

From: [Guinotte, John](#)
To: [Stephen Torbit](#)
Subject: Fwd: Conversation with Noreen about Wolverine Genetics
Date: Tuesday, April 4, 2017 3:27:32 PM
Attachments: [Wolverine Genetic Studies Outline.docx](#)

Hi Steve, I spoke to Betty. Do you need more than this for the genetics from Betty?
Best, John

----- Forwarded message -----

From: **Grizzle, Betty** <betty_grizzle@fws.gov>
Date: Tue, Mar 14, 2017 at 4:38 PM
Subject: Re: Conversation with Noreen about Wolverine Genetics
To: Stephen Torbit <Stephen_Torbit@fws.gov>
Cc: "Shoemaker, Justin" <justin_shoemaker@fws.gov>, Marjorie Nelson <marjorie_nelson@fws.gov>, John Guinotte <john_guinotte@fws.gov>

Steve - I was hoping to hear back from John Wenburg (Alaska) today as I would have liked to discuss with him my proposed needs. However, given the time constraints we are under, I am attaching to this email my preliminary thoughts on the two main needs - 1) genetic diversity and population genetic structure of wolverine in North America (with two options, the SNPs method is more robust) and 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.

It is my understanding that the Conservation Genetics Lab in Alaska has the capability of preparing these types of analyses and in a very short time frame (though a SNPs analyses will take longer and cost more than an re-analysis of RMRS results). But I have not confirmed this with John Wenburg. You might want to wait until I can speak with him before making a decision about this work.

Betty

On Mon, Mar 13, 2017 at 3:02 PM, Stephen Torbit <Stephen_Torbit@fws.gov> wrote:

I had a good conversation with Noreen today about wolverines. She is willing to start asking for financial support for the genetics work among the regions and states. But first, she wants a clear idea of what specific questions we need answers to and what the costs will be to analyze the hair samples the states have collected. I have calls into Mike Schwartz in Missoula and just now to Bob Inman to understand exactly what the states have requested of Schwartz. I can then develop a cost based on what we need and what kind of genetic analysis Mike needs to do.

However, I need Betty to drive this information request because she has done the most thinking about what she needs for the DPS, for effective population size, etc. So, we could get on the phone soon and discuss, but at the end of the day, I need the details from Betty of what she needs. Hopefully, I can get some insights from the states about what they have

asked for and what the analysis will look like.

Steve

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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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