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To: [Jeffrey Olsen](#); [John Wenburg](#)
Subject: Wolverine genetics
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Attachments: [Wolverine Genetic Studies Outline.docx](#)

Jeff and John - I am attaching a document I prepared for Region 6, outlining information needs for our listing determination. In short, there are two main needs:

- 1) understanding the genetic diversity and population genetic structure of wolverine in North America (with two options, the SNPs method being more robust)
- 2) developing a revised effective population size estimate for lower 48 US wolverine population, and possibly for Canada/Alaska, in the context of "small population size" as a potential threat to wolverine.

Addressing both of these needs will also inform our DPS analysis and I understand you have evaluated other species in the context of our DPS Policy.

The Regional Office has asked me to provide a time frame and potential costs for this effort. I understand that the quickest path forward would be to request the completed analyses from Rocky Mountain Research Station and evaluate those data rather than request their samples and run new analyses.

Let me know if you have any questions. Very much appreciate your assistance with this listing process.
Betty

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Wolverine Genetic Studies

Background/Context: The District Court’s ruling in April 2016 indicated the Service should evaluate “the threat posed to the wolverine by small population size and lack of genetic diversity.”

Part I. Population Genetics of Wolverine (genetic diversity within and among populations): What is the genetic diversity and population genetic structure of wolverine populations in North America?

Two possible methods to pursue:

Option 1. Use existing microsatellite loci using samples and analysis already completed by the Rocky Mountain Research Station (Schwartz’s lab) (both U.S. and Canada, maybe Alaska if samples are available) to examine the current genetic structure of populations of wolverines in North America. This method uses repetitive DNA; no coding involved.

Analyses of these types of study results generally use the STRUCTURE software program. This program was used by Koskela (2013) in a study of wolverine population genetics in Finland. It assigns individuals probabilistically to one of several assumed populations, characterized by their allele frequencies, using a Bayesian clustering method. Cegelski et al. (2006) also used STRUCTURE (and GENECLASS) to “detect migration and the degree of reproductive discreteness among sampling locations” to evaluate population genetic structure for wolverines in North America (except Alaska).

In addition, Koskela used another software program – DIYABC 1.0.4.46 [citing Cornuet et al. 2010] as a way to infer the time at which population clusters had split from an ancestral population, and to also infer the effective population sizes (N_e) of the assumed populations.

Option 2. Conduct a genomic analysis using next generation sequencing (or other high-throughput technique) to genotype single nucleotide polymorphisms (SNPs). This type of genomic information can provide a greater resolution than microsatellites (especially for delineating DPS “units”) because a much larger number of loci are used. Genetic connectivity also should be assessed among populations.

[From Funk et al. 2012] Selection of the loci to be used requires careful consideration. Options include neutral loci, (presumably) adaptive loci, specific genes of known function, or all loci. Generally, neutral and adaptive loci are recommended, but should be determined by considering which evolutionary processes affect variation for the different classes of markers.

The second consideration for this method is to decide on the type of analyses used to delineate population groups. Options include multivariate statistical approaches such as principle component analysis (PCA), or STRUCTURE and GENELAND (both of the latter use Bayesian clustering algorithms). Selection should consider whether the genetic marker being used (e.g., neutral or adaptive loci) meets the assumptions for the type of analysis chosen.

Note: Mitochondrial DNA studies have also been used to evaluate wolverine populations, but these types of studies are best suited for phylogenetic and/or phylogeography studies (see Tomasik and Cook, 2005; Zigouris et al. 2013).

Part II. Determining Effective Population Size (and, relatedly, minimum viable population)
Background: There is much confusion and debate about the use of the 50/500 rule (developed over 30 years ago). Should a minimum viable effective population size (N_e) be genetically derived to set the threshold for a minimum viable population? In this case, the rule is interpreted as 50=short term (inbreeding depression) and 500=long term (retention of genetic variation). Or should the N_e of 500 (or larger census population size (N_c) equivalent) be viewed as a long-term goal for maintaining healthy and genetically robust populations? (not as a threshold trigger that predicts extinction risk or used to allocate resources for conservation). In addition, the 500 value refers to a global rather than a local N_e ; thus, it is not necessary to maintain a local N_e of 500 as long as there is some gene flow into a population (Jamieson and Allendorf 2012). Finally, Frankham et al. (2014) have recommended this rule be modified to 100/1000.

For wolverine, a new estimate of a minimum viable effective population size (N_e) should be prepared using different tools and methods than those used by Schwartz et al. (2009). This type of analyses can be used not only to address “small population size” as a potential threat for wolverine, but also for assessing discreteness for a distinct population segment analysis. In addition, a revised N_e (and N_e/N_c ratio) could be incorporated in a population viability analysis where scenarios can be modeled to assess long-term viability of wolverine populations (see, for example, Nilsson 2014 – PVA for lynx, wolverine, bear for Scandinavia).

One approach is to follow methods used by Koskela (2013), who developed estimates for wolverines in Finland. Those methods (including software programs) are summarized below: “The effective population size (N_e) was estimated using LDNe 1.31 [citing Waples and Do 2008]. The program estimates contemporary effective population sizes using a biased correction for the Hill method [citing Hill 1981; Waples 2006] based on linkage disequilibrium data. Three estimates of N_e are given based on the lowest allele frequency used (0.01, 0.02 and 0.05).” Also used by Koskela – the software program BOTTLENECK 1.2.02 [citing Cornuet and Luikart 1996] to detect recent reductions in effective population size. {this study found a low N_e in both Finland wolverine populations}

FYI – Schwartz et al. (2009) previous study of wolverines (Rocky Mountain region) analyzed DNA sample at 16 microsatellite loci. (That study also used mtDNA data as “an independent test of our least-cost path corridor analysis, which was developed based on microsatellite data.”) Their publication also reported estimates of the effective population size (and 95% credible limits of the estimate) using summary statistics and approximate Bayesian computation methods using ONE-SAMP [citing Tallmon et al. 2008].

Final note

Ecological, phenotypical, and environmental information should also be used to complement genomic data to assist in interpreting the strength of conclusions or inferences of spatial patterns of adaptation (or adaptively divergent populations).

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