Assessment of coyote–wolf–dog admixture using ancestry-informative diagnostic SNPs

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Abstract

The evolutionary importance of hybridization as a source of new adaptive genetic variation is rapidly gaining recognition. Hybridization between coyotes and wolves may have introduced adaptive alleles into the coyote gene pool that facilitated an expansion in their geographic range and dietary niche. Furthermore, hybridization between coyotes and domestic dogs may facilitate adaptation to human-dominated environments. We genotyped 63 ancestry-informative single-nucleotide polymorphisms in 427 canids to examine the prevalence, spatial distribution and the ecology of admixture in eastern coyotes. Using multivariate methods and Bayesian clustering analyses, we estimated the relative contributions of western coyotes, western and eastern wolves, and domestic dogs to the admixed ancestry of Ohio and eastern coyotes. We found that eastern coyotes form an extensive hybrid swarm, with all our samples having varying levels of admixture. Ohio coyotes, previously thought to be free of admixture, are also highly admixed with wolves and dogs. Coyotes in areas of high deer density are genetically more wolf-like, suggesting that natural selection for wolf-like traits may result in local adaptation at a fine geographic scale. Our results, in light of other previously published studies of admixture in *Canis*, revealed a pattern of sex-biased hybridization, presumably generated by male wolves and dogs mating with female coyotes. This study is the most comprehensive genetic survey of admixture in eastern coyotes and demonstrates that the frequency and scope of hybridization can be quantified with relatively few ancestry-informative markers.

Keywords: admixture, *Canis*, diagnostic markers, hybridization, single-nucleotide polymorphism

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Introduction

Hybridization is of immense evolutionary importance as a source of new adaptive genetic variation. Unlike novel mutations, introgressive hybridization with wild species simultaneously introduces many alleles that have already passed through the filter of natural selection. Alternatively, hybridization with domestic species can introduce many alleles that have passed through the filter of artificial selection; this may produce

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adverse consequences in natural populations. Although hybridization has generally been perceived negatively in the conservation and resource management communities (e.g. Rhymer & Simberloff 1996; Allendorf *et al.* 2001; Wolf *et al.* 2001; Oliveira *et al.* 2008), its value in enhancing the adaptive potential of parental lineages is gaining recognition (e.g. Seehausen 2004; Kyle *et al.* 2006; Kays *et al.* 2010a). Hybridization has played an important evolutionary role in past range expansions and adaptation to changing environments (Willis *et al.* 2006), and may be vital for the future survival of some taxa under rapidly changing conditions due to anthropogenic land use or climate change. Despite this critical

role that hybridization plays in evolution and conservation biology, it remains unclear how habitat variation at the landscape and regional scales affects the flow of introgressed alleles (but see Fitzpatrick & Shaffer 2007). This is particularly true in cases of anthropogenic hybridization, in which stable contact zones may not exist or hybrid swarms may establish.

Modern populations of North American wolf-like canids are known to be admixed in some areas. Coyote-derived DNA was first found in wolf populations of the Great Lakes region in the early 1990s (Lehman *et al.* 1991). A growing body of evidence indicates that the introgressive hybridization among North American *Canis* is very complex, with genetic exchange occurring in varying degrees among western gray wolves (*Canis lupus*), eastern wolves (also known as Great Lakes wolves, *C. lupus lycaon* or *C. lycaon*), Mexican wolves (*C. lupus baileyi*), red wolves (*C. rufus*), coyotes (*C. latrans*) and domestic dogs (*C. familiaris*) (Kyle *et al.* 2006 and references therein; Hailer & Leonard 2008; Kyle *et al.* 2008; Leonard & Wayne 2008; Koblmüller *et al.* 2009; Wheeldon & White 2009; Wilson *et al.* 2009; Bohling & Waits 2011; vonHoldt *et al.* 2011). Although admixture is widely accepted, researchers differ in the interpretations of molecular data and their implications for taxonomic recognition and conservation. Most of the research emphasis has been placed on the wolf side of the admixture story because of ongoing debate regarding the validity of the Great Lakes wolf and red wolf recovery programs, while less attention has been given to the causes and consequences of admixture in eastern coyotes (but see Kays *et al.* 2010a; vonHoldt *et al.* 2011). Hybridization with wolves is thought to have aided coyotes in their colonization of eastern forests by allowing them to rapidly evolve larger body size, including wider skulls, which made them more effective deer hunters (Kays *et al.* 2010a). Hence, we hypothesized that individuals living in areas of high deer density are genetically more wolf-like than those living in areas of lower deer density.

Steadily improving molecular tools and geographic sampling have refined our understanding of this hybridization story. For two decades, the extent of the molecular data was limited to restriction fragment length polymorphisms (Lehman *et al.* 1991), sequences of mitochondrial DNA (mtDNA) usually coupled with genotypes of a few nuclear microsatellites (e.g. Wayne & Lehman 1992; Roy *et al.* 1994; Koblmüller *et al.* 2009; Wilson *et al.* 2009; Rutledge *et al.* 2010), and sequences of one gene of the major histocompatibility complex (Hedrick *et al.* 2002). Still, the results of these studies, or more specifically their interpretations, were conflicting. This may be due, in part, to the low resolution offered by a small number of segregating loci in the context of a complex hybridization scenario. Microsatellites accumulate homoplasy quickly and thus have low statistical power for inferring population structure when samples are drawn from an admixed population, especially when admixture proportions are high (Haasl & Payseur 2010). More recently, vonHoldt *et al.* (2011) published the largest genomic study aimed at addressing the complex evolutionary history of wolf-like canids, taking advantage of the thousands of single-nucleotide polymorphisms (SNPs) ascertained from the dog genome project. They used a SNP microarray to assay genomic variation in more than 48 000 loci genotyped in a panel of 277 wolves and coyotes and several hundred dogs. Although this was the most extensive genetic survey of any wild vertebrate group, the geographic sampling of coyotes was limited, with only 13 individuals from north-eastern North America and three from Ohio, thus limiting inferences about admixture and population subdivision in eastern coyotes.

Here, we present data on ancestry-informative SNPs carefully selected and genotyped in a broad geographic sample of 425 eastern coyotes and two suspected immigrant wolves, and compare these genotypes to those of 40 western coyotes, 34 western wolves and 17 eastern wolves from vonHoldt *et al.* (2011). This represents the largest survey of genomic variation in eastern coyotes to date. Our objectives in this study were to use ancestry-informative SNPs to (i) assess the prevalence and spatial distribution of admixture in eastern coyotes, (ii) estimate coyote versus wolf ancestry of individuals, (iii) test for sex-biased hybridization and (iv) investigate the ecological context of admixture.

Methods

Study area and sampling

Our study area was located in north-eastern North America (Fig. 1, Table S1). All samples genotyped in this study ($N = 427$) are archived and vouchered in the New York State Museum, Albany, NY, and were collected with the assistance of local hunters and trappers since 1999. All samples were fresh tissue, except for 10 faecal samples that came from previous scat surveys in New York (Gompper *et al.* 2006; Kays *et al.* 2008); the faecal samples generated high-quality DNA as judged by spectrophotometry and mtDNA sequence quality. Two samples (zm14276 from Saratoga County, New York, and zm15083 from Orleans County, Vermont) were suspected wolves based on morphology and preliminary genetics (USFWS 2004, 2007); stable isotope data indicate that these two wolves were natural immigrants rather than escaped pets (Kays & Feranec 2011). No IACUC ethics review was required for this study
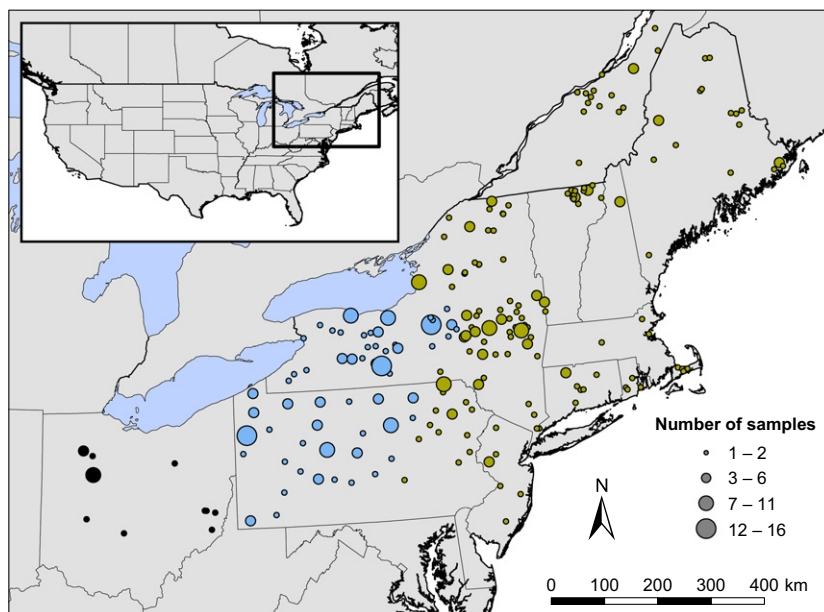
Fig. 1 Study area and sampling localities of coyotes in north-eastern United States and south-eastern Canada. Circle size represents sample size per locality. Circle colour represents geographic zone as in Kays *et al.* (2010a): black, Ohio; blue, contact zone; gold, north-east zone.

because DNA samples came from scat or were salvaged from animals killed for reasons other than research.

Selection of ancestry-informative SNPs

We selected molecular markers based on a previous study that used the Affymetrix Canine Mapping Array to genotype 60 584 autosomal SNP loci in coyotes, western gray wolves, eastern wolves and dogs (vonHoldt *et al.* 2010, 2011). We used two independent but complementary tests to select ancestry-informative SNPs. First, we used the program EIGENSTRAT (Patterson *et al.* 2006; Price *et al.* 2006) to perform a principal component analysis (PCA) of the genetic variance of western coyote, western wolf, and eastern wolf reference populations (Table S1, Supporting information) at all 60 584 loci; we then ranked all SNPs based on their contributions to the first and second principal components. Second, we computed pairwise F_{ST} per locus among the three reference populations and ranked all SNPs based on their degree of differentiation. We selected SNPs that were present both in the top 1% of loci loading the principal component that separates each pair of putative source populations and in the top 1% of an analogous F_{ST} comparison (Fig. S1, Supporting information).

The goal of our SNP selection process was to obtain a relatively small number of loci with maximum information content to discriminate among three putative parental populations of eastern coyotes: western coyotes, western wolves and eastern wolves. By choosing SNPs that have a very high F_{ST} and PCA score, we genotyped SNPs whose alleles are not shared by eastern wolves and western coyotes or by eastern wolves and

western wolves. Although we acknowledge that contemporary eastern wolves are admixed themselves, our approach to selecting SNPs gives the ability to distinguish the relative contributions of eastern and western wolf populations in the genome of eastern coyotes. Despite historic and recent hybridization with both coyotes and western gray wolves, the genomic and geographic distinctiveness of eastern wolves is widely accepted (Rutledge *et al.* 2010, 2012; vonHoldt *et al.* 2011). Also, the genetic profiles of extant wolves from the western Great Lakes region (i.e. western Ontario, Minnesota and Wisconsin) are similar to those of historic samples, suggesting that current eastern wolves are representative of the historic population (Wheeldon & White 2009; Fain *et al.* 2010). Further, admixture between wolves and coyotes very likely occurred in the western Great Lakes region (Kays *et al.* 2010a,b). For these reasons, our sample of eastern wolves included animals from Minnesota, Wisconsin and Algonquin Park in Ontario (Table S1). Although there is controversy over the systematics of eastern wolves (vonHoldt *et al.* 2011; Rutledge *et al.* 2012), our inclusion of eastern wolves as a reference population does not address their taxonomic status.

We designed a custom GoldenGate genotyping assay for the Illumina (San Diego, CA, USA) BeadXpress platform. GoldenGate is a medium-throughput, PCR-based method of genotyping many loci in one multiplex reaction and was recently used to survey genetic variation in wild canids (Sacks *et al.* 2009, 2011). We tested in silico the multiplex compatibility of those SNPs that met the abovementioned criteria by downloading from dbSNP (www.ncbi.nlm.nih.gov/SNP) at least 60 bp of

flanking sequence on each side of the polymorphism and submitting the sequences to Illumina for processing with Illumina's Assay Design Tool (ADT). ADT executes an iterative process that evaluates candidate loci and outputs an Illumina score for each SNP that could vary from 0 to 1. SNPs with scores >0.7 have a high likelihood of being amplified and genotyped successfully in the multiplex assay. In an initial set of 138 submitted SNPs, the ADT score varied from 0.17 to 0.99. We selected SNPs with Illumina scores >0.7, resulting in a final panel of 63 unlinked SNPs distributed across 25 autosomes. All SNPs are nongenic, except rs22491491, which is located in the intron region of the *CASP2* gene. The 63 loci were thus carefully selected to resolve the ancestry of the admixed coyote populations: 21 SNPs diagnostic between western coyote and western wolf, 21 diagnostic between western coyote and eastern wolf, and 21 diagnostic between western wolf and eastern wolf (Table 1).

Laboratory methods

We extracted total genomic DNA using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions or as described in Kays *et al.* (2010a). We cleaned and concentrated genomic DNA using a modified QIAamp DNA Micro Kit (Qiagen) protocol with SpinSmart PCR Purification columns (Denville Scientific, South Plainfield, NJ, USA). We determined final DNA concentrations using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and prepared five 96-well plates with genomic DNA aiming to attain uniform concentration (average: 44 ng/μL).

Genotyping was performed at the Center for Genomics and Human Genetics in The Feinstein Institute for Medical Research, Manhasset, NY, USA. The Golden-Gate assay was performed in accordance with the manufacturer's protocols. We processed the raw data using the genotyping module of Illumina's GenomeStudio software suite (version 2011.1) and followed Illumina's recommendations for evaluating overall data quality, locus performance and sample performance. We removed from all downstream analyses low-performing samples with call rates <90%. GenomeStudio automatically generates genotypes from raw fluorescent signal intensities. All SNPs were biallelic, so there are three possible genotypes per locus – AA, AB and BB – each forming three distinct clusters when signal intensity is plotted. For any particular locus, some samples did not

Table 1 Ascertainment and F_{ST} analysis of 63 ancestry-informative SNPs. SNP rs names correspond to the CanFam2.0 dog genome assembly

Western coyote – Western wolf SNPs				Western Coyote – Eastern wolf SNPs				Western wolf – Eastern wolf SNPs			
SNP	Cla allele	Clu allele	F_{ST}	SNP	Cla allele	Cly allele	F_{ST}	SNP	Clu allele	Cly allele	F_{ST}
rs24175585	G	A	0.97	rs22927609	G	C	0.96	rs22011433	C	T	0.74
rs22333390	A	G	0.97	rs22416514	G	A	0.95	rs23651611	T	C	0.72
rs9150379	G	A	0.97	rs23054155	G	C	0.94	rs24207725	A	G	0.70
rs22877057	A	C	0.97	rs22691222	C	T	0.92	rs21906101	T	G	0.70
rs24471781	T	G	0.95	rs22659787	C	G	0.90	rs22976400	G	A	0.69
rs23367849	A	G	0.91	rs22436136	A	G	0.90	rs21962387	A	G	0.62
rs24514093	T	C	0.91	rs22491491	G	A	0.90	rs22128776	A	G	0.62
rs24543100	C	T	0.90	rs22488932	C	T	0.90	rs21972855	G	A	0.62
rs23617324	T	C	0.88	rs22494347	T	G	0.89	rs24617980	T	C	0.61
rs22161480	C	T	0.88	rs22582321	C	T	0.87	rs23245491	G	C	0.61
rs8612074	A	G	0.88	rs9073720	C	T	0.87	rs23653965	T	A	0.60
rs23909187	A	G	0.87	rs24489243	G	A	0.86	rs22817050	T	C	0.59
rs23278100	C	T	0.86	rs24373496	G	A	0.85	rs22767921	A	G	0.58
rs23037622	T	C	0.86	rs24447332	C	T	0.85	rs23070823	G	T	0.57
rs22928481	C	A	0.86	rs9029227	G	A	0.85	rs24401025	A	G	0.55
rs23050823	G	A	0.86	rs24218607	T	C	0.85	rs8747831	T	C	0.54
rs23006689	G	A	0.85	rs23126832	T	A	0.84	rs24427396	A	G	0.54
rs24517393	T	C	0.85	rs8666298	G	A	0.84	rs22521423	G	A	0.54
rs22645721	C	T	0.85	rs23410089	G	T	0.83	rs24260906	A	G	0.53
rs22409691	G	A	0.84	rs22350704	C	T	0.83	rs23966574	G	A	0.53
rs23001750	C	A	0.84	rs23486713	G	A	0.83	rs24312148	G	C	0.53

Cla, western coyote; Clu, western wolf; Cly, eastern wolf.

conform to any of the three possible genotype clusters, so we also manually removed these ambiguous genotypes.

Analyses of admixture

We used two independent approaches to evaluate admixture. First, we used PLINK 1.07 (Purcell *et al.* 2007; http://pngu.mgh.harvard.edu/purcell/plink) to perform multidimensional scaling analysis (MDS). MDS is a data-agnostic multivariate method that allows one to explore and visualize the variation and dominant relationships in genetic data. MDS in PLINK is based on the pairwise identity-by-state distance matrix, and the results are comparable to those of PCA. Second, we used STRUCTURE 2.3 (Pritchard *et al.* 2000) to quantify admixture and estimate ancestry in our sample in relation to the three reference populations. STRUCTURE implements a Bayesian clustering algorithm to infer the ancestry of admixed individuals by calculating the posterior mean estimates of K proportions of the genome inherited from ancestors in K populations. By employing these two complementary approaches, we address the criticisms of each. For example, STRUCTURE and similar Bayesian clustering methods are powerful analytical tools, but assume a population genetics model that may be violated in natural populations. MDS and similar ordination methods simply provide a scatter plot that one subjectively examines for interesting patterns, but the data are not required to meet biological assumptions. Therefore, using both complementary approaches strengthens the interpretability of the results (Patterson *et al.* 2006; Rutledge *et al.* 2010).

We used a set consisting of 40 western coyotes, 34 western wolves and 17 eastern wolves from vonHoldt *et al.* (2011) as reference parental populations to estimate the ancestry proportions of 427 eastern canids genotyped in this study and 10 additional coyotes genotyped by vonHoldt *et al.* (2011) – nine north-eastern coyotes plus one Ohio coyote known to be admixed (Table S1, Supporting information). To ensure that the results of admixture analyses are not largely affected by our choice of priors in STRUCTURE's Bayesian framework, we first tested the ability of our 63 ancestry-informative SNPs to distinguish among the three reference populations using six combinations of allele frequency and ancestry models available in STRUCTURE 2.3. We also performed these tests using 63 random SNPs; we used the website www.random.org to generate 63 random numbers between 1 and 48 036 to extract the genotypes of 63 random SNPs from the phased 48K-SNP data set of vonHoldt *et al.* (2011). In these confirmatory tests, we utilized 50 000 burn-in and 500 000 MCMC iterations, and set the number of populations (K) to 3.

After validating the diagnostic capability of the 63 ancestry-informative SNPs and their robustness to various modelling settings, we estimated the ancestry proportions of each individual in our sample of eastern coyotes using STRUCTURE and the same parameters as described above, following Bohling & Waits (2011). Specifically, for individuals without prior population information (i.e. the 437 'unknown' samples), we used the admixture model and set STRUCTURE to infer α (a Dirichlet parameter for degree of admixture) using a separate α for each population. Even though the ancestry estimate for each unknown individual depends only on the reference set and not on the other unknown hybrid individuals, Vähä & Primmer (2006) concluded that STRUCTURE may be somewhat sensitive to the proportion of hybrids in the overall sample. We tested whether the posterior ancestry estimates would be affected by this proportion by analyzing only the Ohio coyotes ($N = 24$) with the reference set ($N = 91$) and comparing their ancestry estimates with those generated when the full sample ($N = 437$) is analyzed with the reference set.

Although domestic dogs were not included in our SNP ascertainment panel or in the initial STRUCTURE reference set, vonHoldt *et al.* (2011) discovered substantial levels of admixture with dogs, especially in Ohio and eastern coyotes. To test for the degree of dog admixture in our samples of Ohio and eastern coyotes, we conducted a post hoc analysis by adding a fourth reference population of domestic dogs, choosing ten modern dog breeds based on their size, presence in North America, and potential to mate with coyotes and wolves (Table S1, Supporting information). We carried out this analysis in STRUCTURE using the same parameters as above, but setting K to 4. For all STRUCTURE analyses, we conducted five replicate runs and used CLUMPP (Jakobsson & Rosenberg 2007) to align and average the five replicate cluster membership coefficient matrices.

To investigate the broader ecological context of admixture, we tested the hypothesis that individuals living in areas of high deer density are more wolf-like genetically than those living in areas of low deer density. We obtained white-tailed deer (*Odocoileus virginianus*) densities from a Quality Deer Management Association 2008 map that depicts deer densities across management units or counties and summarizes the data provided by state wildlife agencies (www.qdma.com/shop/qdma-white-tailed-deer-density-map). Deer density was treated as a categorical ordinal variable with four density bins (<15, 15–30, 30–45 and >45 deer per square mile) because that is how Quality Deer Management Association standardized data across wildlife agencies. We assigned to each individual canid the deer density that corresponds to its sampling locality. We analyzed the association of wolf ancestry to deer

density using a nonparametric Kruskal–Wallis test in R (R Development Core Team 2012). We used the combined western + eastern wolf ancestry component estimated when dogs were included in the analysis, thus eliminating the *latrans* and *familiaris* components of individuals' ancestry.

Results

Genotyping results

We attempted to genotype 480 samples at 63 SNP loci. Our quality control measures filtered out 53 samples: 51 that performed poorly with call rates <90% and two that were mislabelled. Thus, we report the results for the remaining 427 samples. Out of 26 901 expected genotypes, 1372 were not called or were manually removed due to ambiguity, for a total genotyping call rate of 94.9%. Across all 63 SNPs, call rates ranged from 83% to 100% and averaged 95.5% ($\pm$3.5% SD). Ten of the thirteen scat samples we attempted to genotype amplified successfully with high call rates. Genotyping success did not depend on the source of DNA, whether faecal or tissue sample (chi-squared test for independence: $\chi^2 = 2.158$, df = 1, $P = 0.142$).

Admixture

The distribution of genetic variation among genotyped individuals in relation to the reference populations can be visualized with MDS (Fig. 2). In this analysis using

Fig. 2 Multidimensional scaling plot of the three reference populations and the 427 canids genotyped in this study. Data for all samples are only from 63 ancestry-informative SNPs. Samples genotyped in this study were partitioned into three geographic zones as in Kays *et al.* (2010a): Ohio, contact zone and north-east zone. Black triangles represent the three Ohio coyotes genotyped by vonHoldt *et al.* (2011). Gold squares represent two immigrant wolves from Vermont (left) and New York (right).

only the 63 ancestry-informative SNPs, the three reference populations formed their own distinct clusters in a manner similar to when the ordination is performed on all 60 000+ loci (Fig. 2, Fig. S1, Supporting information). MDS axis 1 separated coyotes from wolves and axis 2 separated the two wolf reference populations (western vs. eastern). Most of the 425 eastern coyote samples formed their own cluster that has little overlap with the western coyote cluster. As expected, eastern coyotes were situated between western coyotes and wolves in MDS space, with large variability in genomic contributions from each reference population; that is, some individuals were genetically more similar to western coyotes while others were much more wolf-like. Most individuals from Ohio were far from western coyotes in MDS space. Instead, all Ohio coyotes were fully within the distribution of contact zone and north-east zone coyotes (Fig. 2), even in higher MDS dimensions (data not shown). The three Ohio coyotes genotyped by von-Holdt *et al.* (2011) – graphed as black triangles in Fig. 2 – were outliers relative to other Ohio coyotes genotyped in this study. In addition, one of the suspected wolf immigrants (zm15083 from Orleans County, VT) clearly clustered with western wolves and the other (zm14276 from Saratoga County, NY) with eastern wolves (Fig. 2).

In all 42 SNPs that distinguish western coyotes from western and eastern wolves (Table 1), the frequency of the rare wolf allele was much higher in eastern coyotes than in western coyotes (Fig. 3). The average frequency of the wolf allele in western coyotes was 0.05, but increased to 0.31 in eastern coyotes. In five loci, the wolf allele became the major allele in eastern coyotes, attaining frequencies in excess of 0.5. In five loci that were monomorphic in western coyotes (i.e. the wolf allele was absent in a sample of 40 diploid individuals), the wolf allele appeared in moderate to high frequencies in eastern coyotes (Fig. 3). In these same 42 SNPs, eastern coyotes had moderate to high dosage of coyote alleles and an average heterozygosity of 0.33 (range: 0.02–0.73; Fig. S2, Supporting information).

We conducted preliminary tests in STRUCTURE with six model combinations of ancestry and allele frequency priors, using only the 91 reference individuals. The 63 ancestry-informative SNPs performed well in all simulations, clearly discriminating the three reference populations (Fig. 4a). Thus, the 'cleanness' of the bar plots in Fig. 4a is not a statistical artefact, and the parameters used as priors in STRUCTURE's Bayesian approach do not strongly affect the results. With 63 randomly chosen SNPs, individuals from all three reference populations were misassigned in the 'No Admixture' simulations, and the reference populations were not clearly partitioned in the 'Admixture' simulations (Fig. 4b).
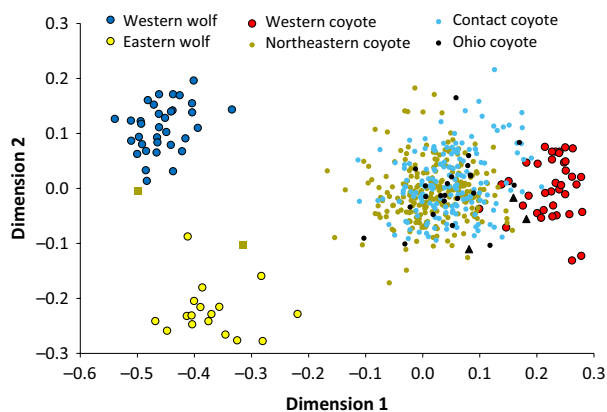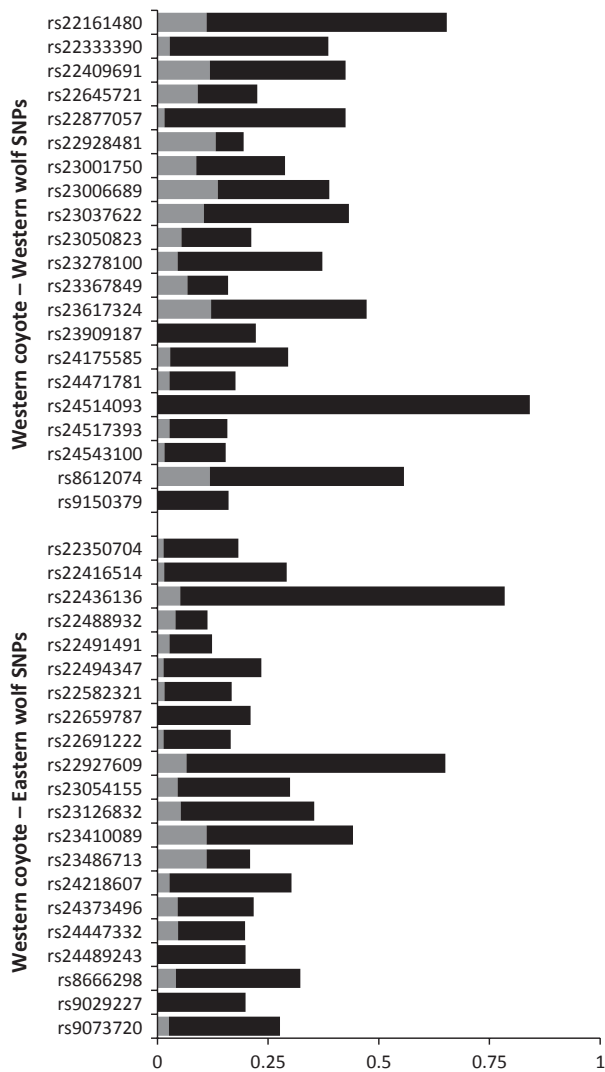
Fig. 3 Frequency of the wolf allele in 42 SNPs that distinguish western coyotes from western and eastern wolves. The length of the gray bar is the frequency of the rare wolf allele in the western coyote reference population. The total length of the bar (gray + black) is the frequency of the wolf allele in the full sample of eastern coyotes; thus, the black portion is the increase in frequency due to introgression.

The random SNPs did not perform well, except in the 'Use Population Info' simulations (Fig. 4b).

After determining that the 63 ancestry-informative SNPs successfully distinguished among western coyotes, western wolves and eastern wolves, and that the results are not much affected by modelling decisions, we used them to assess the ancestry of 437 eastern canids in relation to the three parental populations. Although the 63 ancestry-informative SNPs were not initially selected to distinguish dogs from wild *Canis*, they did so remarkably well (Table 2). This allowed us to also estimate the proportion of dog ancestry in

eastern coyotes. All coyotes examined showed a signal of admixture, but there were some subtle geographic differences in the degree of wolf and dog introgression (Fig. 5, Table 2). Coyotes from the north-east zone were significantly more wolf-like than Ohio and contact zone coyotes (Kruskal–Wallis $\chi^2 = 26.45$, $P < 0.001$ with $K = 3$; Kruskal–Wallis $\chi^2 = 22.54$, $P < 0.001$ with $K = 4$). There was a widespread signal of dog ancestry, with about 10% of eastern coyotes' genome assigned to dog, but north-eastern coyotes had significantly higher dog ancestry (Kruskal–Wallis $\chi^2 = 6.66$, $P = 0.010$). Ohio coyotes had, on average, 31% wolf ancestry (western + eastern), which is not significantly different from contact and north-east zone coyotes (Kruskal–Wallis $\chi^2 = 3.26$, $P = 0.071$). When we compared the ancestry estimates of the Ohio coyotes when analyzed alone versus when analyzed with the other 413 canids, the differences were negligible; thus, the STRUCTURE analyses were not sensitive to the proportion of hybrids in the overall sample.

Ten coyotes sampled from the highest deer density habitats (>45 deer/mile²) were genetically more wolf-like than coyotes sampled from habitats with lower deer density (Fig. 6, Kruskal–Wallis $\chi^2 = 7.21$, $P = 0.007$). The average pairwise relatedness of these ten individuals was $r = 0.13$ (2×Lynch & Ritland 1999 estimator). We note here that the Kruskal–Wallis statistic and corresponding P-value test the equality of medians and not a causal relationship; that is, the statistical hypothesis was that wolf ancestry varies in areas of different deer density, not that deer density 'shapes' wolf ancestry.

Discussion

Admixture in North American canids

We analyzed 63 ancestry-informative autosomal SNPs in 437 north-eastern canids and found that admixture is pervasive across the region. The ancestry of all coyotes we sampled showed a clear signal of hybridization with various *Canis* groups: western wolves, eastern wolves and domestic dogs. Recent evidence from mitochondrial and Y-chromosome DNA supports our finding of extensive hybridization among western gray wolves, eastern wolves, coyotes and dogs in eastern North America (Wilson *et al.* 2012; Wheeldon *et al.* 2013). This coyote–wolf–dog hybrid swarm extends into the Midwestern United States. Contrary to our expectations from an earlier finding of no wolf mtDNA in Ohio coyotes (Kays *et al.* 2010a), these same individuals were, on average, 66% coyote and 24% wolf in their nuclear genome (Table 2). The extension of wolf introgression into Ohio was unexpected because vonHoldt *et al.* (2011) found

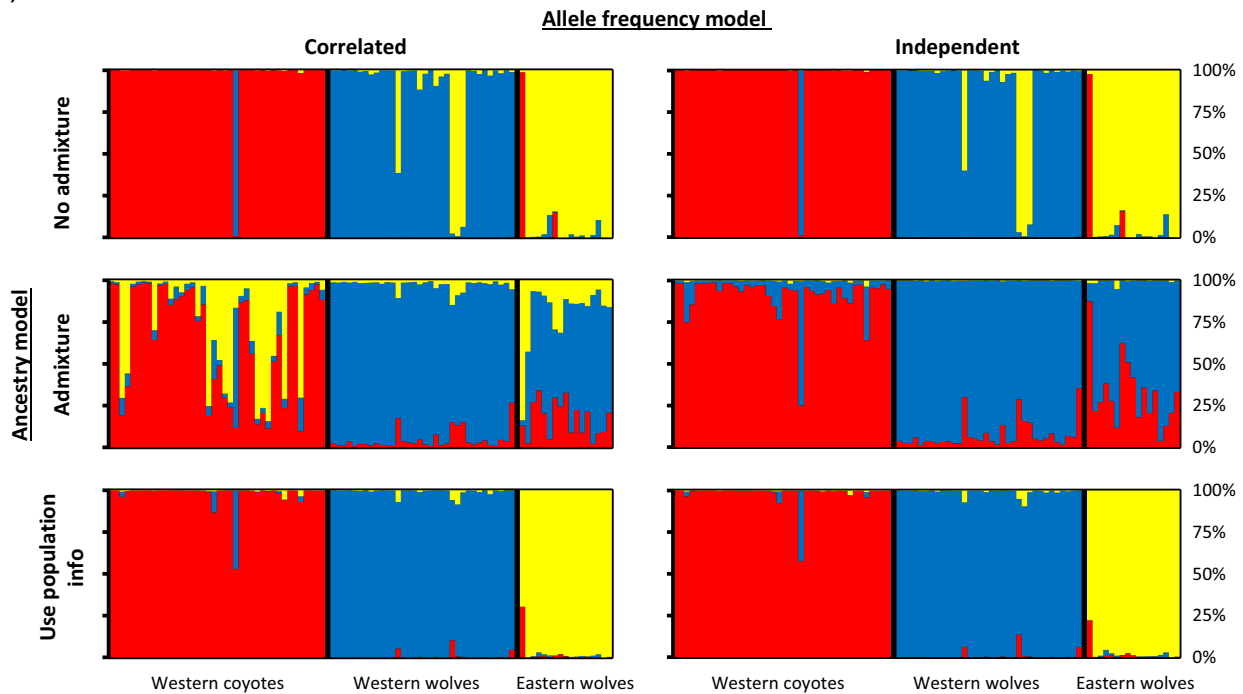**(a) 63 diagnostic SNPs**
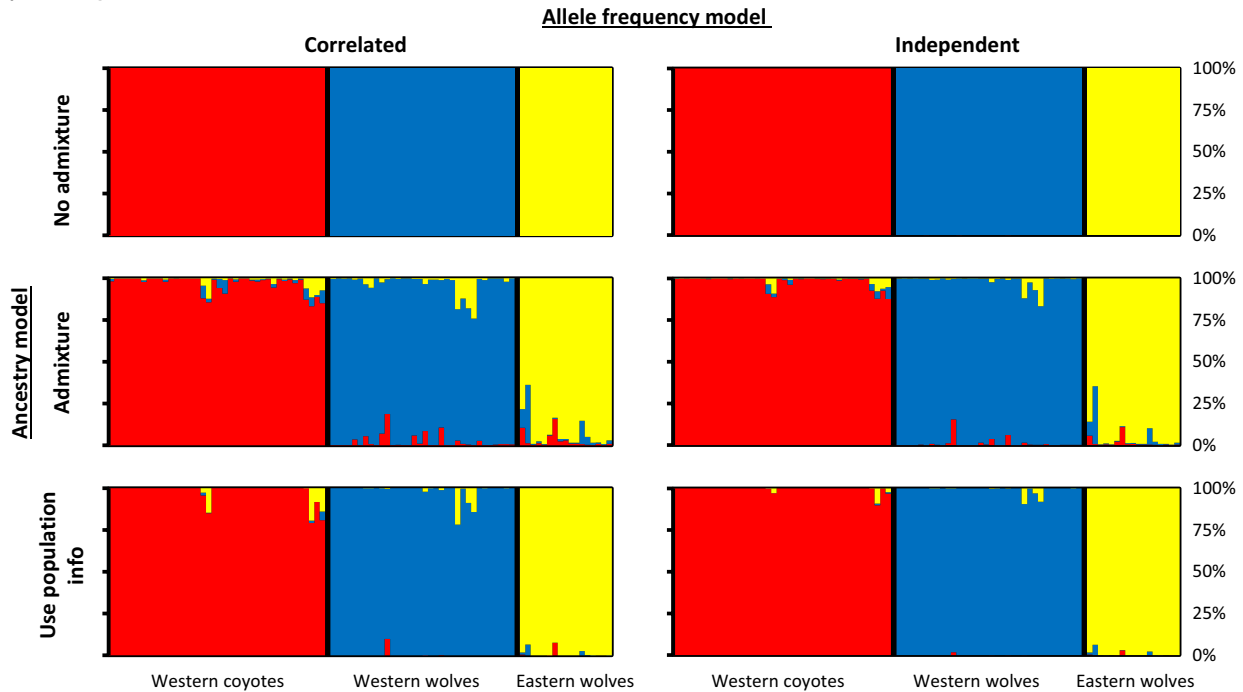
(b) 63 random SNPs

Fig. 4 Ability of 63 ancestry-informative SNPs (a) versus 63 random SNPs (b) to distinguish among 40 western coyotes (red), 34 western wolves (blue) and 17 eastern wolves (yellow) using six combinations of three ancestry models and two allele frequency models available in STRUCTURE 2.3. The purpose of this analysis was to validate the diagnostic capability of our carefully selected SNPs and to test the sensitivity of the results to the priors in STRUCTURE's Bayesian framework.

that Midwestern/southern coyotes were genetically distinct from hybrid north-eastern coyotes, and that admixture in Midwestern/southern coyotes was

primarily with dogs. In their analyses, Midwestern/ southern coyotes had, on average, 7.5% dog ancestry and 2.4% wolf ancestry. However, their inference came

Table 2 Ancestry analysis of 437 admixed canids in relation to three or four parental populations (in bold)

		Three parental populations			Four parental populations			
	N	% Cla	% Clu	% Cly	% Cla	% Clu	% Cly	% Cfa
Western coyotes	40	99.59	0.05	0.37	99.44	0.03	0.27	0.26
Western wolves	34	0.05	99.32	0.63	0.03	99.30	0.46	0.21
Eastern wolves	17	0.20	0.58	99.22	0.13	0.40	99.30	0.17
Dogs	10	—	—	—	0.01	0.06	0.08	99.85
Ohio coyotes	24	68.94	14.62	16.43	66.23	11.67	12.36	9.74
Contact coyotes	167	68.64	15.56	15.80	65.77	12.31	11.66	10.26
North-eastern coyotes	244	64.69	17.10	18.21	61.73	13.62	13.58	11.07
North-eastern wolves	2	23.22	49.80	26.99	20.99	44.98	19.88	14.16

Cla, western coyotes; Clu, western wolves; Cly, eastern wolves; Cfa, domestic dogs. Ohio, contact and north-eastern subdivisions correspond to geographic zones in Fig. 1. Values indicate mean ancestry estimate of N samples.
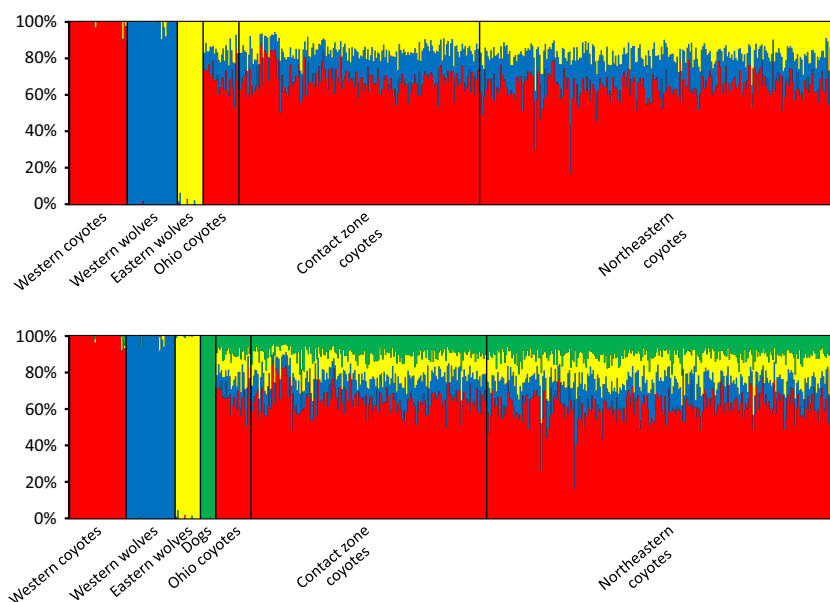

Fig. 5 Ancestry analyses of 437 admixed canids in relation to the three (top) or four (bottom) parental populations. STRUCTURE bar plots depict each individual as a vertical bar divided into three or four posterior mean estimates of its admixed ancestry, that is, the estimated proportion of its genome inherited from western coyote, western wolf, eastern wolf or dog ancestors. Ancestry-informative genetic markers were selected to make the parental populations as distinct as possible. The two north-eastern individuals with high wolf ancestry are immigrant wolves sampled in New York and Vermont. Average ancestry estimates per group are given in Table 2.

from a limited sample of 13 north-eastern and 19 Midwestern/southern coyotes, only three of which were from Ohio. Those three Ohio coyote samples are on the periphery of the statistical distribution of other Ohio coyotes genotyped in this study (Fig. 2). This is not surprising given that those three coyotes were selected for genotyping with the SNP microarray because they were morphologically peculiar, having unusual pelage and craniodental phenotypes.

How did wolf-derived DNA arrive in Ohio? We propose three hypotheses that require further investigation: (i) coyote–wolf hybrids, descendants of the northern expansion front, circled around the Great Lakes and back westward into Ohio; (ii) coyote–wolf hybridization occurred in Minnesota or western Ontario (Kays et al. 2010b) and the initial colonizers of Ohio were admixed; (iii) coyote–wolf hybrids from southern Ontario moved into the southern peninsula of Michigan and then south

into Ohio. These three and any other hypotheses must be able to account for the disparate patterns in mitochondrial and nuclear DNA.

In theory, it is possible that north-eastern coyotes evolved to be more wolf-like genetically due to natural selection, genetic drift, or both, thus appearing admixed in the absence of actual hybridization. We reject this hypothesis on several grounds. First, early studies were highly suggestive of a hybrid origin for north-eastern coyotes, long before the availability of any molecular data needed to confirm this (Monzón 2012). The hybridization hypothesis was proposed by various authors entirely on the basis of morphology (Lawrence & Bossert 1969) and captive rearing experiments (Silver & Silver 1969; Kolenosky 1971; Mengel 1971). Second, molecular evidence has unequivocally confirmed coyote–wolf admixture in the Great Lakes region and further east since the early 1990s (Lehman et al. 1991;
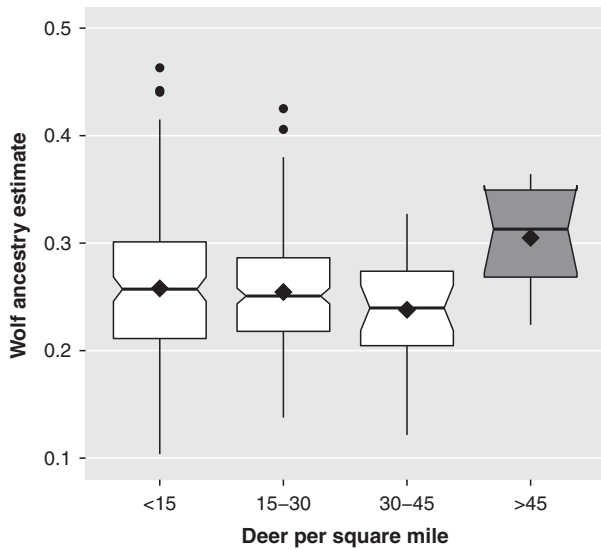
Fig. 6 Wolf ancestry plotted against deer density. Canids in areas of very high deer density are significantly more wolf-like than in lower density classes, as denoted by the dark gray box. Wolf ancestry on the vertical axis is the combined western + eastern wolf ancestry proportion estimated when dogs were included in the STRUCTURE analysis, thus eliminating the *latrans* and *familiaris* components. The lower and upper boundaries of each box correspond to the first and third quartiles, respectively; black line is the median, black diamond is the mean and circles are outliers. Nonoverlapping notches on the sides of boxes indicate strong evidence that the medians differ.

Wayne & Lehman 1992), including recent evidence of wolf mitochondrial DNA introgressing north-eastern coyotes (Koblmüller *et al.* 2009; Kays *et al.* 2010a). Third, genetic drift alone would make some rare wolf alleles become more frequent at a few loci but become extinct at most other loci, and selection alone would make rare wolf alleles more frequent at a few loci but remain constant at most other loci. However, our results show that the rare wolf alleles universally became more frequent in eastern coyotes than in western coyotes, even if they were absent in the western coyote parental population (Fig. 3), demonstrating the rapid influx of wolf DNA from introgressive hybridization.

During the design phase of our study, there was little evidence that hybridization with domestic dogs is prevalent in the Northeast. Way *et al.* (2010) found no dog mtDNA in 67 coyotes from eastern Massachusetts, and Kays *et al.* (2010a) found only one dog mtDNA haplotype in a region-wide sample of 715 eastern coyotes. Consequently, we did not select any SNPs to be diagnostic of dog ancestry when designing our study, but were able to consider this using a post hoc analysis. Our findings are consistent with those of vonHoldt *et al.* (2011), who found that north-eastern coyotes have on average 9.1% dog ancestry; we found that region-wide

(including Ohio) coyotes have on average 10.7% (±3.3% SD) dog ancestry. Using twelve autosomal microsatellites, Wheeldon *et al.* (2013) recently found that coyotes in south-eastern Ontario have on average 2.3% dog ancestry. Together, these results suggest a limited, but appreciable, amount of coyote–dog hybridization in the recent past (11–24 generations, estimated by vonHoldt *et al.* 2011). Since then, the dog components of the genome have been diluted and integrated into the wild gene pool through generations of backcrossing with eastern coyotes. We found no evidence for ongoing coyote–dog hybridization; the homogeneity and low proportion of the dog component in our large sample of wild eastern coyotes suggest that coyote–dog hybridization is infrequent, although the wild population is so abundant that coyote–dog F1 hybrids may appear at a frequency below the detection power of our sample. On the other hand, the dosage of coyote versus wolf alleles and the fraction of heterozygous loci (Fig. S2, Supporting information) suggest that at least some individuals are first- or second-generation coyote–wolf hybrids backcrossed to coyote (vonHoldt *et al.* 2013).

Our data reveal a complex pattern of admixture among coyotes, dogs, and two distinct wolf populations. We do not believe the common name 'Coywolf', proposed for north-eastern coyotes by Way *et al.* (2010), captures this complexity. Similar patterns of three- and four-way hybridization have been observed in North American *Canis*. Hailer & Leonard (2008) found some degree of hybridization among sympatric coyotes, Mexican wolves, and red wolves in Texas. Bohling & Waits (2011) detected frequent admixture among coyotes, grey wolves, red wolves, and domestic dogs in North Carolina. Rutledge *et al.* (2010) showed that eastern wolves in Ontario act as a conduit of gene flow between coyotes and western wolves by hybridizing with both. Hybridization in *Canis* extends outside North America: domestic dog genes have introgressed into the wild Australian dingo, European grey wolf, and Ethiopian wolf (*Canis simensis*) populations (Gottelli *et al.* 1994; Elledge *et al.* 2008; Godinho *et al.* 2011).

Sex-biased hybridization

The observation that wolf DNA is present in the nuclear genome but absent in the mitochondrial genome of Ohio coyotes is clear evidence of sex-biased hybridization between male wolves and female coyotes. The first genetic evidence of coyote–wolf interbreeding suggested that hybridization is unidirectional and occurs only with male wolves and female coyotes (Lehman *et al.* 1991). Additional mitochondrial, Y-chromosome, and autosomal microsatellite data revealed that male western wolves tend to cross with female

eastern wolves, and that male eastern wolves tend to cross with female coyotes across Ontario (Rutledge *et al.* 2010). A recent survey of several populations across eastern North America found a surprisingly high frequency of western wolf Y-chromosome haplotypes in eastern coyotes, despite an absence of western wolf mtDNA (Wilson *et al.* 2012). Evidence for interspecific crosses between male coyotes and female wolves is much scarcer. Only 1 in 70 Texas coyotes surveyed carried maternal gray wolf DNA (Hailer & Leonard 2008); and although hundreds of eastern coyotes carried maternal eastern wolf DNA (Kays *et al.* 2010a), nearly all carried the same haplotype, possibly the result of a single hybridization event. However, it should be noted that the genetic ancestry of wolf is expected to be lost much faster from the mitochondrial genome compared to the nuclear genome if hybridization is followed by subsequent backcrosses with coyote. For example, if the F1 hybrid son of a male coyote and female wolf backcrosses with a female coyote, his offspring will have 25% wolf ancestry in the nuclear genome but 0% wolf ancestry in the mitochondrial genome.

Similarly, the observation that dog DNA is present in the nuclear genome but absent in the mitochondrial genome of eastern coyotes reveals that male dogs mated with female coyotes, but not *vice versa*. A recent analysis of maternally inherited mtDNA and paternally inherited Y-chromosome markers revealed that the asymmetric introgression of dog genes into north-eastern coyotes is mediated by male dogs (Wheeldon *et al.* 2013). Hybridization of European and African wolves with dogs is also consistently mediated by male dogs and female wolves (Gottelli *et al.* 1994; Vilà *et al.* 2003; Godinho *et al.* 2011, but see Hindrikson *et al.* 2012 for a rare exception). It is conceivable that our coyote study and the Old World wolf studies failed to sample the hybrid progeny of wild males and domestic females as these pups would likely be reared by bitches in domestic settings or eliminated by dog owners. In contrast to this general pattern of sex-biased hybridization, Adams *et al.* (2003) documented about 10% of south-eastern coyotes with a dog mtDNA sequence, but they postulated a more unnatural cause: young male coyotes from Texas were periodically trapped and released in the south-east for sport hunting before the main front of coyotes colonized the region; a male coyote that escaped had no female conspecifics and mated with a local dog instead. Our data are consistent with other observations that sexual interactions between wild and domestic canids generally involve male dogs. It is not uncommon for males of certain dog breeds to be as large, or larger, than female wolves or coyotes.

Overall, our data support the hypothesis that the directionality of coyote–wolf–dog sexual interactions is largely determined by body size, with the males of the larger species mating with females of the smaller, as some dog breeds are larger than coyotes. But at least two other hypotheses may account for the apparent directionality of hybridization in *Canis*. First, wolf populations, usually being sparse, may be subject to Allee effects. One such effect may be the perception by male wolves of heterospecific females as potential mates. Male wolves may encounter lone female coyotes much more frequently than male coyotes encounter lone female wolves. Second, strong maternal effects in *Canis* may preclude F1 progeny of domestic mothers and wild fathers to enter the wild population, as mentioned earlier. The body size, Allee effects and maternal effects hypotheses are not mutually exclusive, but one way to start testing them is to look for coyote and wolf introgression in rural and feral dogs. For example, the size hypothesis predicts greater introgression of wild alleles in litters of smaller female dogs than in litters of larger females. Alternatively, the maternal effects hypothesis predicts the presence of wild diagnostic alleles in hybrid sons of domestic mothers, but dog diagnostic alleles in hybrid sons of wild mothers.

Ecological context of hybridization

Coyotes living in areas of high deer density are more wolf-like genetically (Fig. 6), supporting the idea that introgressive hybridization with wolves facilitated the colonization of eastern forests and introduced adaptive genetic variation that allowed coyotes to exploit a prey base rich with ungulates (Kays *et al.* 2010a). Broadly comparing regional populations, north-eastern coyotes eat more deer than their western and southern counterparts. Many studies (reviewed by Parker 1995 and Gompper 2002) from western, Midwestern and southern areas of its range have shown the coyote to be an insignificant predator of deer. On the other hand, deer are the most important component of the diet of north-eastern coyotes, especially for reproductive adults and coyotes in areas with dense forest and severe winters (Harrison & Harrison 1984; Major & Sherburne 1987; Brundige 1993). At a more local scale, predation of white-tailed deer, especially of fawns, is much greater in areas of high deer density versus areas of low deer density (Blanton & Hill 1989). Wolf ancestry of admixed canids in southern Ontario also is positively associated with moose density (Benson *et al.* 2012). The strong dependence of north-eastern coyotes on ungulate prey presents the possibility that heterogeneity in prey density exerts differential selection pressures on coyotes' ability to hunt them. Wolves are better adapted to kill ungulates; thus, habitats rich in deer and moose may be favourable to hybrid individuals with a greater

degree of wolf ancestry, resulting in fine-scaled local adaptation.

The positive association of wolf-likeness with deer density observed in this study is driven solely by a small sample of 10 individuals from two areas of very high deer density in New Jersey; these two areas measure 1380 and 842 km^2. The association is not clinal and disappears when the two highest levels of deer density are compared with the two lowest. Further research is needed to confirm the local adaptation hypothesis with more sampling sites of high deer density, or better, finer-grained, continuous deer density data and genetic markers linked to traits of known function, such as genes related to body size and skull morphology. Further, an analysis of skulls may reveal that coyotes living in areas of high deer density appear more wolf-like morphologically. Coyote–wolf hybrids in undisturbed landscapes of south-eastern Ontario indeed have a more wolf-like morphology and diet, while those in nearby fragmented and disturbed landscapes have a more coyote-like form and diet (Sears *et al.* 2003). These and our results preliminarily indicate that natural selection for wolf-like versus coyote-like traits may be occurring at a fine geographic scale based on landscape characteristics, such as prey availability and human land use.

Methodological matters

Several methodological issues are noteworthy. First, our use of ancestry-informative markers allowed us to quantify the relative genomic contributions of four putative parental populations to eastern coyotes. Fitzpatrick & Shaffer (2004, 2007) employed a similar approach using 8 diagnostic restriction fragment length polymorphisms to ascertain the degree of introgression from an introduced salamander's genes into the gene pool of the threatened California tiger salamander (*Ambystoma californiense*). Talbot *et al.* (2011) likewise developed an assay to distinguish among four poplar (*Populus*) species and their hybrids using 26 diagnostic SNPs. vonHoldt *et al.* (2013) developed an assay to detect recent wolf-dog hybridization using a panel of 24 diagnostic SNPs with divergent allele frequency distributions. These studies demonstrate how readily hybridization can be quantified with just a few carefully chosen fixed or nearly fixed diagnostic markers. Indeed, a simulation study suggested that 12 loci with an average F_{ST} of 0.21 have sufficient power to detect hybrids of two parental populations, although the hypothetical loci were multiallelic (Vähä & Primmer 2006). The 63 markers we used in this study have very high F_{ST} values (average: 0.79, range: 0.53–0.97; Table 1) compared to genome-wide F_{ST} (western coyote–western wolf: 0.14, western coyote–eastern wolf: 0.11, western

wolf–eastern wolf: 0.05; vonHoldt *et al.* 2011). As a result, we were able to still use the admixed eastern wolves as a reference population to assess their relative contribution to eastern coyotes. Even though none of the loci investigated in this study were in coding regions, it is possible that they are linked to functional genes; this is likely because of their high divergence across parental populations. If wolf alleles are favourably selected in north-eastern coyotes as we propose, then the admixture proportions may be somewhat overestimated.

Second, several recent studies have employed STRUCTURE or similar Bayesian clustering programs to assess hybridization (e.g. Oliveira *et al.* 2008; Bohling & Waits 2011; Godinho *et al.* 2011; vonHoldt *et al.* 2011; Sacks *et al.* 2011). Most of these studies avoid using prior population information (the usepopinfo ancestry model in STRUCTURE) that may bias the posterior probabilities of assignment. However, the usepopinfo model is necessary when using a sample of reference populations to assess admixture in another population. In STRUCTURE's user manual, Pritchard *et al.* (2010) caution the user to also run the program without population information to ensure that the predefined populations are in rough agreement with the genetic information because this model assumes that the predefined populations are usually correct. We ran our reference samples through the program with and without the usepopinfo model and observed that it is unwise to use prior population information if the genetic markers are polymorphic but not diagnostic. If, on the other hand, diagnostic markers are chosen to maximize differentiation among groups, user decisions concerning biological assumptions are less influential (Fig. 4).

Third, using faecal samples for population-level genetic analyses became popular in the 1990s, but has some technical complications because scat-derived DNA tends to be highly fragmented and degraded (Kohn & Wayne 1997; Kohn *et al.* 1999). The Illumina GoldenGate assay we used worked as well with scat samples as with tissue samples, most likely because the PCR amplicons are short (100–120 bp). Sacks *et al.* (2011) obtained similar positive results using the same method. Thus, we recommend that researchers working with faecal DNA use a method that amplifies similarly short sequences, such as assays of SNPs and small indels or next-generation sequencing procedures that produce short reads.

Fourth, SNPs are the new vogue in population and conservation genetics (Morin *et al.* 2004; Kohn *et al.* 2006). The completion of the dog genome project enabled researchers to discover and interrogate many SNPs in wild canids. Despite the technological advances, several pioneering studies were fraught with laborious SNP discovery and genotyping methods, small sample sizes, and low genotyping rates (Seddon *et al.* 2005; Andersen *et al.*

2006; Sacks & Louie 2008). As in all molecular studies, researchers must balance the costs of genotyping many individuals versus many loci. We navigated these issues by choosing a mid-throughput genotyping method and judiciously selecting SNPs with the highest information content for admixture analyses. This inclined us to genotype many samples versus many SNPs. The resulting dense geographic sampling gives unparalleled resolution to understand admixture dynamics and facilitates future investigations of cryptic population structure and local adaptation (Sacks *et al.* 2004, 2005; Monzón 2012, in review).

Philosophical matters and implications for coyote and wolf management

Although hybridization is increasingly accepted as a natural phenomenon, even among vertebrate groups, it is often problematic for conservation practitioners. Many conservation policies have the biological species concept as their foundation, thus assuming that 'species' are reproductively isolated. Yet, 1 million years of divergence from a common ancestor and thousands of years of intense artificial selection in dogs have been insufficient for reproductive isolation to fully evolve in *Canis*. In many cases, even when reproductive isolation has not fully evolved, there tends to be some outbreeding depression from the loss of locally adaptive genotypes (gene × environment interactions) or the disruption of coadapted gene complexes (gene × gene interactions) (Edmands 1999). The opposite outcome, hybrid vigour, appears to be the case in North American *Canis*. A more diverse genome, with genes from both wolves and dogs, likely allowed north-eastern coyotes to survive in new habitats, both forested and human-dominated. The introgression of adaptive genetic variation via hybridization with wolves presumably permitted admixed coyotes to rapidly colonize the Northeast (Kays *et al.* 2010a). In fact, the movement of coyotes into the Northeast did not occur until after they began hybridizing with wolves about 154–190 years ago or 86 ± 9 coyote generations ago, assuming a 2-year generation time (vonHoldt *et al.* 2011). Admixed north-eastern coyotes have higher genome-wide heterozygosity than nonadmixed populations (vonHoldt *et al.* 2011), and modern admixed Great Lakes wolves have more mitochondrial and Y-chromosome haplotypes than western coyotes and gray wolves (Koblmüller *et al.* 2009).

One phenomenon that is of particular concern is hybridization between a domestic species and its wild relatives. Some examples among vertebrates include bison (Halbert & Derr 2007), wolves (Godinho *et al.* 2011) and wildcats (Oliveira *et al.* 2008). It is worrisome that some wildlife may lose its wildness and untamed nature by hybridizing with a domestic species. Indeed, genetic evidence from a domesticated line of foxes (*Vulpes vulpes*) reveals that domestication leads to marked differences of gene expression in brain regions that modulate emotions and behaviour (Lindberg *et al.* 2005). The possibility that introgression of dog alleles has made eastern coyotes more adapted to human-dominated environs warrants further research.

The type of hybridization documented in this study may be perceived in a negative or a positive way. Seehausen *et al.* (2008) noted that hybridization may result in a net loss of species numbers, effectively reversing speciation; they used eastern coyote–wolf hybrids to exemplify how two species may coexist in sympatry in some parts of their range, but merge as a hybrid swarm in another more disturbed area. But they could have also used eastern coyote–wolf hybrids to exemplify the rescuing of local biota or the colonization of a new niche via hybridization. As mentioned above, admixed coyote and wolf populations in north-eastern North America are more genetically diverse than their parental populations, and this enhanced genetic diversity may be adaptive. In a way, hybridization between coyotes and wolves and the subsequent colonization of eastern forests have yielded a net increase in local species diversity by restoring a large wolf-like canid into the north-east United States. It is unclear whether this admixed canid will prevent the recolonization of true, full-sized wolves. The two wolves genotyped in this study were likely natural dispersers from Canada (Kays & Feranec 2011). One of them had a genetic profile of western *Canis lupus* and likely dispersed from northern Quebec where wolves remain distinct from Great Lakes wolf populations.

Wolf and coyote management policies should consider the ecological importance of large predators. With 16–20 million white-tailed deer in the United States, the direct and indirect socio-economic costs of overpopulated deer are staggering: annual estimates of deer damage are reported to exceed $2 billion nationwide, including $1 billion/year in car damages (Rondeau & Conrad 2003). There is no question that we need to restore natural predator–prey dynamics, lest we allow deer populations to be regulated by cars. Because eastern North American canids form a taxonomically complex group characterized by reticulate evolution, we argue that management policies in the region should aim at conserving natural ecological and evolutionary processes (Ennos *et al.* 2005; Kyle *et al.* 2006), such as trophic dynamics and local adaptation.

Acknowledgements

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Data accessibility

SNP genotypes, sample locations, PLINK and STRUCTURE input/output files are deposited in the Dryad Digital Repository doi:10.5061/dryad.1bh5q/1.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1. Principal component analysis of initial reference populations of western coyote, western wolf and eastern wolf genotyped at 60 584 SNPs (vonHoldt *et al.* 2011).

Fig. S2. Plot of dosage of coyote alleles and observed heterozygosity.

Table S1. Populations of 538 wild and domestic canids whose genotypes at 63 ancestry-informative SNPs were analyzed in this study.