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## Notes and Discussion

### Coyotes in Wolves' Clothing

**ABSTRACT.**—We report on three presumed wolf pups captured in Michigan's northern Lower Peninsula, potentially representing the first documented case of wolf reproduction in the Lower Peninsula since wolves were extirpated there a century ago. The pups, two females and one male, were assumed to be wolves based on physical characteristics. Genetic profiles assigned all three pups as coyotes but revealed evidence of maternal introgression from a Great Lakes wolf in their pedigree. These findings suggest that Great Lakes wolves are capable of interbreeding with coyotes when conspecifics are rare.

#### INTRODUCTION

Wolves recently re-colonized Wisconsin and the Upper Peninsula of Michigan (UPMI), after having been extirpated there during the last century (FWS, 2007). The recovery of wolves in these states is a success story for wildlife conservation and serves as a testament to the power of the protection offered by the Endangered Species Act. As the recovered wolf population continues to expand, it is expected that wolves will disperse and recolonize areas to the south, including the Lower Peninsula of Michigan (LPMI). Gehring and Potter (2005) estimated between 2200–4200 km<sup>2</sup> of favorable wolf range in northern LPMI and suggested that this area could support between 40–105 wolves. Although there have been reports of wolves in LPMI (Roell *et al.*, 2010), researchers have yet to document wolf reproduction in-situ there.

Recent genetic studies of wolves in the western Great Lakes region (WGLR; hereafter referred to as Great Lakes wolves) have revealed that hybridization with coyotes (*Canis latrans*) is rare but that Great Lakes wolves have a hybrid heritage from gray wolves (*C. lupus*) and eastern wolves (*C. lycaon*) (Fain *et al.*, 2010; Wheeldon *et al.*, 2010a). The limited evidence for wolf-coyote hybridization in the WGLR contrasts with the more extensive hybridization observed between Algonquin Provincial Park (APP) wolves and southeastern Ontario (SEON) coyotes (Sears *et al.*, 2003; Rutledge *et al.*, 2010). Given the phenotypic variability of hybridizing *Canis* and the high mobility of both wolves and coyotes, dispersing wolves and coyotes are expected to occasionally appear in previously unoccupied areas, sometimes confusing wildlife researchers and managers trying to identify them.

We report on the phenotypic classification and genetic testing of three presumed wolf pups captured in northern LPMI, potentially representing the first documented case of wolf reproduction in LPMI since wolves were extirpated there circa 1910 (Beyer *et al.*, 2009).

#### METHODS

In Mar. 2010, U.S.D.A. Wildlife Services (WS) biologists observed two sets of wolf-sized tracks in Cheboygan County, northern LPMI. Photos from trail cameras placed in the area captured images of a large canid, identified as a wolf. In Jul. and Aug. 2010, WS attempted to trap and radio-collar a wolf in this area as part of the Michigan Department of Natural Resources wolf-monitoring program. The first of three presumed wolf pups was captured on 19 Jul. 2010; it was a male. The animal was physically restrained, ear tagged, weighed, and measured. Measurements included total length, tail length, hind foot length, leading edge of ear length, shoulder height, chest girth, and upper right canine length. A blood sample for DNA analysis was collected by veni-puncture and several drops of blood were transferred to FTA<sup>TM</sup> paper (Whatman, Maidstone, Kent, UK) and stored at ambient room temperature. A coyote trapper captured the second and third presumed wolf pups on 10 Oct. 2010, less than 0.4 km from the first capture site; both were females. These individuals were immobilized with an approximate 3:1 (2.6–2.8 ml dose) combination of 100 mg/ml ketamine (Ketaset<sup>®</sup>; Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) and 100 mg/ml xylazine (X-Ject E<sup>TM</sup>; Butler Schein Animal Health, Dublin, Ohio, USA), then they were ear-tagged, weighed, measured, and blood sampled as described above.

TABLE 1.—Sex, weight, and morphological measurements of three pups from LPMI: male pup captured 19 Jul. 2010 and female pups captured 10 Oct. 2010

Field Code	Sex	Weight <sup>1</sup> (kg)	Total Length (cm)	Tail Length (cm)	Hind Foot Length (cm)	Ear Length (cm)	Shoulder Height (cm)	Chest Girth (cm)	Upper Right Canine Length (mm)
ET0426	M	10.4	109.2	35.6	20.3	10.2	48.3	50.8	5
ID#1602	F	22.2	137.2	48.3	22.2	12.7	66.0	62.2	21
ID#1603	F	19.1	134.6	50.8	21.6	12.1	66.0	59.7	19

<sup>1</sup> Body condition of each pup at time of capture was good

Genomic DNA was extracted from blood samples, then mitochondrial DNA (mtDNA) control region sequences, a Y-chromosome haplotype (male only), and genotypes based on twelve autosomal microsatellite loci were generated as in Wheeldon *et al.* (2010a).

The autosomal microsatellite genotypes of the pup samples were analyzed in the Bayesian clustering program STRUCTURE (v2.3, Pritchard *et al.*, 2000; Hubisz *et al.*, 2009) with genotypes of highly assigned individuals (*i.e.*,  $Q$ -value >0.9) from reference populations: WGLR coyotes ( $n = 47$ ; Wheeldon *et al.*, 2010a); SEON coyotes ( $n = 98$ ; not previously analyzed); APP wolves ( $n = 67$ ; Rutledge *et al.*, 2010); UPMI wolves ( $n = 82$ ; Wheeldon *et al.*, 2010a). The admixture model of STRUCTURE was run ten times at  $K = 4$  for  $10^6$  iterations following an initial burn-in of 250,000 iterations, using default settings with FREQSCORR = 1 and ANCESTDIST = 1 (90% probability intervals). The individual admixture proportions (*i.e.*,  $Q$ -values) of the pups were taken from the run having the highest posterior probability and lowest variance. A non-model based factorial correspondence analysis (FCA) of the genotype data was performed using GENETIX (v4.05, Belkhir *et al.*, 2004), and two factorial components, FC-1 and FC-2, which accounted for 7.17% and 4.29% of the total inertia respectively, were plotted to visualize the clustering of the pup samples relative to the reference populations. An exclusion test using 10,000 simulated genotypes and the frequencies-based method (Paetkau *et al.*, 1995, 2004) was conducted on the pup genotypes in GeneClass (v2, Piry *et al.*, 2004) to determine the probability of them originating from each reference population: this test does not require the assumption inherent to STRUCTURE that the true population of origin has been sampled in the dataset.

#### RESULTS

The WS biologist identified the first animal captured as a wolf pup based on dentition (Mech, 1970), size (especially length of legs and size of the feet and toes) and weight (Table 1). At the time of capture, tracks of what appeared to be an adult-sized wolf and pups were observed at the trap site. On 20 Jul. 2010, the WS biologist observed fresh adult wolf-sized tracks returning to the capture site and tracks of the adult and pup leaving on the same path. The following day he observed three presumed wolf pups on the edge of an old beaver (*Castor canadensis*) flooding about 0.4 km from the capture site. A Michigan Department of Natural Resources wildlife research assistant handled the second and third pups captured by the coyote trapper. He identified both individuals as wolf pups based on dentition (Mech, 1970), size and weight (Table 1).

The STRUCTURE analysis assigned all three pups as SEON coyotes with minimal admixture to the other groups, but the 90% probability intervals of the  $Q$ -values for the pups indicated statistical uncertainty in assigning membership between the two coyote groups (Table 2; Fig. 1). The FCA revealed that the pups clustered with coyotes and away from wolves (Fig. 2). The GeneClass analysis excluded (*i.e.*,  $P < 0.01$ ) both APP wolf and UPMI wolf groups as probable populations of origin for the pup samples but did not exclude either of the coyote groups (Table 2). The concordance among the results of the genetic analyses performed clearly indicates that the pups had a closer genetic affinity to coyotes than wolves.

The three pups had the same eastern wolf mtDNA haplotype (C3), and the male pup had a Y-chromosome of coyote origin (CR) (Wheeldon *et al.*, 2010a; Table 2). The microsatellite genotypes of the pups revealed extensive allele sharing across loci (67–79% alleles shared in pairwise comparisons), suggesting that they were putatively siblings from the same litter, as also suggested by their being captured in the same area.

TABLE 2.—Genetic profiles of three pups from LPMI, including STRUCTURE admixture proportions to four reference populations (90% probability intervals in brackets) and GeneClass exclusion test probabilities ( $P = X$ )

Field Code	mtDNA Haplotype	Y-chromosome Haplotype	WGLR coyotes	SEON coyotes	APP wolves	UPMI wolves
ET0426	C3	CR	0.091 (0 to 0.875) $P = 0.36$	0.876 (0.001 to 1) $P = 0.46$	0.024 (0 to 0.186) $P < 0.01$	0.009 (0 to 0.050) $P < 0.01$
ID#1602	C3	n/a	0.134 (0 to 0.999) $P = 0.25$	0.833 (0 to 1) $P = 0.18$	0.019 (0 to 0.142) $P < 0.01$	0.013 (0 to 0.087) $P < 0.01$
ID#1603	C3	n/a	0.075 (0 to 0.612) $P = 0.12$	0.796 (0 to 1) $P = 0.17$	0.119 (0 to 0.609) $P < 0.01$	0.010 (0 to 0.061) $P < 0.01$

DISCUSSION

The genetic assignments of the three pups as coyotes (Table 2; Figs. 1, 2) revealed an unexpected discordance with their phenotypic assignments as wolves. The sizes and weights of the female pups (Table 1) are at the upper limits reported for eastern coyotes and their weights overlap the range for APP wolves (Sears *et al.*, 2003; Way, 2007; Rutledge *et al.*, 2010). Considering that they were not fully developed, these coyote pups were unusually large.

Eastern wolf mtDNA haplotype C3 is common in Great Lakes wolves but rare in APP wolves and it has not been observed in coyotes (Koblmuller *et al.*, 2009; Kays *et al.*, 2010; Fain *et al.*, 2010; Rutledge *et al.*, 2010; Wheeldon *et al.*, 2010a, b), indicating probable maternal introgression from a Great Lakes wolf into the pups' pedigree. Although it is conceivable that a dispersing female APP wolf was the origin of the introgression, the relatively large distance between LPMI and APP wolves compared to that between LPMI and Great Lakes wolves makes the latter a much more likely source of the introgression. Considering the observed lack of admixture to UPMI wolves in the pups (Table 2; Fig. 1), we suggest that a female Great Lakes wolf hybridized with a male coyote and descendant female offspring backcrossed with male coyotes for an undetermined number of generations, culminating in the pups' birth. In contrast to the evidence presented by the microsatellite genotypes, the observation of adult wolf-sized tracks at the capture site of the male pup suggests that the pups may have been the F1 progeny of a wolf-coyote mating. Considering the potential inaccuracies of hybrid detection in STRUCTURE (Vaha and Primmer, 2006), the pups being F1 hybrids is possible, but the concordance of the genetic assignments of the pups among analyses (Table 2; Figs. 1, 2) suggests that it is unlikely. Rather we suggest that the large tracks observed at the capture site of the male pup were from an unusually large adult coyote or wolf-coyote hybrid.

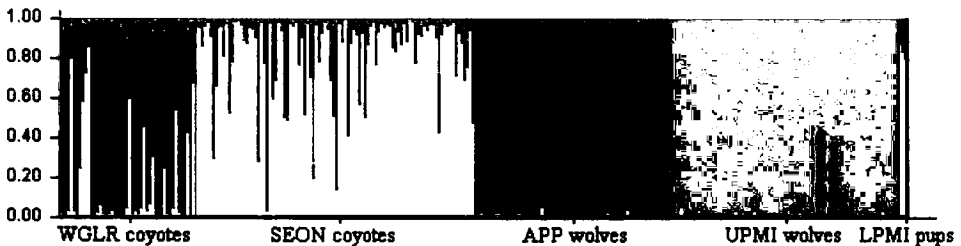


FIG. 1.—Plot of the individual proportional memberships to the  $K = 4$  reference populations analyzed in STRUCTURE

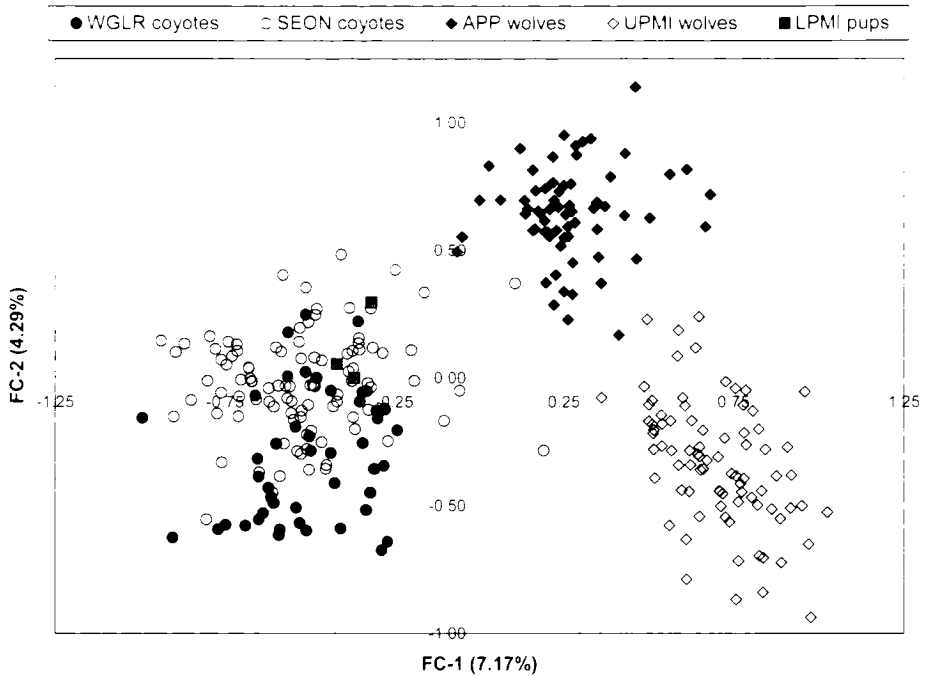


FIG. 2.—Factorial correspondence analysis of the autosomal microsatellite genotypes of the LPMI pups and reference population samples

The wolf-coyote hybridization could have occurred in UPMI with a resulting descendant later dispersing into LPMI. Alternatively, the wolf-coyote hybridization could have occurred in LPMI if a recolonizing wolf encountered a coyote and mated with it in the absence of a conspecific. The latter scenario seems more probable considering that: (1) wolves are common in UPMI and thus more likely to encounter a conspecific and (2) wolf-coyote hybridization is rare where Great Lakes wolves are well established (*e.g.*, Minnesota, Wisconsin, UPMI; Wheeldon *et al.*, 2010a).

Our findings stress the importance of employing multiple genetic markers for identification of *Canis*, because if we had relied solely on mtDNA we would have misclassified the pups as wolves. The discordance between phenotypic and genetic assignment observed in this study highlights the phenotypic variability in hybridizing *Canis*, which complicates management and enforcement. Continued monitoring of LPMI may yet reveal recolonization by wolves in the form of in-situ conspecific reproduction, but genetic testing should be used to validate phenotypic classifications until wolves have re-established themselves in LPMI in significant numbers.

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