



Invited reply

Cite this article: Rutledge LY, Devillard S, Hohenlohe PA, White BN. 2016 Considering all the evidence: a reply to Sefc and Koblmüller (2016). *Biol. Lett.* **12**: 20151009. <http://dx.doi.org/10.1098/rsbl.2015.1009>

Received: 22 December 2015

Accepted: 12 January 2016

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The accompanying comment can be viewed at <http://dx.doi.org/10.1098/rsbl.2015.0834>.

Evolutionary biology

Considering all the evidence: a reply to Sefc and Koblmüller (2016)

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In their comment on our recent article [1], Sefc & Koblmüller [2] acknowledge the need for eastern wolf (*Canis lycaon*) conservation, regardless of evolutionary origin. This is an important consolidating statement for endangered species policy that demonstrates agreement among scientists of what should be conserved. They also suggest, however, that eastern wolves from Algonquin Park may have originated from an ancient grey wolf (*C. lupus*) × western coyote (*C. latrans*) hybridization event followed by drift over many generations. Their evidence includes: (i) skull morphology suggesting plausible wolf–coyote hybridization [3], (ii) results of our own f_3 tests [1] and (iii) a successful experimental cross between western grey wolves and western coyotes [4]. We acknowledge that alternative scenario in the original article and agree that our data reject a recent hybrid origin for eastern wolves. Their comment speaks more to academic disagreement on species definitions [5] than to the applied nature of wolf conservation. Here, we point out that the overall evidence remains weak for an ancient hybrid origin for eastern wolves, and we caution against biological interpretation of a single statistical test to the exclusion of all other information.

Our objective was to test the eastern wolf species hypothesis based on the genetic clusters species concept [6], to which we show ‘support for the eastern wolf centralized in Algonquin Provincial Park as a distinct genomic cluster’ [1, p. 3]. We resolve hybrid origins within North American *Canis* by demonstrating that Great Lakes-boreal wolves and eastern coyotes arose from recent hybridization that includes eastern wolves as a key genomic component of that admixture. To further clarify our position, the unified species concept [7] used to identify a new species of African golden wolf (*C. anthus*) [8], is also applicable to the eastern wolf [9,10]. Although eastern wolf ancestry is confounded by contemporary gene flow [11] and introgressed mtDNA and Y-chromosomes [12,13], they remain morphologically distinct from neighbouring *Canis* types [14].

The craniological analysis of Nowak [3] supports the emergence of a small wolf during Late Rancholabrean. However, the targeted eradication of wolves within Algonquin Park through the 1950s likely resulted in interbreeding with surrounding grey wolves and invading coyotes. Nowak’s analysis, while thorough, is insufficient to assess ancestry in a system complicated by ancient hybridization followed by widespread extirpation and habitat alteration leading to contemporary admixture.

Both f_3 and LD statistics assess historical admixture in a population. The f_3 statistic uses allele frequencies and the LD-statistic is inferred from linkage disequilibrium [15]. Our f_3 results suggest grey wolf × western coyote admixture in eastern wolves. Conversely, the LD-statistic found no evidence of this admixture. Also, an f_3 test failed to identify admixture in eastern coyotes, which clearly has

wolf (grey or eastern wolf) and western coyote ancestry [12,13]. These conflicting results warrant cautious interpretation. A *de novo* assembly of a western coyote genome may resolve these discrepancies because we excluded 14.1% of potentially informative loci that could not be mapped to the dog genome.

The experimental cross of western grey wolves and western coyotes [4] provides little support for ancient hybridization. Natural breeding did not occur, and multiple attempts of artificial insemination resulted in few pregnancies and even fewer surviving litters. Furthermore, there is no evidence for grey wolf \times western coyote hybridization in wild populations [16], and there is a behavioural barrier to reproduction [17]. What remains to be explained by the two-species model is why we see extensive hybridization, ongoing admixture, and persistent introgression in eastern North America and not in western North America. If the eastern evolutionary patterns

arose from simply grey wolf \times western coyote hybridization, one would expect similar events to also occur in the west, especially where dwindling grey wolf populations live sympatrically with thriving coyotes.

Conservation policy has long overlooked the role that hybridization plays in speciation of mammal populations. We suggest that endangered species legislation be updated to reflect the potential conservation value of hybrids, despite their origins [18]. The rapid rate at which humans are altering the evolutionary trajectory of animal populations [19] brings a sense of urgency to these changes. In short, regulation of endangered species needs to reflect the realization that hybridization, in its many forms, is an important part of adaptation, evolution and speciation.

Competing interests. We have no competing interests.

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