Non-genetic Data Supporting Genetic Evidence for the Eastern Wolf

L. David Mech*

Abstract - Two schools of thought dominate the molecular-genetics literature on *Canis* spp. (wolves) in the western Great Lakes region of the US and Canada: (1) they are hybrids between *Canis lupus* (Gray Wolf) and *Canis latrans* (Coyote), or (2) they are hybrids between the Gray Wolf and *Canis lycaon* (Eastern Wolf). This article presents 3 types of non-genetic evidence that bears on the controversy and concludes that all 3 support the second interpretation.

Introduction

Wilson et al. (2000) presented molecular genetic evidence for a new interpretation of the taxonomy of North American *Canis* species and proposed that the *Canis lupus* L. (Gray Wolf) subspecies *lycaon,* is a separate species, similar to *Canis rufus* Audubon and Bachman (Red Wolf), that should be named *Canis lycaon* Schreber (Eastern Wolf). The study was based on analyses of 8 microsatellite loci and mitochondrial DNA (mtDNA) control-region sequences from wolves of southeastern Ontario from the 1960s. None showed Gray Wolf mtDNA, and estimates were that mtDNA sequences from both the Eastern Wolf and Red Wolf diverged from *Canis latrans* Say (Coyote) 150,000–300,000 years ago, as compared to divergence from the Gray Wolf around 2 million years ago. Based on this evidence, Wilson et al. (2000) suggested that both the Red Wolf and Eastern Wolf evolved in North America along with the Coyote, as opposed to the Gray Wolf, which evolved in the Old World. Wilson et al. (2000) also compared their microsatellite allele frequencies with published frequencies of wolves and Coyotes from other areas (Roy et al. 1994, 1996), and found both microsatellite evidence and mtDNA evidence of Eastern Wolves as far west as Manitoba.

Previously, Lehman et al. (1991) and Wayne and Lehman (1992) had considered the same mtDNA haplotypes as evidence of Gray Wolf x Coyote hybridization, and the Wayne school continued to interpret them that way (Koblmuller et al. 2009, Leonard and Wayne 2008). Wilson et al. (2000) based their interpretation on the fact that those haplotypes have not been found in extant non-hybridizing Coyote populations and on other considerations. Wayne and Vila (2003:235, 236) agreed that the Wilson et al. (2000) interpretation should be further tested.

Kyle et al. (2006) examined in detail whether the genetic data support the Eastern Wolf as a distinct species and considered the alternate hypotheses that the Eastern Wolf is a subspecies of the Gray Wolf or derived from hybridization between the Gray Wolf and Coyote. Kyle et al. (2006) rejected the subspecies and hybrid hypotheses

*US Geological Survey, Northern Prairie Wildlife Research Center, 8711 37th Street, SE, Jamestown, ND 58401-7317; mailing address - US Geological Survey, The Raptor Center, 1920 Fitch Avenue, University of Minnesota, St. Paul, MN 55108; mechx002@umn.edu; david_mech@usgs.gov.

and accepted Eastern Wolf as a species, which other authors have also since accepted (Fain et al. 2010, Murray and Waits 2007, Rutledge et al. 2009, Way et al. 2010, Wheeldon 2009, Wheeldon and White 2009, Wheeldon et al. 2010).

Leonard and Wayne (2008) reported on divergent mtDNA haplotypes in historic Great Lakes wolves and suggested they belonged to a distinct mtDNA lineage referred to as the "Great Lakes wolf" that originated from ancient hybridization between Coyote females and male Gray Wolves. However, Wheeldon and White (2009) showed that 2 of these haplotypes were similar to, or the same as, haplotypes of the Eastern Wolf. Meanwhile, Koblmuller et al. (2009) interpreted genetic data from Great Lakes wolves without distinguishing between Coyote and Eastern Wolf lineages, instead referring only to Gray Wolf and Coyote lineages, but found all 3 Eastern Wolf mtDNA haplotypes of Wilson et al. (2000) among both historical and modern wolves of the western Great Lakes region. Furthermore, each school of molecular genetics (the Wilson school and the Wayne school) mustered additional genetic data and arguments in support of their interpretation. The most apparent conflicting issue is the lack of consensus on whether the Coyote-like mtDNA sequences in hybridized wolves are those of actual Coyotes or of the Eastern Wolf.

Regarding the current wolf population in the Great Lakes region, from northeastern Ontario west across Ontario, Michigan, Wisconsin, Minnesota, and at least part of Manitoba, the disparate schools of thought are that those wolves are either hybrids between Gray Wolf x Eastern Wolf (Wheeldon 2009, Wilson et al. 2009) or Gray Wolf x Coyote (Koblmuller et al. 2009, Lehman et al. 1991). The Gray Wolf has been on the US Endangered Species List since 1967 and is protected by the Endangered Species Act of 1973. Although it has been delisted a few times, legal issues have forced it back on the list. In addition, claims have been made, based on genetic analyses, that the native Great Lakes wolves were not restored (Leonard and Wayne 2008, but cf Mech 2009, Wheeldon and White 2009) and that the Great Lakes wolves are a "unique population or ecotype of Gray Wolves" (Koblmuller et al. 2009, but cf Cronin and Mech 2009).

Therefore, it is important to try to determine the correct interpretation of the Coyote-like mtDNA haplotypes by examining evidence other than the genetic data. This article attempts to do that.

Discussion

There are 3 types of non-genetic evidence relevant to the question of whether the Coyote-like mtDNA haplotypes in hybridized wolves are those of Coyotes (the Wayne interpretation) or those of the Eastern or Red Wolf that putatively evolved with Coyotes (the Wilson interpretation): (1) morphological data, (2) reproductive information, and (3) behavior. Some of this evidence has been discussed before, but is included here for the sake of completeness.

Phenotypically the Gray Wolf, the putative Eastern Wolf and the Red Wolf, and the Coyote are similar, with body and skull sizes decreasing from the Gray Wolf to the Coyote. There seems to be agreement that the Eastern Wolf (formerly *C. l. lycaon)* and the Red Wolf appear intermediate to the Gray Wolf and the Coyote (Kolenosky and Standfield 1975, Mech 1970). However, the picture is further confounded by the fact that, in eastern North America, hybridization occurred between

western Coyotes and the Eastern Wolf (Kays et al. 2009, Kolenosky and Standfield 1975, Kyle et al. 2006, Way et al. 2010, Wheeldon et al. 2010, Wilson et al. 2009).

The crux of distinguishing between the Wayne and Wilson interpretations is determining whether Coyotes have ever hybridized with the Gray Wolf or whether they even can (Mech 2010). Morphological evidence that would help distinguish whether phenotypic wolves with Coyote-like mtDNA are Gray Wolves that have hybridized with Coyotes or with Eastern Wolves would be (1) the existence of *Canis* that generally appeared intermediate between Gray Wolves and Coyotes or that generally appeared intermediate between Gray Wolves and Eastern Wolves, or (2) skulls that appear similarly intermediate.

As for the former, Great Lakes (Minnesota, Wisconsin, Michigan) wolves look like Gray Wolves both in appearance and size, although females in some areas are up to 12% lighter weight, and males up to 15% lighter weight than Gray Wolves (Mech and Paul 2008). Similarly, skulls of 1970–1976 Great Lakes wolves are similar to those of the Gray Wolf (Nowak 2009), although a sample taken later possess narrower rostra (Mech et al., in press). In both body mass and skull measurements, the Great Lakes wolves are more similar to the Gray Wolf than to the Coyote, providing evidence that they have resulted not from Gray Wolf x Coyote but rather from Gray Wolf x Eastern Wolf. Furthermore, the only animals or skulls that have been recorded that appeared to be a product of mating between Coyote and any kind of wolf were those in eastern Canada, which according to the Wilson interpretation would have resulted from matings between Coyotes and Eastern Wolves (Kolenosky and Standfield 1975, Sears et al. 2003). Not only do these hybrids only occur in eastern Canada and the northeastern US, but their sizes and skulls are intermediate between Eastern Wolves and Coyotes, not between Gray Wolves and Coyotes (Kolenosky and Standfield 1975:Fig. 5–2).

Table 1. Evidence of wolves killing Coyotes in the Great Lakes region. No. = number of wolf-killed Coyotes.

Location	No.	Source
Michigan	1+	B. Roell (Department of Natural Resources, South Marquette, MI, pers. comm.)
Wisconsin	3	R.P. Thiel (2006) and (Department of Natural Resources, Babcock, WI, pers. comm.)
	2	R. Schultz (Department of Natural Resources, Woodruff, WI, pers. comm.)
	1	J. Evrard (Department of Natural Resources, Grantsburg, WI, pers. comm.)
Minnesota	1	M.E. Nelson (US Geological Survey, Eli, MN, pers. comm.)
	1	L.D. Mech (unpubl. data)
	2	Berg and Chesness 1978
Eastern Ontario	0[A]	B. Patterson, (Ontanrio Ministry of Natural Resources, Petersborough, ON, Canada, pers. comm.)
	0	J.B. Theberge and M.T. Theberge (1998, 2004) and (University of Waterloo, Waterloo, ON, Canada, pers. comm.)
	0	G. Kolenosky (Ontanrio Ministry of Natural Resources, Petersborough, ON, Canada, pers. comm.)
Quebec	0	M. Villemure (2003)

[A]"We had Coyote-like animals killed by wolves, but they were in 'wolf' packs and functioning like wolves."

It seems highly relevant that, although hybrids of Eastern Wolves and Coyotes have been recorded for years in eastern Canada, no such phenotypic hybrid between Gray Wolves and Coyotes has been found west of there. Neither has genetic evidence of Coyotes been found in Gray Wolves from Montana, Wyoming, or Manitoba (Carbyn 1982, Paquet 1992, Pilgrim et al. 1998), where no one disputes that the wolves are Gray Wolves and have long been sympatric with Coyotes. In Minnesota, Wisconsin, and Michigan alone, over 2000 wolves have been examined (Beyer et al. 2009, Mech and Paul 2008, Nowak 2009, Wydeven et al. 2009) with no one reporting an apparent Gray Wolf x Coyote hybrid. This finding supports the Wilson interpretation.

The second line of relevant non-genetic evidence that might help distinguish between the Wayne and Wilson interpretations would be reproductive experiments. A wolf from eastern Canada (putative Eastern Wolf) has successfully bred in captivity with a Coyote (Kolenosky 1971), but no one has attempted to mate a Gray Wolf from the West with a Coyote. If such an experimental mating were accomplished, it would lend some support to the Wayne interpretation.

The third type of non-genetic evidence relevant to the Wayne-Wilson difference in interpretation is behavioral. If a Gray Wolf mated with a Coyote, there had to be some tolerance between the 2 species. Here the record is clear. From Michigan westward, Gray Wolves kill Coyotes (summary by Ballard et al. 2003, Berger and Gese 2007), whereas I could find no record of wolves east of Michigan killing Coyotes, despite considerable field work there on both species (Table 1).

In the Great Lakes area, the extant wolf population, considered Gray Wolf x Coyote under the Wayne interpretation and Gray Wolf x Eastern Wolf under the Wilson interpretation, does kill Coyotes (Table 1), although a few observations of wolves and Coyotes tolerating each other have been made (Thiel 2006). On balance, however, Great Lakes wolves kill Coyotes as do wolves farther west, a fact that makes it unlikely that the 2 species would mate. This is further evidence that the Coyote-like mtDNA sequences found in some Great Lakes wolves are not derived from Coyotes, a finding that leaves the alternative—that they derive from the Eastern Wolf—more plausible.

In summary, non-genetic evidence based on morphology, reproduction, and interspecific relations all support the contention that Gray Wolf x Coyote hybridization is rare to non-existent from approximately Michigan westward. This finding then lends support to the Wilson (2000, 2009) hypothesis that the Coyote-like genetics found in wolves of the Great Lakes region represent the Eastern Wolf rather than the Coyote.

Acknowledgments

This study was supported by the US Geological Survey. I thank T. Wheeldon and S.R. Fain for critiquing an earlier draft and making helpful suggestions for improving it.

Literature Cited

Ballard, W.B., L.N. Carbyn, and D.W. Smith. 2003. Wolf interactions with non-prey. Pp. 259–271, *In* L.D. Mech and L. Boitani, (Eds.). Wolves: Behavior, Ecology, and Conservation. University of Chicago Press, Chicago, IL.

Berg, W.E., and R.A. Chesness. 1978. Ecology of coyotes in northern Minnesota. Pp. 229–247, *In* M. Bekoff (Ed.). Coyotes: Biology, Behavior, and Management. Academic Press, New York, NY.

Berger, K.M., and E.M. Gese. 2007. Does interference competition with wolves limit the distribution and abundance of coyotes? Journal of Animal Ecology 76:1075–1085.

Beyer, D.E., Jr., R.O. Peterson, J.A. Vucetich, and J.H. Hammill. 2009. Wolf population changes in Michigan. Pp. 65–85, *In* A.P. Wydeven, T.R. Van Deelen, and E.J. Heske (Eds.). Recovery of Gray Wolves in the Great Lakes Region of the United States. Springer, New York, NY.

Carbyn, L.N. 1982. Coyote population fluctuations and spatial distribution in relation to wolf territories in Riding Mountain National Park, Manitoba. Canadian Field Naturalist 96:176–183.

Cronin, M.A., and L.D. Mech. 2009. Problems with the claim of ecotype and taxon status of the wolf in the Great Lakes Region. Molecular Ecology 18:4991–4993.

Fain, S.R., R.J. Straughan, and B.F. Taylor. 2010. Genetic outcomes of Eastern Timber Wolf recovery in the western Great Lakes States. Conservation Genetics 11:1747–1765.

Kays, R., A. Curtis, and J.J. Kirchman. 2009. Rapid adaptive evolution of northeastern Coyotes via hybridization with wolves. Biology Letters, Available online at doi:10.1098/rsbl.2009.0575. Accessed 23 September 2009.

Koblmuller, S., M. Nord, R.K. Wayne, and J.A. Leonard. 2009. Origin and status of the Great Lakes wolf. Molecular Ecology 18:2313–2326. Available online at doi:10.1111/j.1365-294X.2009.04176.x. Accessed 17 December 2009.

Kolenosky, G.B. 1971. Hybridization between wolf and Coyote. Journal of Mammalogy 52:446–449.

Kolenosky, G.B., and R.O. Standfield. 1975. Morphological and ecological variation among Gray Wolves (*Canis lupus*) of Ontario, Canada. Pp. 62–72, *In* M.W. Fox (Ed.). The Wild Canids: Their Systematics, Behavioral Ecology and Evolution. Dogwise Publishing, New York, NY.

Kyle, C.J., A.R. Johnson, B.R. Patterson, P.J. Wilson, K. Shami, S.K. Grewal and B.N. White. 2006. Genetic nature of eastern wolves: Past, present, and future. Conservation Genetics 7:273–287.

Lehman, N., A. Eisenhawer, K. Hansen, L.D. Mech, R.O. Peterson, P.J.P. Gogan, and R.K. Wayne. 1991. Introgression of Coyote mitochondrial DNA into sympatric North American Gray Wolf populations. Evolution 45:104–119.

Leonard, J.A., and R.K. Wayne. 2008. Native Great Lakes wolves were not restored. Biology Letters 4:95–98. Available online at doi:10.1098/RSBI.2007.0354. Accessed 17 December 2009.

Mech, L.D. 1970. The Wolf: The Ecology and Behavior of an Endangered Species. Natural History Press, Doubleday Publishing Co., New York, NY. (Reprinted in paperback by University of Minnesota Press, May 1981).

Mech, L.D. 2009. Crying Wolf: Concluding that wolves were not restored. Biology Letters 5:65–66.

Mech, L.D. 2010. What is the taxonomic identity of Minnesota wolves? Canadian Journal of Zoology 18:129–138.

Mech, L.D., and W.J. Paul. 2008. Wolf body-mass cline across Minnesota: Related to taxonomy? Canadian Journal of Zoology 86:933–936.

Mech, L.D., R.M. Nowak, and S. Weisberg. In press. Use of cranial characters in Minnesota wolf taxonomy. Canadian Journal of Zoology.

Murray, D.L., and L. Waits. 2007. Taxonomic status and conservation of the endangered Red Wolf: A response to Kyle et al. (2006). Conservation Genetics 8:483–1485.

Nowak, R.M. 2009. Taxonomy, morphology, and genetics of wolves in the Great Lakes region. Pp. 233–250, *In* A.P. Wydeven, T.R. Van Deelen, and E.J. Heske (Eds.). Recovery of Gray Wolves in the Great Lakes Region of the United States. Springer, New York, NY.

Paquet, P.C. 1992. Prey use strategies of sympatric wolves and Coyotes in Riding Mountain National Park, Manitoba. Journal of Mammalogy 73:337–343.

Pilgrim, K.L., D.K. Boyd, and S.H. Forbes. 1998. Testing for wolf-Coyote hybridization in the Rocky Mountains Using Mitochondrial DNA. Journal of Wildlife Management 62:683–686.

Roy, M.S., E. Geffen, D. Smith, E.A. Ostrander, and R.K. Wayne. 1994. Pattern of differentiation and hybridization in North American wolflike canids revealed by analysis of microsatellite loci. Molecular Biology and Evolution 11:553–570.

Roy, M.S., E. Geffen, D. Smith, and R.K. Wayne. 1996. Molecular genetics of pre-1940s Red Wolves. Conservation Biology 10:1413–1424.

Rutledge, L.Y., K.I. Bos, R.J. Pearce, and B.N. White. 2009. Genetic and morphometric analysis of sixteenth-century *Canis* skull fragments: implications for historic Eastern and Gray Wolf distribution in North America. Conservation Genetics 11:1273–1281. Available online at doi:10.1007/s10592-009-9957-2. Accessed 17 December 2009.

Sears, H.J., J.B. Theberge, M.T. Theberge, I. Thornton,.and G. Douglas. 2003 Landscape influence on *Canis* morphological and ecological variation in a Coyote-wolf *C. lupus* x *latrans* hybrid zone, southeastern Ontario. Canadian Field-Naturalist 117:589–600.

Thiel, R. P. 2006. Conditions for sexual interactions between wild Grey Wolves, *Canis lupus*, and Coyotes, *Canis latrans*. Canadian Field-Naturalist 120:27–30.

Theberge, J.B., and M.T. Theberge. 1998. Wolf Country: Eleven Years Tracking the Algonquin Wolves. McClelland and Stewart, Inc., Toronto, ON, Canada.

Therberge, J.B., and M.T. Theberge. 2004. The Wolves of Algonquin Park: A 12-year ecological study. Department of Geography, University of Waterloo, Toronto, ON, Canada.

Villemure, M. 2003. Ecologie et conservation du loup dans la region du Parc National de la Mauricie. M.Sc. Thesis, Faculte Des Sciences Universite De Sherbrooke, Sherbrooke, QC, Canada.

Way, J.G, L. Rutledge, T. Wheeldon, and B.N. White. 2010. Genetic characterization of eastern Eoyotes in eastern Massachusetts. Northeasteastern Naturalist 17:189–204.

Wayne, R.K., and N. Lehman. 1992. Mitochondrial DNA analysis of the eastern Coyote: Origins and hybridization. Pp. 9–22, *In* A.H. Boer (Ed.). Ecology and Management of the Eastern Coyote. Wildlife Research Unit, University of New Brunswick, Fredericton, NB, Canada.

Wayne, R.K., and C. Vila. 2003. Molecular genetic studies of wolves. Pp. 218–238, *In* L.D. Mech and L. Boitani (Eds.). Wolves: Behavior, Ecology, and Conservation. University of Chicago Press, Chicago, IL.

Wheeldon, T. 2009. Genetic characterization of *Canis* populations in the western Great Lakes region. M.Sc.Thesis, Trent University, Peterborough, ON, Canada.

Wheeldon, T., and B.N. White. 2009. Genetic analysis of historic western Great Lakes region wolf samples reveals early *Canis lupus/lycaon* hybridization. Biology Letters 5:101–104.

Wheeldon, T., B.R. Patterson, and B.N. White. 2010. Colonization history and ancestry of northeastern Coyotes. Biology Letters 6:246–247.

Wilson, P.J., S. Grewal, I.D. Lawford, J.N.M. Heal, A.G. Granacki, D. Pennock, J.B. Theberge, M.T. Theberge, D.R. Voigt, W. Waddell, R.E. Chambers, P.C. Paquet, G. Goulet, D. Cluff, and B.N. White. 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, P.J., S. Grewal, F.F. Mallory, and B.N. White. 2009. Genetic characterization of hybrid wolves across Ontario. Journal of Heredity 100:S80–S89.

Wydeven, A.P., J.E. Wiedenhoeft, R.N. Schultz, R.P. Thiel, R.L. Jurewicz, B.E. Kohn and T.R. Van Deelen. 2009. History, population growth, and management of wolves in Wisconsin. Pp. 87–105, *In* A.P. Wydeven, T.R. Van Deelen, and E.J. Heske (Eds.). Recovery of Gray Wolves in the Great Lakes Region of the United States. Springer, New York, NY.