differentiation by distance. The farther apart two populations are the more different they should be. However, it does not explain much of the difference of variation for the mitochondrial data or for microsatellite data. What does explain the difference is the vegetation and climate – they explain 73% of the variation. Leslie Carmichael first described this. It is ecology that explains genetic patterns. Even wolves even though they disperse long distances are finding habitats that are similar to their natal habitats that contain their natal prey base. Carmichael figure (slide) from Molecular Ecology. I want to emphasize that all the wolves recolonized these areas from the American West 10-15,000 years ago following the last glaciation. In that time, they have set up genetic groupings that Carmichael found through structure analysis that correspond with ecology – boreal forest wolves, high arctic wolves, eastern barren wolves, tundra di Triaga wolves that follow the caribou, and coastal wolves – are all genetically distinguishable. More sound evidence for genetic differentiation and adaptation. This seems to me a more natural means to set up a taxonomy if you need one. This literature is now immense here (Jennifer Leonard, work at UCLA) and in Europe (Pilo). This is the dominant literature for canids' taxonomy and *Canis lupus*. It is mentioned peripherally in Chambers et al. and dismissed even though concordance concept allows for it.

Another example: Pacific Coast rain forest wolves are phenotypically distinct. They are smaller feed mostly on salmon and deer, very different from the inland wolves that feed on caribou and are bigger. These rain wolves and have a distinct genotype. Chambers dismisses these differences because they have a scenario of invasions into North America. But I don't think the fossil record supports this theory well. What has likely happened is that there has been colonization since the last glaciation. Differentiation and local adaptation are all important concepts that fall under genealogical concordance concept. [Refer to final table from publication in slide deck] describes beyond genetics to include differentiation on morphology, prey differentiation.

Chambers ignores 10-15 years of discussion of genetic partitions in N. America based on microsatellite data further supported by SNP data more recently by Bridget van Holtz.

Dr. Courtney Are you stating that there is consistency across whole range of characters and approaches - morphological, ecological, behaviors as well as a subset of genetics,

Dr. Wayne Chambers relies overly, heavily on evidence mitochondrial in the DNA where one wouldn't have expected. All these areas have been recently colonized. To achieve reciprocal monophyly requires time, something like 4N generations. This hasn't happened. That expectation is flawed. The use of mitochondrial DNA data is inappropriate. Discussion on *C. lycaon*, we agree is premature. In terms of the subspecific taxonomy, the lines were drawn around *C. l. nubilus* and *C. l. occidentalis* geographic ranges are really odd shaped. This is strange since wolves move all around the place. Chambers attributes it to patterns of invasions and then replacements. Replacement implies competition. We know wolves add mix more than having a different mitochondrial haplotype. Vila et al. suggested that Mexican wolf may represent an invasion. Another paper – maybe too recent for their analysis, even at the rate of morphology, it is really the ecological variance as with gray wolf in the Great Lakes region (the Journal of Morphology by F. Robin O'Keefe).

Dr. Fallon Building off of Dr. Wayne, part of the problem with Chambers subspecies classifications is that it relies heavily on Nowak classification previously that was based on morphology and then superimposes genetics on top. When they could have used genetics from the beginning or some of the new approaches described earlier. I agree it is a problem in the *C. l. nubilus* and *C. l. occidentalis* recognition. It is not clear.

The other issue is the Coastal type of wolf – this comes up in Chambers and in the rule in different ways. It is relevant to gray wolves in the Pacific Northwest. Chambers goes by the Nowak classification says they are *C. l. nubilus* but the rule talks about ecologic uniqueness in that area, but says that wolves in the Pacific Northwest are not going to be discrete or distinct from the wolves of the Northern Rocky Mountains. The data that Dr. Wayne just showed makes it clear that there is a distinct coastal type of wolves that are likely to inhabit the Pacific Northwest.

- Dr. Wayne** It was considered a subspecies at one time. I think it is notable that Chambers does criticize Nowak's analysis, because it a discriminate analysis because you presumes a number of groups and the membership of those groups, and then analyzes whether they are distinct or not. PCA takes a more unbiased view of variability. Nevertheless he relies heavily on Nowak for his taxonomy.
- Dr. Courtney** Break soon and we will send Dr. Wayne's slides to the USFWS.
- Dr. Wilson** No substantial additions on the *Canis lupus* subspecies designation.
- Dr. Murdoch** I can't address the issues substantively. The Chambers paper strikes me as a process paper. I checked the authorship of the paper and they are four people who work for the Service. I don't know how the USFWS works in general, but if you are trying to say "this is the best available scientific evidence" and then have the paper written internally? I am not even sure if it was published in a peer-reviewed journal? It seems to me that you would want the definitive document to reflect what the scientific community is arguing about it. So, even if they can't resolve the issues, they could lay them out in an evenhanded way. The process of creating something that is meant to be the "best scientific evidence" -- this strikes me as shaking ground scientifically, seems like you would want something with provenance scientifically.
- Dr. Wayne** I agree it is problematic. I would want the USFWS to have successful policy based on science. It is curious why they only involved FWS scientists and it be published in *Flora and Fauna*. I think it is a journal that hasn't had an issue since 1991. I understand that this is a large monograph, and what journals publish monographs now? But, they could have, and I think they should still distill out the core of their analysis that supports the rule and publish them in one of the naturalist oriented journals. Just to solidify it so that this question isn't out there. *Flora and Fauna* is a USFWS journal. It is not going to have the same legitimacy as if they had gone out to an independent journal.
- Dr. Courtney** The USFWS wants to use the "best science".
- Dr. Wayne** I agree and this may call into question the science because it hasn't gone through maybe the rigorous peer review process. Just calls into question the "best science"

Dr. Courtney I am impressed with the level of discourse this morning. Thank you. Break for lunch.

[Break for Lunch]

Dr. Courtney The Mammalogy paper and the Dr. Wayne's presentation have been delivered to Dr. Wilson and the USFWS. We are going to expand on what we covered before lunch and then move on to talk about question 4 from the USFWS. I want to make sure we have time to talk about the other things that we need to do – to complete the NCEAS review process, other reviewers, and possible "what next" steps.

But this is a panel driven process, so if you think we need to cover other things, please let me know. Let's open the floor back up on Question 3 and Chambers et al. as best available science.

Dr. Fallon One thing that I wanted to bring up earlier on Chambers. We talked about the conclusions about the rule being reasonable. One thing to flag is that the rule says that the results of recent molecular genetic analyses (Tyler, Rutledge, etc.) are the basis for their determination that the gray wolf did not occur in the eastern United States. It is not even referencing Chambers. I want to flag this because a lot of the scientists cited here have all said that whether or not *Canis lycaon* is recognized as a separate species, that does not preclude the existence of the gray wolf in the eastern United States. They have signed a letter and submitted it to the Service during the Midwest delisting rule. I have talked to Linda Rutledge again as part of this ruling process and she still feels that way. I want to flag it.

Dr. Courtney Since this is pers comm, I don't think we can submit it. I think we can flag it as perhaps the USFWS would want to follow up these folks to discuss further.

Dr. Wayne Perhaps citing these references in support of the presence of *Canis lycaon* in the 22 east coast states is incorrect. It is contingent on accepting the theory that the eastern wolf

had a large geographic range but these molecular analyses themselves only pertain to where their samples are from. Dr. Wilson do you have a perspective on this?

Dr. Wilson I agree with that. But more important, accepting a model of *Canis lycaon* doesn't exclude the presence of *Canis lupus* in eastern N. America. I don't think any statement was made to that it was to the exclusion of *C. lupus*. One of the papers I circulated on the Y-chromosomes indicates some evidence that those Y-chromosomes are wolf or dog. I don't believe those statements were made that *C. lupus* and *C. lycaon* are mutually exclusive in the eastern states of the US.

Dr. Wayne So, that statement seems incorrect. And, it occurs to me that the whole premise for the ruling is predicated on the gray wolf is not on the east coast. But, if there is no scientific evidence that the gray wolf is entirely absent from the eastern US, I don't see how this ruling. It seems like a fundamental flaw

Dr. Courtney A strong and accurate statement would be - You are all of the opinion that the presence of *C. lycaon* doesn't rule out the presence of *C. lupus*. This seems to be a confused issue in the Service's development of the ideas. This needs at the very least clarification. And, if it is based on a false premise, which you are of the opinion that it is at least a plausible idea, you are suggesting that this needs to be looked at.

Dr. Wayne The opinion of the panel is that this is an incorrect statement and that none of these papers exclude the presence of *C. lupus* from the east coast.

Dr. Courtney This is a useful pointer into something the Service may want to take a look at. Seems like it is a unanimous opinion from the three of you (Dr. Murdoch did not have an opinion). Everyone concurs. Dr. Fallon started the discussion here and everyone agreed with her. Dr. Wilson you are a specialist in the eastern taxon, do you want to weigh in here with regards to *C. lycaon*.

Dr. Wilson I can share what we know, beyond Chambers et al. The results are still consistent with an eastern evolved canids that's independent of the gray wolf is largely on the non-recombining molecule. So even Y-chromosomes up to the point of Chambers and the all the mitochondrial DNA – a lot of work has been done around those. There seems to be a

pattern where there is an eastern specificity, regardless of what animals they are in right now. There seems to be an eastern geographic separation and a divergence that would be consistent with a model of a *lycaon* entity. That is trying to reconstruct history based on some complex admixtures. I do think some of the Y-chromosome results right now paint a picture of that seems more complicated than just a lineage of the *C. lycaon* as an eastern wolf independent of the gray wolf. It doesn't preclude having some contact with *C. lupus* in the past. At this point given the data on the table (post Chambers) the big unknown to me, is what relationship the progenitor of these molecules on the east what relationship it had with the gray wolf in the past? So whether it is some level of wolf and then a gray wolf moved some genetic material in there both historically or recent, or whether this was a canid that through reticulate evolution picked up some *C. lupus*. While we have the eastern specificity in some of the markers I would tag as "new world" evolved that would put them closer to coyotes; there is a predominance of Y-chromosomes on the east, that is evident in the red wolf captive program, that are of *C. lupus* origin. A number of those are common within the dog breeds. I don't think that is a full test of what their origin is. I think there is strong evidence for some eastern entity. Whether you classify *C. lycaon* as wolf or otherwise, there is something different. The mitochondrial DNA and Y-chromosomes, in terms of the eastern specific haplotypes are divergent. I think some of the Y-chromosome work is opening up what some alternatives to what some of the relationships might have been: whether recent from dog introgression from when the Europeans arrived, or whether this represents something more historic, Pleistocene exchange. Once you have those Y-chromosomes assigned to a *C. lupus* old world lineage, I don't agree with the Chambers report where they assign those to dog and they cite the 2005 paper. In my mind, those haven't been explicitly tested. In comparison to other literature out there I think one issue comes up about what gets packaged up in reference databases that is used. With the vast number of the SNIPS that have been used and reading Roland Kays' paper (early online in *Micron Ecology*) they try to come up with a breakdown of ancestries. I think this is a great approach. But what I would like to see moving forward, is someone needs to tackle some explicit test that are about a *C. lycaon* that is not just a *C. lycaon* up and around the Great Lakes. There needs to be some reference database to test some of that explicitly. In the past work, I am not sure that some ancestries have been accurately assigned given some of these tests haven't been done. Chambers et al. doesn't describe that some of these tests haven't been done. I am not sure that Chambers can get to the conclusions they do without doing some of these tests. Some of my recommendations are post-Chambers, but they are the ones that have flagged these issues. Some of these issues are unresolved and need to be tested.

Dr. Fallon

I would love to see the new data on Y-Chromosomes. To me the question about the existence of *C. lycaon*, is that I haven't seen a strong nuclear signal for the species. The

species descriptions hinge on a couple of mitochondrial haplotypes and Y-chromosome haplotypes that are of different historic origin, some more divergent than others. It is difficult, especially when you are trying to distinguish between the existence of the species and the existence of hybridization between two known species, whether that hybridization occurred historically or more recently. I find it difficult to distinguish between those two if you are only looking at a few mitochondrial or Y-chromosome signals. For me the more convincing data has been the nuclear data. The nuclear genome has shown, particularly in the Great Lakes region, a greater affinity with what we traditionally call gray wolves. I understand that there are degrees of introgression with this *C. lycaon* type across the United States. For me, that is where a lot of the issue has been.

Dr. Wilson I agree. To get to the nuclear components is the critical next step. However, we need to test for specific hypothesis about a new world evolved wolf. I haven't seen it explicitly tested even with the large datasets of SNIPS. To me the strongest evidence is with the non-recombining molecules. I look at the maps in the paper – to me there is an Eastern diversification of those two non-recombining molecules. While those two molecules have been around for some time, I am not sure that are any less informative. The lack of recombination and their differentiation and divergence are part of the characteristics that make them interesting. Based on the Y-chromosome and mitochondrial DNA I wouldn't reject the hypothesis of *C. lycaon*. I would like to see the SNIPS genome scans more accurately test the hypotheses. There is a reliance on the Great Lakes samples as being a representative of *C. lycaon* and that certainly is part of the story. But that isn't really dealing with the relevant parts where the *C. lycaon* may have interacted more with the coyotes in the east. While I think the approach is reasonable, I don't think a representative database examining these diagnostic SNIPS have been explicitly included as terms of some of those alternatives. I wouldn't hang my hat on the two markers given there are Y-chromosomes from a *C. lupus* origin. There are only a couple ways to explain that – from an ongoing hybridization between an old world and a new world wolf, or that *C. lupus* did have some major role in forming in whatever is on the east coast. I don't think the data we have on the table can distinguish those two alternatives.

Dr. Wayne The most critical element is that the data do not seem to definitively support any particular origin hypothesis. I think the nuclear data is convincing in some ways in that it represents 48,000 independent loci. That paper was rebutted by Rutledge in the Biological Conservation paper. Their basic finding was that Rutledge's model of origin could not be excluded by the nuclear data. I think that is the agreement among us now is that no model of origin can be excluded by the current data. That's why to find the

Chambers et al. paper support so definitively one version about the eastern wolf disturbs us so much.

Dr. Wilson I agree. That is a fair comment.

Dr. Wayne The Rutledge paper does a reanalysis of the nuclear data and comes to a conclusion – the conclusion is not that the eastern wolf is a distinct species that uniquely evolved in the new world. The conclusion is that it can't rule out that hypothesis. I think this is a very different conclusion than that of the Chambers paper.

Dr. Courtney For clarification. False precision – stating something more precisely than is warranted. Are you all of an opinion that there is a communication of a degree of certainty that simply is not congruent with the information that is actually there.

Dr. Wayne The data in total give conflicting signals. And, the more we learn the more complex the scenario seems to be. Hopefully that will be sorted out with more nuclear data and more sampling. That is why we view this is so premature. We don't believe the data is sufficient to support one camp or another. That being said, there are mistakes in the Chambers paper on the SNPS data. The ruling suggests that SNPS may be under selection therefore they should be rejected. And, this is simply incorrect. Chambers et al. and the ruling seem to both suggest that the SNPS genotyping is an experimental method or it is rife with interpreted difficulties. As if it hasn't been standardized. It (SNPS) is the leading method for human genetic variation. There have been dozens of papers published using this same method to identify genetic variation. These are proven methods with its biases. However, it is not an experimental approach. It can be criticized in a scholarly way. However, the criticism in the ruling and Chambers was not balanced.

I am hoping in one years time we will all be in agreement on C. lycaon one way or another.

Dr. Wilson I think we are getting there, I really do. With regards to the imbalance in terms of presentation of criticism in the document, I agree. There are some pretty sweeping recommendations out of Chambers. Perhaps C. lycaon might have been a sweeping recommendation but we have been at it for a decade or so. The thinking is matured.

With Chambers there is a big leap in some of the recommendations in terms of the taxonomy where the thinking hasn't matured. In the big picture, the science is always moving. The reality is some of that thinking is converging.

Dr. Courtney We are all trying to help the Service. Is it another 10 years of mayhem before we can come up with some certainty? It seems that it is becoming more complex. I would be interested to hear from you, are we moving to something that is more like a consensus position in one year, five years?

Dr. Wayne I think we are and I don't think it will be 5 years, more like a few. In a year, we will be much closer than we are now.

Dr. Wilson I think in a year we will be a lot farther ahead than when Chambers was published. I am not going to say we will be at consensus.

Dr. Wayne I think we will find out more things for sure in a short period of time.

Dr. Fallon It is one thing of having an entire genome at your disposal, but sampling has always been an issue - both the geographic sampling of the currently canids on the landscape as well as the historical sampling. Do you think the sampling is available to tease this issue apart?

Dr. Wayne We have looked at the issue of historic samples used in the past, like those that Ron Nowak used. We've had some issues in getting permission but there are much better technics now available for extracting DNA and characterizing the nature of the material - so this could certainly be revisited. I don't know if we have plans or grants to do that. If we could get our hands on historical material we could certainly try the new approaches.

Dr. Wilson I would agree with that. There is another aspect that. There are a lot of intensive studies going on local studies like Roland's group in New York. They have characterized a lot of eastern coyotes and some other work here with Tyler. Some other graduate students are doing a lot of on-the-ground characteristics of what is wolfi-ness and what is coyote-

ness. Like Dr. Wayne was showing before lunch, where the ecology is driving some of the subspecies of gray wolves. There may be a way to elucidate some of the important chunks of different chromosomes in some of these intensely studied areas. While the historic stuff is going to be valuable, there still might be material on the ground. As long as everyone understands the complexities of how things might have gotten there. Some light might be shed on how these things may be adapted to more coyote or wolf-like characteristics.

Question 4 from the USFWS

Does the proposed rule utilize the best available scientific information and draw reasonable and scientifically sound conclusions concerning the status of the gray wolf in the Pacific Northwest United States; the gray wolf subspecies *Canis lupus nubilus*; the gray wolf subspecies

C. l. occidentalis; and the gray wolf subspecies *C. l. baileyi*?

Dr. Fallon I feel like we did talk about the Pacific Northwest and the subspecies of *C. l. occidentalis* and *C. l. nubilus*, but we haven't talked about *C. l. baileyi*. The reason we haven't talked about the Mexican wolf is that it is one of the lesser controversial taxonomy and seems to be the most differentiated from other gray wolves.

Dr. Courtney What is the basis for saying that? There is a whole suite of characters we are looking at - ecology, morphology, mitochondrial, nuclear, behavior, eco-type.

Dr. Fallon In terms of its ecology and genetics and morphology.

Dr. Wayne I agree with that. I think there is a problem in the evaluation of the status of the Mexican wolf but I will come back to that. I want to say, the numbers are in the ballpark reasonable but the whole discussion is all predicated on whether their taxonomy is correct. I agree that *C. l. baileyi* is a subspecies, but I don't agree with the other two (*C. l. occidentalis* and *nubilus*). If you instead viewed five or six subspecies, some of them being geographically restricted, like the Coastal wolf, then you might have viewed them as threatened at least and have to evaluate a whole set of impacts. So if you accept the

taxonomy may not be well supported, then you would have to say the status discussion which is based on that taxonomy, which groups these enormous wolf populations together, is not accurate. It assumes this taxonomy is accurate.

With regards to the Mexican wolf, I do think the geographic range is an issue. It is defined based on a limited number of specimens and there are ecological arguments that would indicate that their geographic range was much larger. So in terms of the geographic or historical range of the Mexican wolf that this is well supported or argued. My biggest issue here is that the whole status argument is based on a taxonomy that is not well supported.

Dr. Courtney On the *C. l. baileyi*, Dr. Fallon has indicated that it is the most distinct population, would you agree with that?

Dr. Wayne I would agree with that. It has the most distinct mitochondrial DNA sequence and haplotypes. It is the most distinct in terms of microsatellites. It is also distinct with regard to our SNPS assay. However there was a paper published by Jennifer Leonard about the historic wolves of the American west that found the distribution of this diagnostic mitochondrial DNA haplotype, and it is only one marker, goes beyond the historic range as if there was a large area of admixture between the Mexican wolves that stretched into the Rockies and farther north. The genetic data, which is a strong indication of where the genes are, suggests that the historic range is not adequate. While the admixture is mentioned, it is not taken into consideration when considering the status.

Dr. Courtney Clarification. When it comes to drawing a line on a map that would be a problem. It is not that there's an issue with the entity. But where to draw the line would be difficult.

Dr. Wayne Correct. When you take a typological view that there are an entity that can be defined by a boundary. With the wolves that can be very hard to abide by. To their credit Chambers and the rule mention calls these fuzzy boundaries. More realistically, where there was appropriate habitat in the Southwest, there were probably Mexican wolves. So I think they should move to utilize the historic data of the range for the Mexican wolves and look for habitats that might have been appropriate. You must understand that when sampling if you only have a dozen or two dozen wolf samples that you are

going to base the historic range on, you know you are going to miss populations. I think the reconstruction of the historic range is a real problem with the Mexican wolf.

Dr. Wilson I would agree with Dr. Wayne but I am much less familiar with those issues.

Dr. Wayne In the Leonard et al. paper, we found a southern clade that contained the classic canonical Mexican wolf mitochondrial DNA and also two other related ones. This makes sense because if it was isolated for a long time you would expect some evolution in the tree. Then the southern clade has a larger distribution.

Dr. Courtney Move on. Talk about next steps, then take a break and think about what you would like to go on the record as your closing statements.

NCEAS was tasked with getting a set of opinions on several aspects of wolf biology. We have heard both a diversity of opinions and previous views on genetics. I want to compliment you on how it has played out and how much agreement there has been. It has been very productive. I think there are still a few missing pieces. I would like to suggest that our product, I would like to add a step to the process. I was hoping to have all our work done today. I am suggesting that if we can get our write up done of what we have done today, and each of you to write your reviews – maybe we want to show our results to some other people. I would like to reach out to a few more geneticists, partially because the level of concordance surprises me. I want to make sure that when we go forward with that level of concordance, it is seen as more than just the three people I happen to have selected. I also think we might want to find the other pieces like taxonomic experts.

Do you think that is a good idea? Are there additional fields we should look at? Do you think it is a waste of time since we got such concordance?

Dr. Wilson What additional information some reviewers will bring to the table other than this needs to go forward and some of these questions need to be addressed. I think that's we are in concordant is that it is open and we have to get additional information for consideration. I guess I don't see the value of other reviewers. Are we supposed to then respond to their opinions?

Dr. Murdoch You have three people here who know about the genetic aspects of this and they come from different areas and backgrounds. I guess I don't see why you would need additional input in this area unless you know there is a whole slug of geneticists who are not going to agree with these three. I am not sure how you can be a taxonomist without knowing something about genetics. If you think that taxonomists have a really orthogonal view of all this and might disagree with all of this, then you might want to get another opinion on what's been done. Dr. Wayne does taxonomy. If you think they might have a different view then it might be useful.

Dr. Wayne I could see if you had disagreement, seeking other opinions might add depth to the disagreement. But this leads me to ask then what would be the conditions when you might not need additional input? You have three experts leading players in Canis taxonomy who are in agreement. I am always one who would say, yes let's bring in more opinions. But we are in agreement amongst ourselves. I would lean toward "no" too.

Dr. Fallon I agree that had there been disagreement, then it would be good to bring in additional people to review and weigh in. But there is agreement, and as Dr. Wilson said, that the agreement is that there this issues that are unresolved, therefore not resolved as Chambers had depicted. I think that Chambers is controversial because it is asserting a new taxonomy. It is not that we agreed to assert a different taxonomy that would then need to be reviewed by someone else. We are just saying it is premature to go as far as this.

Dr. Murdoch And ill-founded is a word that comes to mind. The Chambers paper is not based on what is available in the way of science.

Dr. Wilson I think it is important to articulate what we are in agreement on. It is not like we have come to an agreement on a new model. As Dr. Wayne said before lunch, we are agreeing that there is a controversy still. That is the reality.

Dr. Courtney That's real progress. Time to take a break.

[Break]

Dr. Courtney I am going to ask each of you to comment on the process and how you feel about the technical materials we have discussed. Now is your last opportunity to be heard by the group. I remind you that you can write anything you wish in your individual report. I will summarize your reports, Ms French will write up her notes from our discussion today. You have agreed that we don't need to circulate our collective report. I will write up a process report. It is all due by January 31, 2014.

Dr. Murdoch It has been a very good process. I am not an expert on the topic, and have enjoyed listening to the discussion. I feel that you have covered all the critical issues. I think the whole thing has worked well. The three scientists who have expertise on the issues have brought up topics that sound to me to be need attention. I know a bit about biology and the ESA and these seem to be substantive issues that you can't just skim over. I do have this problem with the Chambers document. If you want to have a document that is the current scientific statement on a controversial topic, you would want it done in a way that everyone else will acknowledge is appropriate. If you do it in house and publish it in a journal that is not really peer-reviewed, a non-standard journal - this strikes me as not a strong enough way to go about it.

Dr. Wayne First, with regard to the process, I think it is great that there is an independent panel like this and you have run it well. A number of the most pertinent issues were raised so I am pleased with the process.

With regards to the proposed rule and Chambers et al. they are comprehensive in regards to taxonomy and it represents a lot of work and a difficult task. The USFWS should be acknowledged for the effort. And it is an important issue. These are the two things that are right with both. Things that were troubling:

The taxonomic framework and the over reliance on the Avis and Ball's genealogical concordance approach where that is predicted to be unlikely given history of *C. lupus* and the recent expansion into Canada and elsewhere. The differences between populations are not likely to be mitochondrial bases because there hasn't been enough time for reciprocal microphyly but are likely to be in the differences of allele frequencies or haplotype frequencies and that kind of work has been done. It shows that there are distinct differences that mostly accord with ecology in North America for *Canis lupus*. So if one accepts their framework, the question is whether *C. lycaon* is a subspecies. The

scenario is complex. No single hypothesis can be advocated without contrary evidence. So I think we all feel that it is still controversial so this is not the time to be so definitive on taxonomic scenario. I know these papers, and none of them suggest that *Canis lupus* is absent from the eastern United States. The whole argument for the ruling hinges on that, so if you can't provide definitive evidence, then one can't buy the argument that *Canis lupus* as a listed taxon isn't valid

With regard to the subspecies issues. With the indicated taxonomy and three subspecies (*C. l. nubilus*, *occidentalis*, and *baileyi*) with *C. l. occidentalis* and *C. l. nubilus* having a very large expansive geographic range with no subspecies or even DPS's that merit protection is not accurate given state of genetic data that has been derived from Kirk Strobach's group, our group, Jennifer Leonard's group and even the information from Europe. These suggest that there is genetic units often accords with ecology that could be incorporated into a taxonomic framework. Especially pertinent is the coastal wolf along the coast of British Columbia often called the rain wolf which has phenotypical evidence, ecologic evidence and genetic evidence with regard to microsatellite loci and with SNIPS that would identify it as a distinct taxonomic entity. And identifying it simply as part of a larger subspecies is incorrect. Finally, with regard to the Mexican wolf, we have evidence that its supposed geographic range and therefore its status is not correct because historic evidence suggests that it had a larger geographic range. This makes an important point about its status, if that status is predicated on the taxonomic framework put forward in Chambers et al. at the subspecies level, then the status at that level is also questioned.

Dr. Fallon I am happy to summarize in a written report. I think the process has been fine, I think it is essential to have both Dr. Wayne and Dr. Wilson participating. I think it is telling that there was consensus opinion about the topics we discussed. I think this supports the idea that Chambers et al. is not accepted as consensus scientific opinion or best available science for supporting the wolf ruling.

Dr. Wilson I must admit that I was not sure of what to expect with the process and I was surprised with the flow and discussion. Not to get into any specifics about the Chambers et al. paper, but my read, that moment in time, their interpretation and assumptions even those that they recognize – they can't close the loop on it, even with their own identification of those issues. Our consensus here is that it is a complex issue. We haven't left it open-ended saying that science is always evolving. We have two individuals who have studied wolf genetics saying that it is within reach. I would hope that the USFWS would take advantage of a narrow window of agreement. But moving

forward, some of these things need to be put into place before some of the recommendations could be put into place. I do think there are some sweeping recommendations in Chambers and if they are going to set that benchmarks it need to be done appropriately and they need to build on what has already been done. I do also hope that as part of this discussion, it might be viewed that whatever iteration or document that comes out of it, that there is an arms length constructive review of the genetics and the issues, outside the USFWS biologists. I am not criticizing, and I agree with Dr. Wayne that they did a comprehensive overview and it is a complex issue. But, from the optics and to even be effective, to have that arms length, building on what's we've discussed in this meeting would be beneficial in the sort term. I don't think it would be open ended or a long dragged out processes, there would be an end in sight.

Dr. Courtney The USFWS service participants are welcome to come back online at this time. I hope you have all found the day as useful as I have, as comprehensive, productive, straight forward, and with a surprising consensus product. I know it is hard to be on the a conference call all day. I want to thank the four panelists for a collegial process. I want to thank NCEAS for providing all the resources that were harnessed on short notice. We will try to get the final product to the folks at the Service as quickly as is possible. I want to say thank you to all of you and congratulate you on a good process.

Ms Constantino

Thank you for extending the opportunity for us to listen in today. I hope that the one clarifying point that we provided was helpful. I did find it useful to listen in today. Thank you.

Peer Review Statement of Work

National Center for Ecological Analysis and Synthesis (NCEAS) Sponsored Independent Expert Peer Review of the Scientific Findings in the Proposed Rule: Removing the Gray Wolf (*Canis lupus*) from the List of Endangered and Threatened Wildlife and Maintaining Protections for the Mexican Wolf (*Canis lupus baileyi*) by Listing It as Endangered

September 23, 2013

1. Introduction/Background

The U.S. Fish and Wildlife Service (Service) evaluated the classification status of gray wolves (*C. lupus*) currently listed in the contiguous United States and Mexico under the Endangered Species Act (ESA). Based on our evaluation, we published a proposed rule on June 13, 2013 (78 FR 35664), to remove the gray wolf from the List of Threatened and Endangered Wildlife but to maintain endangered status for the Mexican wolf by listing it as a subspecies (*C. l. baileyi*). We proposed these actions because we determined that the best available scientific and commercial information indicates that the currently listed entity is not a valid species under the ESA and that the Mexican wolf (*C. l. baileyi*) warrants listing as an endangered subspecies.

In the course of making these determinations, we recognized three wolf species with ranges in the contiguous United States: *Canis lupus*, *C. rufus*, and *C. lycaon*. We interpreted the results of recent molecular genetic analyses and morphometric studies to show that the mid-Atlantic and southeastern states historically were occupied by the red wolf (*C. rufus*). We accepted and relied upon a recent taxonomic review and synthesis that concluded that the gray wolf subspecies, *Canis lupus lycaon*, which occurs in southeastern Canada and historically occurred in the northeastern United States and portions of the upper Midwest United States, should be recognized as a separate species, *Canis lycaon*. In light of the above, we found that the best available scientific information indicated that *C. lupus* did not occur in the eastern United States.

The proposed rule also constituted the completion of a status review for gray wolves in the Pacific Northwest initiated on May 5, 2011. Finally, the proposed rule replaced our May 5, 2011, proposed action to remove protections for *C. lupus* in all or portions of 29 eastern states. Upon publication of the proposed rule (June 13, 2013, 78 FR 35664), the Service opened the public comment period on the proposal. Public comments will be accepted through December 17, 2013. Specific guidance on how to submit comments is posted on our public website (<http://www.fws.gov/home/wolfrecovery/>). The Service intends to reopen the public comment period on the proposal in early 2014 in conjunction with the submission and posting of the peer review report. The Service expects to make a final determination regarding the proposed rule by December 2014.

In accordance with the Service's July 1, 1994 peer review policy (59 FR 34270) and the Office of Management and Budget's December 16, 2004, Final Information Quality Bulletin for Peer Review, the Service is subjecting this proposal to independent expert peer review. The purpose of seeking independent peer review is to ensure use of the best scientific and commercial information available and to ensure and maximize the quality, objectivity, utility, and integrity of the information upon which the proposal is based, as well as to ensure that reviews by qualified experts are incorporated into the rulemaking process.

The National Center for Ecological Analysis and Synthesis (NCEAS), a research center located at the University of California, Santa Barbara, has agreed to sponsor this peer review and an NCEAS associate, Dr. Steven Courtney, has offered to donate his time to organize and manage the peer review process at no cost to the Service. This Statement of Work describes the nature of the peer review process needed by

the Service.

2. Description of Analyses/Service

The purpose of this review is to provide an objective, independent, external scientific peer review of the information in the proposed rule. The proposed rule is 246 pages long and synthesizes the existing best available scientific and commercial information regarding the status of various gray wolf populations and subspecies that occur within portions of the lower 48 States where the species is currently listed. Factors to be addressed in the peer review include the scientific merit of the proposed rule's primary analysis components (i.e., gray wolf taxonomy and status) which provide the basis for the proposal. The peer reviewers should confirm that any scientific uncertainties are clearly identified and characterized by the Service, and the potential implications of the uncertainties for the technical conclusions drawn are clear. Specific factors and questions the reviewers must evaluate, at a minimum, are listed under Item 3 below.

The estimated start date for the peer review is December 1, 2013. The peer review needs to be completed, and a draft peer review report provided to the Service, in accordance with the signed purchase order. Following receipt of the draft peer review report, the Service will have the opportunity to seek clarification, through the Panel Coordinator (PC), of any peer review comments. Following that, the PC will submit a final peer review report, by January 31, 2014, in a format suitable for posting on the FWS public web site.

Peer reviewers should prepare individual memoranda summarizing their opinions and conclusions; these memoranda will be incorporated into a peer review report provided by the PC. The PC should provide a summary narrative of the comments and issues contained in the peer review memorandums, but the PC is not required to analyze the similarities and differences in the individual peer review memorandums. The Service will be available to answer questions from the PC as needed to clarify the proposed rule.

3. Methods, Protocols and/or Scientific Standards

It is important that the peer review be managed and conducted by individuals with appropriate and relevant expertise who are independent (i.e., not under the control or influence) of the U.S. Fish and Wildlife Service. The independent peer reviewers shall be experienced senior scientists, who have previously conducted similar reviews or regularly provided reviews of research and conservation articles for the scientific literature. The PC will be responsible for leading this review and for the selection of 5-7 well-qualified, independent reviewers.

The peer reviewers shall include individuals with professional qualifications and relevant expertise in at least one of the following areas: conservation biology, management of large carnivores, wildlife management, and mammalian taxonomy/systematics. The PC should assemble a group of peer reviewers that includes representative expertise and experience that covers all of these areas, with particular attention to individuals with experience applying these disciplines to conservation of the gray wolf. The PC must also vet peer reviewers to verify and document that they are able to provide an objective review of the proposal and that they have no financial, professional, or other conflict of interest with the outcome or implications of the Service's ESA listing determination. Attachment A is provided as guidance to the PC in selecting peer reviewers that have no conflict of interest, are independent of the Service, and are capable of providing an objective scientific peer review, but the selection and vetting of peer reviewers is to otherwise occur completely outside the influence of the Service.

Peer reviewers will be asked to provide a thorough, objective peer review and to focus their review on aspects of the proposed rule that are within their area of expertise. Peer reviewers should distinguish between matters related to legal interpretation and application of agency policy and those related to the analysis and consideration of scientific information; we ask that they focus their review and comment on

the latter.

Peer reviewers are asked to comment specifically on the quality of any information and analyses used or relied on in the document; identify oversights, omissions, and inconsistencies; provide advice on reasonableness of judgments made from the scientific evidence; ensure that scientific uncertainties are clearly identified and characterized, and that potential implications of uncertainties for the technical conclusions drawn are clear; and provide advice on the overall strengths and limitations of the scientific data, analyses, and conclusions presented in the document.

Peer reviewers are asked to answer questions pertaining to the logic of our assumptions, arguments, and conclusions and to provide any other relevant comments, criticisms, or thoughts. The peer reviewers should consider and respond to the four questions listed below, at a minimum, in their reviews. Peer reviewers should also be mindful of the questions contained in the proposed rule.

(1) Did the Service consider the best available scientific information, including the scientific literature, in developing this proposal? Is there additional biological, commercial, trade, or other information relevant to our analysis of the current *C. lupus* listed entity that we did not consider, but should?

(2) Are the assumptions, analyses, and conclusions reflected in the proposed rule reasonable in light of the best available information?

(3) The Service determined that the synthesis and conclusions of Chambers *et al.* (2012. *North American Fauna* 77:1-67) reflect the best available scientific information regarding taxonomy of wolves in North America. In doing so, does the proposed rule draw reasonable and scientifically sound conclusions concerning the taxonomy of the eastern wolf, *Canis lycaon*? (We are not requesting information on the status of *C. lycaon* because we are conducting a status review for this species and peer review of that document will occur separately.)

(4) Does the proposed rule utilize the best available scientific information and draw reasonable and scientifically sound conclusions concerning the status of the gray wolf in the Pacific Northwest United States; the gray wolf subspecies *Canis lupus nubilus*; the gray wolf subspecies *C. l. occidentalis*; and the gray wolf subspecies *C. l. baileyi*?

The PC should advise peer reviewers that their reviews (without specific attribution), their names, and affiliations will be included in the administrative record of our final determination regarding this proposal, and will be available to the public by posting on the FWS Office of the Science Advisor peer review web page and on www.regulations.gov once all reviews are completed. The Service will summarize and respond to the issues raised by the peer reviewers in the record supporting our final rulemaking determination.

The peer review must be conducted in a manner that satisfies OMB and Service guidance for peer review of influential scientific information (OMB 2005, FWS 2012). Until the final peer review report is made public by the Service, no information from the peer review may be released by the PC without express written permission from the Service.

4. PC Responsibilities and Required Deliverables

The PC will execute the responsibilities and produce the deliverables listed below per requirements and guidance in this Statement of Work. Specifically the PC will:

- a) Select peer reviewers.
- b) Organize, structure, lead, and manage the scientific reviews and products.

- c) Manage and produce a draft and final report.
- d) Coordinate response to any follow-up questions from the Service following review of the draft report.
- e) Maintain and produce an official administrative record for the peer review.
- f) Respond to questions, inquiries, or other related requests after the final acceptance of the peer review report, as needed. Inquires or requests are limited to the products provided, and work performed under the MOU. Responses include, but are not limited to phone calls, written responses, and/or meetings.

5. Information Sources

The Service will provide the proposed rule and copies of references cited to the PC.

6. Point of Contact

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7. List of Enclosures/Attachments

Attachment A: Guidance for Selection of Peer Reviewers to Avoid Conflicts of Interest and to Facilitate an Independent, Objective, and Unbiased Scientific Peer Review

Attachment A: Guidance for Selection of Peer Reviewers to Avoid Conflicts of Interest and to Facilitate an Independent, Objective, and Unbiased Scientific Peer Review

In accordance with the Service's July 1, 1994 peer review policy (59 FR 34270) and the Office of Management and Budget's December 16, 2004, Final Information Quality Bulletin for Peer Review, the Service will subject the proposed rule, "Removing the Gray Wolf (*Canis lupus*) from the List of Endangered and Threatened Wildlife and Maintaining Protections for the Mexican Wolf (*Canis lupus baileyi*) by Listing It as Endangered" to peer review. Peer review is one of the important procedures used to ensure that the quality of published information meets the standards of the scientific and technical community (OMB 2005).

The purpose of this review is to provide an objective, independent, external scientific peer review of the information in the proposed rule. To accomplish this, it is necessary for the peer review to be conducted by individuals, and managed by an entity, that are: 1) independent of FWS; 2) lacking any real or perceived conflict of interest; and 3) able to provide an objective review of our proposed rule.

The following information will serve as guidance to the Panel Coordinator (PC) in selecting peer reviewers that have no conflict of interest, are independent of the Service, and are capable of providing an objective scientific peer review. The PC will also be advised to consult the four documents cited below for additional clarifying information.

Independence – "In its narrowest sense, independence in a reviewer means that the reviewer was not involved in producing the draft document to be reviewed. However, for peer reviewer of some documents, a broader view of independence is necessary to assure credibility of the process. Reviewers are generally not employed by the agency or office producing the document." [Excerpted from (OMB 2005)]

Conflict of Interest – The National Academy of Sciences defines "conflict of interest" as any financial or other interest that conflicts with the service of an individual on the review panel because it could impair the individual's objectivity or could create an unfair competitive advantage for a person or organization (NAS 2003).

Objectivity and Lack of Bias – "Questions of lack of objectivity and bias ordinarily relate to views stated or positions taken that are largely intellectually motivated or that arise from the close identification or association of an individual with a particular point of view or the positions or perspectives of a particular group. ... Potential sources of bias are not necessarily disqualifying for purposes of committee service. ... Some potential sources of bias, however, may be so substantial that they preclude committee service (e.g., where one is totally committed to a particular point of view and unwilling, or reasonably perceived to be unwilling, to consider other perspectives or relevant evidence to the contrary)." [Excerpted from NAS (2003)]

Peer Reviewers must be capable of providing an objective review of the proposal. Peer reviewers are ideally free of bias with respect to the proposed rule. If selection of unbiased peer reviewers is not possible, then to ensure the panel is fully competent, the PC should appoint peer reviewers in such a way as to represent a balance of potentially biasing backgrounds or perspectives (NAS 2003).

Citations

National Academy of Sciences. 2003. Policy and Procedures on Committee Composition and Balance and Conflicts of Interest for Committees Used in the Development of Reports. May 2003. Available online at: <http://www.nationalacademies.org/coi/index.html>

Office of Management and Budget. 2005. Final Information Quality Bulletin for Peer Review. December 16, 2004. Available online at: <http://www.whitehouse.gov/omb/memoranda/fy2005/m05-03.pdf>

U.S. Fish and Wildlife Service and National Marine Fisheries. 1994. Endangered and Threatened Wildlife and Plants: Notice of Interagency Cooperative Policy for Peer Review in Endangered Species Act Activities. 59 FR 34274, July 1, 1994. <http://www.nwr.noaa.gov/Publications/FRNotices/1994/loader.cfm?url=/commonspot/security/getfile.cfm&pageid=20831>

U.S. Fish and Wildlife Service. 2012. Information Quality Guidelines and Peer Review. Revised June 2012. Available online at: <http://www.fws.gov/informationquality/>