

NEWS AND VIEWS

REPLY

More is better

STEPHAN KOBLMÜLLER,* MARIA NORD,†
ROBERT K. WAYNE‡ and JENNIFER
LEONARD§,¶

*Department of Zoology; Karl-Franzens University Graz,
Universitätsplatz 2, 8010 Graz, Austria, †Department of Ecology,
Swedish University of Agricultural Sciences, Box 7044, 750 07
Uppsala, Sweden, ‡Department of Ecology and Evolutionary
Biology, University of California, Los Angeles, CA 90095-1606,
USA, §Department of Evolutionary Biology, Evolutionary
Biology Centre, Uppsala University, Norbyvägen 18d, SE-75236
Uppsala, Sweden, ¶Estación Biológica de Doñana-CSIC, Avd.
Americo Vespucio s/n, 41092 Sevilla, Spain

Keywords: *Canis*, Great Lakes wolves, hybridization

Received 28 August 2009; revision received 17 October 2009;
accepted 18 October 2009

Cronin & Mech (2009) suggested that in our recent study on the genetics of the Great Lakes wolves (Koblmüller *et al.* 2009) we misrepresented the literature in two cases and engaged in 'indefinite use of terminology'.

Misrepresentation of literature

Nowak (2002) used morphological data to assess the status of wolves from Eastern North America. On the basis of this analysis and historical data, he clearly advocates recognizing the Great Lakes wolf as subspecies *Canis lupus lycaon*. For this reason, we find it appropriate to use this citation in support of morphological differentiation between *C. l. lycaon* and other subspecies of gray wolves in North America. For a discussion of morphological and ecological differences in Great Lakes wolves, see Kolenosky & Standfield (1975).

The second 'discrepancy' Cronin & Mech raised concerns the sentence 'Note added in proof' in which we state the genetic data of Wheeldon & White (2009) support our conclusions. Wheeldon & White (2009) sequenced a fragment of the mitochondrial control region from three historic Great Lakes area wolves. They identified two haplotypes, both of which were identical to sequences found in our much larger sample of historic Great Lakes area wolves. For this reason and considering the importance of authen-

ticity in ancient DNA studies, we find our statement appropriate. As this statement was a note in proof, we wanted to limit our comment to a sentence, but thought it was necessary to direct the reader to another relevant, recently published article.

As discussed by Cronin & Mech, Wheeldon & White's (2009) conclusions differ from our own with regard to the history and origin of the Great Lakes wolves. We maintain that the discrepancy in interpretation reflects different a priori assumptions about the phylogenetic relationships among North American canids. Based on phylogenetic analysis of mtDNA control region sequences, previous and recent studies suggest widespread hybridization between coyotes and current and historic Great Lakes wolves (Lehman *et al.* 1991; Wilson *et al.* 2000, 2003; Leonard & Wayne 2008; Kays *et al.* 2009; Rutledge *et al.* 2009). Such hybridization causes reticulations in mtDNA phylogenetic trees and confounds evolutionary inference. Nonetheless, primarily on the basis of a clustering of mtDNA sequences of wolves from the Algonquin Provincial Park and surrounding areas (plus red wolves) with coyotes and a weakly supported genetic similarity based on microsatellites loci, Wilson *et al.* (2000) hypothesized that the Great Lakes wolf (including the red wolf *C. rufus*) is a distinct species that has evolved in North America and is closely related to the coyote. This underlying assumption is maintained in subsequent publications. We (Koblmüller *et al.* 2009) feel that the separate origin hypothesis is controversial and the data are not sufficient to support Wheeldon & White's (2009) a priori assumptions about the phylogenetic position of Great Lakes wolves. The intent of our study was to use more genetic markers (in our case additional autosomal and Y-chromosomal microsatellite loci) to clarify the evolutionary history of these 'problematic' taxa in an objective fashion. As argued in our study, we suggest that these new data support the Great Lakes wolf as a distinct ecotype of the gray wolf.

The strongest argument for a sister group relationship of Great Lakes wolves (plus red wolves) and coyotes has been the finding that historic Great Lakes wolves were dominated by mitochondrial haplotypes closely related to western coyotes. However, as explained by Cronin & Mech in the fourth paragraph of their comment, analyses of a single genetic marker 'do not necessarily reflect species and population status'. Introgressive hybridization leading to complete replacement of mtDNA (through drift or selection) on a regional scale (e.g. Melo-Ferreira *et al.* 2005; Nyingi & Agnèse 2007; Good *et al.* 2008) or even throughout a species' distribution range (e.g. Nevado *et al.* 2009) seems to be more common than previously thought. To obtain a more complete picture of the history of these wolves and their relationships with other wolves and coyotes, more

data, in addition to mtDNA, are required. Wheeldon & White (2009) attempted to satisfy this requirement by typing eight nuclear microsatellites. Unfortunately, those loci were not sufficiently informative (see their PCA scatterplot, figure 1 in Wheeldon & White 2009). Our study included the analysis of 26 independent autosomal microsatellite markers, which allowed us to draw much stronger conclusions, clearly demonstrating that Great Lakes wolves are related more closely to western gray wolves than to coyotes, despite ongoing gene flow in the Great Lakes region. This scenario also is supported by our Y-chromosomal data (although we regret that only four historic Great Lakes wolf samples could be successfully genotyped at all six Y-chromosomal loci).

We strongly believe that all published data should be made available to any interested party. Many of the mitochondrial DNA sequences included in Koblmüller *et al.* (2009) were taken from the literature (for details see Materials and Methods section). The mtDNA haplotypes novel to our study have been submitted to GenBank (GQ849369–GQ849391). No Y-chromosome DNA sequences are available because none was generated in this study. Our Y-chromosome haplotypes are based on length polymorphisms at six microsatellite loci (again, we refer interested parties to the Materials and Methods section of Koblmüller *et al.* 2009 for more details). Requests for data not in public repositories (e.g. there is no generally recognized public repository for length polymorphism data) should be made directly to the authors.

Indefinite use of terminology

Cronin and Mech suggest that instead of clarifying the taxonomy of the wolves from around the Great Lakes, which was one of our goals, we create confusion through ‘undefined terminology and unclear application of taxonomy’ and then suggest that ‘for North American *Canis*, it is wise to avoid typological thinking and designation of formal taxonomic names’. The species level status of gray wolves (*Canis lupus*) and coyotes (*Canis latrans*) have been consistently supported in the literature and genetic studies have found little evidence of hybridization between them outside the Great Lakes area (Pilgrim *et al.* 1998; Hailer & Leonard 2008). Discarding this large body of work and calling them all *Canis sp.* does not clarify the situation, but obscure it. For this reason, we use the well defined species designations of gray wolves and coyotes.

The taxonomy of the Great Lakes wolves is less clear and a number of different evolutionary scenarios have been proposed (see above). Testing among these different hypotheses was the subject of our study, and for that reason, we did not initially advocate a specific taxonomy of Great Lakes wolves. We found that the genetic data were not compatible with the hypothesis that the Great Lakes area wolves are a recently evolved species related more closely to coyotes than to wolves. Rather, our data show Great Lakes area wolves are gray wolves (*Canis lupus*).

Within gray wolves, genetic partitions have been identified that are associated with habitat (i.e. Carmichael *et al.* 2001, 2007; Pilot *et al.* 2006; Leonard *et al.* 2007; Musiani *et al.* 2007; Muñoz-Fuentes *et al.* 2009). These habitat-associated genetic units have not currently been given subspecific status; they have just been referred to as ‘ecotypes’ in previous studies. In gray wolves, the criteria for designating subspecies are not clearly defined, but historically have been based on phenotypic rather than genetic data. For this reason, we refer to the Great Lakes wolf as an ‘ecotype’ to emphasize the association between genetics and ecological factors and do not attempt to define subspecies.

Cronin & Mech say ‘phylogenetic relationships at the species level are seldom definitive’. We operate on the working hypothesis that ‘species’ exist. A philosophical discussion on the existence of species is beyond the scope of this response (see Hey 2006).

We agree with Cronin & Mech that ‘maintaining a fit wolf population is an important management consideration’. The data we present in Koblmüller *et al.* (2009) are all neutral genetic markers. They show that Great Lakes wolf-like canids are closely related to gray wolves that have in the past, and are today, hybridizing with coyotes. These data do not directly allow an assessment of fitness. The observation that the wolves around the Great Lakes began hybridizing with coyotes after a period of persecution and habitat modification by humans may suggest causality. Furthermore, the level of hybridization and introgression was not uniform across the region. For these reasons, we suggested in Koblmüller *et al.* (2009) and maintain here that ‘more information on the variation in ecological factors and the extent of hybridization in the different regions could help determine, which circumstances favour hybridization, and provide guidelines for management to maintain Great Lakes wolf integrity in the future’.

References

- Carmichael LE, Nagy JA, Larter NC, Strobeck C (2001) Prey specialization may influence patterns of gene flow in wolves of the Canadian northwest. *Molecular Ecology*, **10**, 2787–2798.
- Carmichael LE, Krizan J, Nagy JA *et al.* (2007) Historical and ecological determinants of genetic structure in arctic canids. *Molecular Ecology*, **16**, 3466–3483.
- Cronin MA, Mech LD (2009) Problems with the claim of ecotype and taxon status of the wolf in the Great Lakes region. *Molecular Ecology*, doi: 10.1111/j.1365-294X.2009.04431.x.
- Good JM, Hird S, Reid N *et al.* (2008) Ancient hybridization and mitochondrial capture between two species of chipmunks. *Molecular Ecology*, **17**, 1313–1327.
- Hailer F, Leonard JA (2008) Hybridization among three native North American *Canis* species in a region of natural sympatry. *PLoS ONE*, **3**, e3333.
- Hey J (2006) On the failure of modern species concepts. *Trends in Ecology and Evolution*, **21**, 447–450.
- Kays R, Curtis A, Kirchman JJ. 2009. Rapid adaptive evolution of northeastern coyotes via hybridization with wolves. *Biol Lett.*, in press. doi: 10.1098/rsbl.2009.0575
- Koblmüller S, Nord M, Wayne RK, Leonard JA (2009) Origin and status of the Great Lakes wolf. *Molecular Ecology*, **18**, 2313–2316.

- Kolenosky GB, Standfield RO (1975) Morphological and ecological variation among gray wolves (*Canis lupus*) of Ontario, Canada. In: *The Wild Canid* (ed MW Fox). pp. 62–72, Van Nostrand Reinhold, NY.
- Lehman N, Eisenhawer A, Hansen K *et al.* (1991) Introgression of coyote mitochondrial DNA into sympatric North American gray wolf populations. *Evolution*, **45**, 104–119.
- Leonard JA, Wayne RK (2008) Native Great Lakes wolves were not restored. *Biology Letters*, **4**, 95–98.
- Leonard JA, Vilà C, Fox-Dobbs K *et al.* (2007) Megafaunal extinctions and the disappearance of a specialized wolf morph. *Current Biology*, **17**, 1146–1150.
- Melo-Ferreira J, Boursot P, Suchentrunk F, Ferrand N, Alves PC (2005) Invasion from the cold past: extensive introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into three other hare species in northern Iberia. *Molecular Ecology*, **14**, 2459–2464.
- Muñoz-Fuentes V, Darimont CT, Wayne RK, Paquet P, Leonard JA (2009) Ecological factors drive differentiation in wolves from British Columbia. *Journal of Biogeography*, **36**, 1516–1531.
- Musiani M, Leonard JA, Cluff HD *et al.* (2007) Differentiation of tundra/taiga boreal coniferous forest wolves: genetics, coat color and association with migratory caribou. *Molecular Ecology*, **16**, 4149–4170.
- Nevado B, Koblmüller S, Sturmbauer C *et al.* (2009) Complete mtDNA replacement in a Lake Tanganyika cichlid fish. *Molecular Ecology*, **18**, 4240–4255.
- Nowak RM (2002) The original status of wolves in eastern North America. *Southeastern Naturalist*, **1**, 95–130.
- Nyingi D, Agnèse JJ (2007) Recent introgressive hybridization revealed by exclusive mtDNA transfer from *Oreochromis leucostictus* (Trewavas, 1933) to *Oreochromis niloticus* (Linnaeus, 1758) in Lake Baringo, Kenya. *Journal of Fish Biology*, **70** (Supplement A), 148–154.
- Pilgrim KL, Boyd DK, Forbes SH (1998) Testing for wolf-coyote hybridization in the Rocky Mountains using mitochondrial DNA. *Journal of Wildlife Management*, **62**, 683–689.
- Pilot M, Jedrzejewski W, Branicki W *et al.* (2006) Ecological factors influence population genetic structure of European grey wolves. *Molecular Ecology*, **15**, 4533–4553.
- Rutledge LY, Bos KI, Pearce RJ, White BN (2009). Genetic and morphometric analysis of sixteenth century *Canis* skull fragments: implications for historic eastern and gray wolf distribution in North America. *Conservation Genetics*, in press. doi: 1007/s10592-009-9957-2.
- Wheeldon T, White BN (2009) Genetic analysis of historic western Great Lakes region wolf samples reveals early *Canis lupus/lycaon* hybridization. *Biology Letters*, **5**, 101–104.
- Wilson PJ, Grewal S, Lawford ID *et al.* (2000) DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. *Canadian Journal of Zoology*, **78**, 2156–2166.
- Wilson PJ, Grewal S, McFadden T, Chambers RC, White BM (2003) Mitochondrial DNA extracted from eastern North American wolves killed in the 1800s is not of gray wolf origin. *Canadian Journal of Zoology*, **81**, 936–940.

M.N. is interested in conservation biology and is working on her PhD on bird diversity in the Swedish archipelago and the effects of human disturbance. S.K. and R.K.W. apply molecular genetic techniques to study questions in ecology, behaviour, conservation and evolution of animals. J.L. combines genetic and other data to determine how and why populations are structured through time and space, and which factors drive some populations to extinction or expansion.

doi: 10.1111/j.1365-294X.2009.04432.x