

Supplementary Information

We extracted DNA from coyote samples (tissue, blood, hair) collected from southern Ontario between 1974-1984 (n = 103) and 2005-2009 (n = 207), using a Dneasy Blood and Tissue Kit (Qiagen). We amplified a 343-347 bp fragment of the mitochondrial control region using previously described primers and conditions (Wheeldon 2009), and we sequenced a 223-224 bp region on a MegaBACE 1000 (GE Healthcare). We generated a neighbor-joining tree of the mtDNA sequences rooted with a gray wolf sequence (C22, Genbank accession FJ687608) using the Tamura-Nei model employed in MEGA (4.0, Tamura *et al.* 2007) (figure S1). Haplotypes C1 and C13 are of eastern wolf origin (see Wheeldon & White 2009), and the remaining haplotypes are of coyote origin (figure S1, but see below). We identified matching sequences in our samples and those of Kays *et al.* (2009) (table S1).

We note that the species origin of coyote-clustering haplotypes C9 and C17 is ambiguous (i.e. *lycaon/latrans*), given that they are observed in eastern wolves (Grewal *et al.* 2004) and eastern coyotes (this study; Grewal *et al.* 2004; Kays *et al.* 2009; Way *et al.* in press), but are not observed in western coyotes from Texas or Nebraska (Hailer & Leonard 2008). This ambiguity does not change the interpretation of the colonization history and ancestry of northeastern coyotes because the data is still consistent with them being hybrids of eastern wolves and western coyotes.

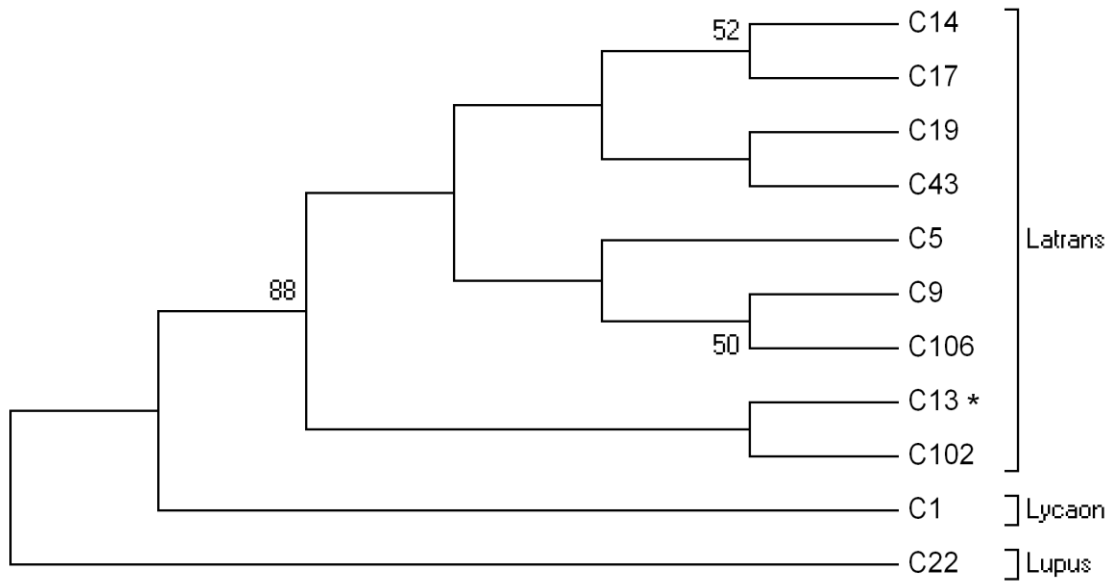


Figure S1. Neighbor-joining tree of mtDNA sequences, $\geq 50\%$ bootstrap support shown. Asterisk indicates coyote-clustering sequence of eastern wolf origin.

Table S1. Southern Ontario (SON) mtDNA haplotype frequencies and matching sequences from Kays *et al.* (2009). Multiple haplotype matches exist because Kays *et al.* sequenced a longer fragment that included the sequenced fragment of the C(n) sequences.

SON haplotype	frequency	Genbank accession	matching Kays <i>et al.</i> haplotype
C1	37	AY267718	GL20, GL21, GL22
C9	165	AY267726	cla29, cla42
C19	92	AY267736	cla28
C5	5	AY267722	cla33, cla44
C13	1	AY267730	
C14	6	AY267731	
C17	1	AY267734	
C43	1	FJ889992	
C102	1	GU062764	
C106	1	GU062765	

References

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- Kays, R., Curtis, A. & Kirchman, J.J. 2009 Rapid evolution of northeastern coyotes via hybridization with wolves. *Biol. Lett.* (doi:10.1098/rsbl.2009.0575).
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