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*Original Article*

# Spatial Genetic Analysis of Coyotes in New York State

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**ABSTRACT** The robust dispersal capability of the coyote (*Canis latrans*) would suggest a pattern of widespread gene flow across North America, yet historical legacies, dispersal barriers, and habitat affinities may produce or reinforce genetic structure. In the northeastern United States, some coyotes carry genetic signatures from past hybridization events with eastern wolves (*C. lupus lycaon*). These so-called "coywolves" may have differential predation or competitive success compared with the western origin coyotes with whom they share the contemporary landscape. We sampled coyote populations from New York ($n = 156$) and Wyoming, USA ($n = 8$) in 2006–2007 and from South Carolina, USA, in 2010 and confirmed regional genetic structure among these coyote populations. Then, within the putative contact zone between the northeastern and western coyote colonization fronts (New York State), we evaluated evidence for broad- and fine-sale genetic structure, and a genetic gradient among New York coyotes using a suite of spatial genetic analyses. Although broad-scale analyses indicated New York coyotes were highly intermixed, subtle isolation-by-distance was detected, and local spatial autocorrelation indicated potentially shorter dispersal distances and larger group sizes for coyotes in the Northeastern Highlands (Adirondack Mountains and foothills). Yet we failed to detect a distinct contact zone between 2 coyote types in New York, indicating that local abundance and ecological context rather than genetic lineage are likely to determine the local ecological effects of coyotes in this region. We suggest that the contact zone between coyote colonization fronts has either eroded or moved further south. © 2019 The Wildlife Society.

**KEY WORDS** *Canis latrans*, coyote, ecozone, genetics, hybrid, landscape, MEMGENE, natal dispersal, New York.

Widespread generalist species, those capable of long-distance dispersal, should freely exchange genetic material over large geographic domains leading to an expectation of random mating and panmixia. Yet, genetic patterns sometimes emerge even within the most vagile and common of species like coyotes (*Canis latrans*; Sacks et al. 2004). The coyote is a consummate generalist species, readily occupying habitats as diverse as desert chaparral, forests, farmland, and urban centers and moving quite freely among such landscapes (Gese et al. 1988, Person and Hirth 1991, White et al. 1995, Grinder and Krausman 2001). Now ubiquitous across North America and into Central America (Parker 1995, Bekoff and Gese 2003), coyotes were first detected in the northeastern United States in the 1920s in central New York, USA (Parker 1995). What makes the genetic landscape of northeastern coyotes of particular interest to ecologists and managers alike is that the earliest colonizing front that entered New York, from north of the Great Lakes and through Canada, included coyotes hybridized to some degree with the eastern wolf (*C. lupus lycaon*)—a genetic legacy still evident today (Koblmüller et al. 2009, Kays et al. 2010). A later colonizing front entered New York from south of the Great Lakes, adding pure western coyote genes into the northeastern coyote population (Kays et al. 2010). Even for generalist species, past colonization events like that observed for northeastern coyotes can create genetic patterns that may persist for generations (Ellsworth et al. 1994, Croteau et al. 2012). Should body size (e.g., Kays et al. 2010) and behavioral differences be driven by genetic lineage, then

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understanding and managing the ecological effects of coyotes may need to account for spatial genetic patterns.

Historical legacies, behavioral ecology, and landscape physiognomy may play a role in generating and reinforcing genetic structure. For carnivores, physical barriers to movement influence the magnitude of dispersal, and by extension gene flow, over large spatial scales (Proctor et al. 2005, Riley et al. 2006, Frantz et al. 2010). Territoriality and cooperative behaviors tend to aggregate related individuals and create local genetic structure even in seemingly homogeneous environments (Selander 1970, Chepko-Sade and Halpin 1987, Storz 1999, Piertney et al. 2002). Gene flow may further be driven by biases toward natal habitat (Davis and Stamps 2004, Geffen et al. 2004, Sacks et al. 2004, Pilot et al. 2006). These mechanisms may reinforce a genetic boundary for coyotes along the edge between the mostly forested portions of the northeastern United States and primarily agricultural Midwestern United States—an edge that converges in central New York (Kays et al. 2010). Investigations of diet (Boser 2009, Warsen 2012) and space use (Kays et al. 2008, Hansen 2013) in the mostly forested portion of northern New York and agriculture–forest matrix of southern New York suggest marked ecological differences among coyotes from these different ecoregions—differences that may be influenced by genes from wolf ancestors (Monzón et al. 2014, vonHoldt et al. 2016). The ecological specialization and genetic legacy of the northeastern coyote may even justify taxonomic distinction (Way and Lynn 2016), which makes characterizing the historical contact zone among coyote lineages and determining whether coyotes in this area exhibit ecoregion specialization leading to restricted gene flow of management importance.

Although the colonization history of coyotes has been fairly well-documented, genetic evidence for a "contact zone" between northeastern and western coyotes remains equivocal (Parker 1995, Fener et al. 2005). Differences have been detected between coyotes in New York and nearby Midwestern states (Supporting Information S1), with midwestern coyotes grouping closely with coyotes from the southeastern United States (Way et al. 2010, vonHoldt et al. 2011, Rutledge et al. 2012); such differences may have arisen due to spatially clustered sampling (Schwartz and McKelvey 2009). Ancestry-informative markers failed to detect 2 distinct lineages within coyotes spanning northeastern to midwestern states, detecting coyotes with wolf ancestry as far west as Ohio, USA (Monzón et al. 2014); indicating that long-distance dispersal and admixture of eastern coyotes with wolf ancestry may have eroded the contact zone between the 2 coyote lineages. However, for highly vagile species like coyotes, neutral genetic differences will likely remain subtle among similar habitat types and, therefore, difficult to detect. Yet, few studies of eastern coyote genetics have incorporated the spatially explicit information needed to illuminate subtle genetic discontinuities (Manel et al. 2003). Given that genetically similar individuals are often aggregated in space, spatially explicit genetic tools provide more robust results of genetic structure, especially when data are limited and sampling schemes are clustered (Guillot et al. 2005*a*, Schwartz and McKelvey 2009).

We provide a spatial genetic analysis to shed light on the genetic landscape of New York coyotes, the historical core of the contact zone for the 2 coyote colonization fronts in the eastern United States. Using microsatellite data, we explored evidence for genetic differentiation among New York coyotes in light of their colonization history and observed heterogeneity in their diets and space use. Specifically, we sought to determine whether genetic differentiation coincides with the putative contact front between eastern and western coyotes and whether such a boundary may be reinforced by habitat heterogeneity. First, we evaluated the degree to which coyotes in 3 broadly defined ecoregions in New York were differentiated from western (Wyoming, USA) and southern (South Carolina, USA) coyotes. We expected western lineage coyotes to be more closely related to coyotes from South Carolina and Wyoming than to eastern lineage coyotes, with the latter expected to reside in the more forested regions of northern New York. Second, we focused our analyses on individuals in the contact zone and, applying a suite of spatially explicit genetic tools, evaluated whether 2 distinct groups of coyotes existed within New York by assessing structure with spatial genetic clustering and searching for spatial patterns in genotypes (Guillot et al. 2005*b*, Jombart et al. 2008, Galpern et al. 2014). Finally, we examined fine-scale patterns by analyzing genetic autocorrelation in coyotes within and among ecoregions, expecting dispersal to be biased toward natal habitat types, which would reinforce genetic boundaries between ecoregions (Geffen et al. 2004, Sacks et al. 2004, Pilot et al. 2006). Ultimately, we looked for concordance across the various analyses to substantiate genetic patterns, which in turn might provide evidence for, and suggest the cause of, genetic heterogeneity among coyotes in New York.

## STUDY AREA

New York lies within the range of North America's Eastern Temperate Forests that are characterized by a humid climate and diverse forest cover; with a dense human population (Gilliam et al. 2011). Inland New York was characterized by a continental climate with warm summers and cold winters. Average high temperatures in July reach 30° C. Areas closer to the Great Lakes experienced more moderate winters with average low temperatures in January of −3° C compared with the Adirondack Mountains where the average low temperature in January is −9° C. Outside of the coastal region, the forested upland areas of New York contained primarily deciduous hardwoods that replaced the American chestnut (*Castanea dentata*) following the introduction of chestnut blight in the early 1900s: oak (*Quercus* spp.), hickory (*Carya* spp.), beech (*Fagus grandifolia*), and maple (*Acer* spp.). Spruce (*Picea* spp.) and fir (*Abies* spp.) forests occurred in mountainous areas and northern latitudes with pine (*Pinus* spp.)–oak forests in drier areas. Lowlands may have housed hemlock (*Tsuga canadensis*), black spruce (*Picea mariana*), northern white cedar (*Thuja occidentalis*), and white oak (*Q. alba*) communities (New York Natural Heritage

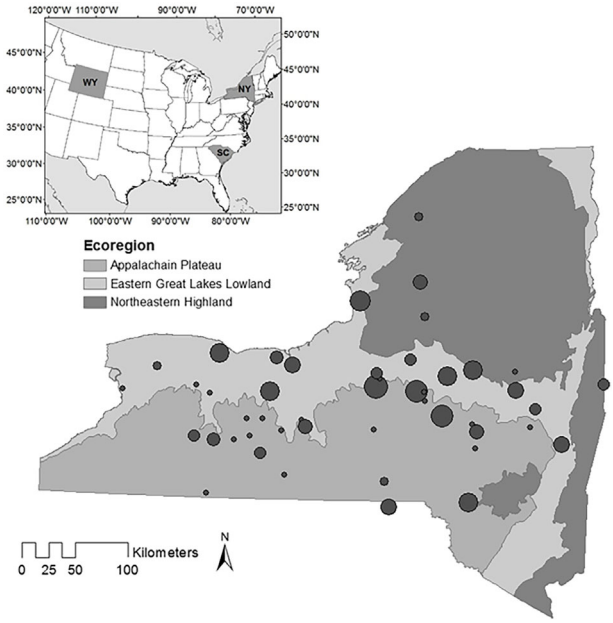


**Figure 1.** The study area in New York, USA, showing 3 broad-scale ecoregions as well as the location of genetic samples from coyotes taken 2006–2007. Point size corresponds to sample size (i.e., logarithm of the no. of coyotes sampled at that location).

Program 2017). We focused on 3 ecoregions in New York (Fig. 1): the Eastern Great Lakes Lowlands (EGLL) that contained relatively flat agricultural land, Appalachian Plateau (AP) that contained rolling relief with a mosaic of forest and agriculture land cover, and Northeastern Highlands (NEHL) that contained the most mountainous area in the state with extensive forest cover (Bryce et al. 2010).

# METHODS

## Sample Collection

We opportunistically collected genetic samples from legally harvested coyotes in New York that spanned the putative contact zone inferred by Kays et al. (2010; Fig. 1). The coyote is a species of "least concern" with an increasing population trend, and managed as a game and furbearer species in many parts of the United States (Gese et al. 2008). New York samples were voluntarily provided by licensed trappers during fur auctions between December 2006 and February 2007 ($n = 172$ coyotes, 48 locations; Fig. 1). Trappers indicated locations of trapped coyotes on a map. Genetic samples were also opportunistically acquired from legally harvested coyotes from Wyoming ($n = 8$ coyotes, 1 location) and South Carolina ($n = 168$ coyotes, 3 locations; Fig. S1, available online in Supporting Information). The South Carolina samples originated from a predator removal study conducted January–April 2010 (South Carolina Department of Natural Resources Research Collection Permit No. 010610-01) by U.S. Department of Agriculture Forest Service researchers on land held by the U.S. Department of Energy (permitted by Interagency Agreement DE-AI09-00SR22188) and following the methods detailed in Kilgo et al. (2014). All genetic samples used in this study were exempted from Institutional Animal Care and Use Committee oversight because specimens were originally collected for reasons unrelated to our study.

For the New York and Wyoming samples, we cut one small piece of dried skin from each coyote hide, typically from the foot. We trimmed samples to 4–6 $mm^2$ in size, mostly removed hair, and scraped or sliced skin to facilitate lysis. We stored samples in 100% ethanol at −20 to 4° C until extraction. We extracted DNA using the Qiagen DNeasy Tissue Kit (Valencia, CA, USA) according to the manufacturer's instructions. We recorded genotypes using a set of 12 microsatellite genetic markers that could be scored consistently (Table 1). For an initial 5 markers (*AHT130*, *CXX.123*, *CXX.213*, *CXX.377*, and *2010*), PCR was accomplished in singleplex using end-labeled primers with *Taq* 2x Master Mix (New England BioLabs, Ispwich, MA, USA), and conditions consisted of 5 min of initial denaturation at 95° C, 33 cycles of 94° C for 1 min,

**Table 1.** Microsatellite loci scored for coyotes from New York, USA, sampled 2006–2007. The number of samples genotyped ($n$), the number of alleles ($n_a$) observed and expected unbiased heterozygosity ($H_O$ and $H_E$), and the fixation index ($F_{IS}$) are given.

| Locus | Source | Lab[a] | $n$ | $n_a$ | $H_O$ | $H_E$ | $F_{IS}$ |
|---|---|---|---|---|---|---|---|
| *AHT130* | Holmes et al. (1995) | SUNY-ESF | 139 | 15 | 0.755 | 0.857 | 0.115 |
| *CXX.123*[b] | Ostrander et al. (1993) | SUNY-ESF | 130 | 6 | 0.423 | 0.592 | 0.282 |
| *CXX.213*[b] | Ostrander et al. (1993) | SUNY-ESF | 151 | 12 | 0.675 | 0.769 | 0.119 |
| *CXX.377* | Ostrander et al. (1995) | SUNY-ESF | 144 | 13 | 0.785 | 0.791 | 0.004 |
| *2010* | Francisco et al. (1996) | SUNY-ESF | 147 | 5 | 0.776 | 0.695 | −0.119 |
| *REN144A06* | Breen et al. (2001)[c] | WGI | 156 | 9 | 0.846 | 0.805 | −0.054 |
| *AHT121* | Holmes et al. (1995) | WGI | 156 | 10 | 0.756 | 0.795 | 0.045 |
| *REN105L03* | Breen et al. (2001)[c] | WGI | 156 | 10 | 0.827 | 0.831 | 0.001 |
| *REN145P07* | Breen et al. (2001)[c] | WGI | 156 | 12 | 0.891 | 0.888 | −0.006 |
| *REN316E23* | Breen et al. (2001)[c] | WGI | 156 | 12 | 0.808 | 0.799 | −0.014 |
| *REN68B08* | Breen et al. (2001)[c] | WGI | 156 | 12 | 0.763 | 0.791 | 0.033 |
| *REN94H15* | Breen et al. (2001)[c] | WGI | 156 | 12 | 0.821 | 0.813 | −0.013 |
| Mean (SE)[d] | | | | | 0.811 (0.013) | 0.808 (0.011) | −0.023 (0.019) |

[a] Samples were analyzed at either the State University of New York College of Environmental Science and Forestry (SUNY-ESF) or Wildlife Genetics International (Nelson, BC, Canada; WGI).
[b] Marker not included in population analyses.
[c] Primers for the markers developed by Breen et al. (2001) were modified by WGI.
[d] Mean and standard error (SE) are given for the 10 markers (in HWE) that were used in downstream analyses.

58.6° C for 45 s, 74° C for 1 min, and a final extension temperature of 74° C for 5 min (Ostrander et al. 1993) on a BioRad iCycler IQ (Hercules, CA, USA). We sent purified PCR product to Cornell University's Biotechnology Core Facility for fragment analysis on an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA). We visualized results and recorded them using ABI Peakscanner software. The remaining 7 loci were genotyped separately by Wildlife Genetics International (WGI; Nelson, BC, Canada; Table 1). Samples from South Carolina were processed by WGI and genotyped using 7 of the same microsatellite loci as the New York and Wyoming coyotes (Table S1, available online in Supporting Information). We excluded insufficiently genotyped (<7 loci) samples from further analysis. We used the program Microchecker to assess null alleles and scoring errors (Van Oosterhout et al. 2004).

We assessed deviations from Hardy–Weinberg Equilibrium within ecoregions (Fig. 1) to account for population genetic structure that may be informative in analyses that rely on optimizing Hardy–Weinberg Equilibrium to delineate genetic groups (Guillot et al. 2005*b*). We used GENEPOP v4.2 to determine both Hardy–Weinberg Equilibrium at each locus in each of the 3 ecoregions and linkage disequilibrium at each locus pair in the entire population and used a sequential Bonferroni correction for multiple comparisons ($\alpha = 0.05$; Raymond and Rousset 1995).

### Regional Differences in Coyote Genetics

We assessed the degree of differentiation among coyotes from New York, Wyoming, and South Carolina using the 7 comparable microsatellite loci genotyped by WGI, expecting differentiation among coyotes from different states as has been observed elsewhere (e.g., vonHoldt et al. 2011; Table 1). For this analysis, we grouped coyotes from New York *a priori* by ecoregion (Fig. 1). If evidence of the 2 colonization fronts persists, we expected coyotes from the NEHL (presumably dominated by coyotes originating from north of the Great Lakes) to show greater differentiation from South Carolina and Wyoming coyotes than coyotes from the AP or EGLL (presumably originating from south of the Great Lakes). We conducted a scaled and centered principal components analysis (PCA) using the bca function and Program R package *adegenet* on samples from all 3 states and, separately, on samples from New York only (Jombart and Ahmed 2011, R Core Team 2014). To further quantify genetic differentiation among ecoregions within New York, and test the strength of large-scale habitat affinities, we calculated pairwise $F_{ST}$ using 999 permutations to determine statistical significance using GENALEX v.6.503 (Weir and Cockerham 1984; Peakall and Smouse 2006, 2012).

### Landscape-Scale Genetic Structure Within New York Coyotes

We applied a suite of spatial analyses to evaluate alternative plausible patterns of genetic structuring within New York coyotes, including methods designed to identify clusters, evaluate large-scale gradients, and explore local structure driven by habitat heterogeneity as described in the sections to follow.

*Distinct groups.*—We used GENELAND to incorporate spatial information into the identification of genetic clusters (Guillot et al. 2005*b*). When genetic structure is weak, GENELAND may better detect genetic discontinuities compared with other algorithms (Safner et al. 2011). We used the uncorrelated allele frequency model in GENELAND because the correlated allele frequency model produced an unrealistic number of clusters (5) with either 1 or 0 locations assigned to ≥1 cluster in all runs, potentially because of model instability caused by the large number of population, locus, and allele specific parameters being estimated (The Geneland Development Team 2012). We ran the more stable uncorrelated allele frequency model 20 times for 400,000 iterations, with a burn-in of 20,000 for $K = 1$–5.

*Spatial genetic patterns.*—For genetic data, PCA finds a few synthetic variables that encompass most of the variability in allele frequencies. However, spatial principal components analysis (sPCA) seeks to optimize the product of genetic variance and spatial autocorrelation (Moran's *I*) in allele frequencies to elucidate spatial pattern of genetic variability (Jombart et al. 2008). This generates an entity score for each individual sample that separates genetic variance that is positively autocorrelated (global structure; e.g., large patches or clines) from variance that is negatively autocorrelated (local structure; e.g., individual attractions or repulsions). The Moran's *I* component depends on a binary neighborhood matrix, which we produced using a minimum spanning tree. When measuring autocorrelation, the allele frequencies of neighbors were taken into account while nonneighbors were disregarded. Statistical significance of spatial structure would be indicated by a significant correlation ($P < 0.05$) among allele frequencies and ≥1 global spatial structure. These spatial structures are represented by Moran's eigenvector maps (MEMs), which are uncorrelated variables that represent different spatial structures in the location data (Jombart et al. 2008). We determined statistical significance using 999 randomizations to create a distribution of the test statistic.

Further analysis of spatial patterns used MEMGENE, which regresses MEM eigenvectors (i.e., variables describing patterns of positive and negative spatial autocorrelation) against genetic distance (Galpern et al. 2014). Forward selection, in which the MEM eigenvectors were added to a regression model until they ceased to improve model fit, determined their statistical significance ($\alpha = 0.05$). Principal component scores of the predicted values are defined as Memgene variables, which can be used to visualize spatial relationships of genetic distance (Galpern et al. 2014). For each analysis, MEMGENE applies random noise, or "jitter," to sample coordinates to properly analyze samples from the same location. Our results changed slightly among replicate analyses due to the jitter. To overcome this inconsistency, we performed 10 analyses with MEMGENE, with each sample location jittered an average of 30 m across the 10 runs. We used Memgene variables from the run with the highest $R^2$ to produce a final map of genetic relationships.

*Isolation-by-distance.*—We determined whether a pattern of isolation-by-distance existed among New York coyotes using a simple Mantel test (Mantel 1967). We calculated individual genetic distances and permutations with Ecodist (Goslee and Urban 2007) and $\log_e$-transformed geographic distances. We employed a 1-tailed test with 1,000 permutations to determine statistical significance ($\alpha = 0.05$).

**Fine-Scale Genetic Structure Within New York Coyotes**

Spatial autocorrelation provides a reliable method for indirectly measuring dispersal (Heywood 1991; Epperson 2005). We examined the degree of spatial autocorrelation ($r$) within and among groups defined *a priori* by ecoregion: EGLL ($n = 71$), AP ($n = 66$), and NEHL ($n = 19$). If coyotes restricted dispersal across ecoregions due to natal habitat bias or ecoregion specialization, we expected to observe larger autocorrelation within versus among ecoregions, and no significant positive or negative autocorrelation in pairwise comparisons across ecoregions. $R$-statistics based on pairs of individuals have well-characterized theoretical and sampling properties (Sokal and Wartenberg 1983; Epperson 1995*a*,*b*). We present values obtained from the first lag distance in detail, which usually obtain high statistical power when measuring spatial genetic structure caused by isolation by distance (Oden 1984). Pairwise comparisons at the first lag distance would include individuals collected at roughly the same location within ecoregions. Consequently, comparisons at the second lag distance (1–25 km), which included those animals collected at different locations within and across ecoregions, would indicate differences of autocorrelation due perhaps to natal-habitat biased dispersal restricted by ecoregion (Sacks et al. 2004). We considered $F_{IS}$ values and heterozygosity within ecoregions to determine whether consanguineous mating may be responsible for any observed patterns. We calculated autocorrelation, heterozygosities, and $F_{IS}$ with GENALEX 6.503 (Peakall and Smouse 2006, 2012).

Finally, we performed a test of genetic differentiation using dbRDA with ecoregion as our predictor variable (Geffen et al. 2004, Pilot et al. 2006). To perform the dbRDA, we used the capscale function in R program *vegan* (Oksanen et al. 2015). We considered each ecoregion a category, with samples coded as 1 when in the specified ecoregion or 0 otherwise (Pilot et al. 2006). We assessed a linear regression model having ecoregion as the sole predictor variable and a model having ecoregion as well as latitude and longitude as covariates ($\alpha = 0.05$). We assessed statistical significance of the models by calculating pseudo-$F$-values with 999 permutations.

## RESULTS

Of the 12 loci genotyped for the New York sample, locus *CXX.123* deviated significantly in the NEHL ecoregion ($P < 0.001$) with a high $F_{IS}$ value ($F_{IS} = 0.675$), indicating violation of Hardy–Weinberg Equilibrium assumptions, and was not included in all following analyses. A second locus (*CXX.213*) was also discarded based on a high $F_{IS}$ value in the New York sample (Table 1) and a preliminary analysis that produced high, outlying $F_{ST}$ values. Scoring errors and null alleles were not detected with Microchecker. No linkage disequilibrium was indicated at the remaining locus pairs, so we performed downstream analyses with the remaining 10 loci (Table 1). Coyote samples from Wyoming and South Carolina were genotyped at 7 loci to determine regional differences among coyotes (Fig. S2, available online in Supporting Information). Sixteen New York samples were excluded from further analysis based on insufficient genotyping (<7 loci genotyped).

**Regional Differences in Coyote Genetics**

As expected, we observed differentiation among coyotes from different states, with New York and South Carolina coyotes differentiated along PCA axis 1 and Wyoming coyotes differentiated from the other 2 sources along axis 2 (Fig. 2a). New York coyotes showed broader differentiation than South Carolina and Wyoming coyotes, along axis 3 (Fig. 2b). Within New York, regional differences were also

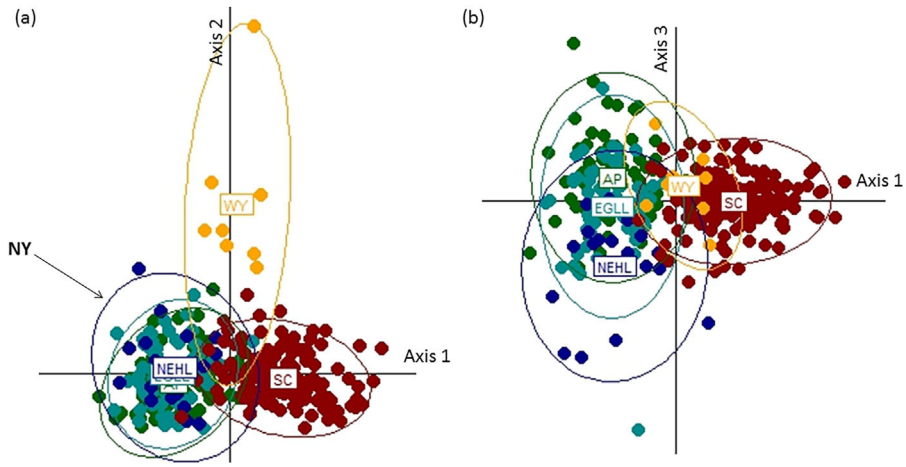


**Figure 2.** Differentiation of coyotes sampled 2006–2007 and 2010 using principal components analysis. Points and 95% inertial ellipses correspond to coyotes within 3 ecoregions of New York (NY; blue, cyan, green), 3 study areas in South Carolina (SC; dark red), and 1 location in Wyoming (WY; orange), USA. Shown are the principal component scores from 7 microsatellite loci for each individual plotted for (a) axis 1 (70% of inertia) versus axis 2 (17% of inertia) and (b) axis 1 versus axis 3 (7% of inertia).

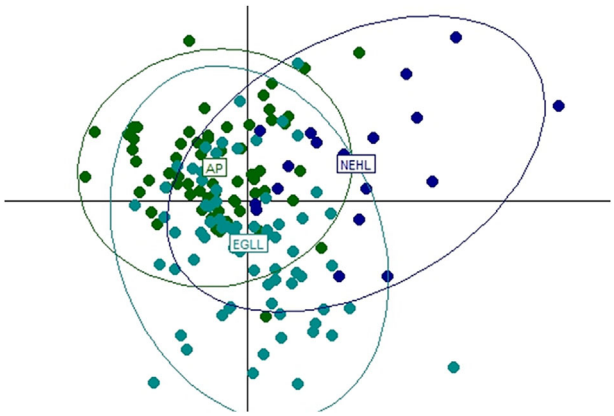


**Figure 3.** Differentiation of coyotes in New York, USA, sampled 2006–2007 using principal components analysis. Points and 95% inertial ellipses correspond to coyotes within 3 ecoregions of New York: Appalachian Plateau (AP; green), Eastern Great Lakes Lowland (EGLL; cyan), Northeast Highland (NEHL; blue).

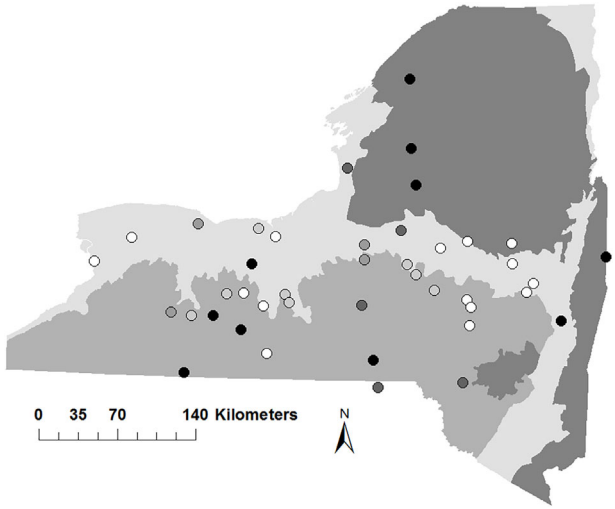


**Figure 4.** Spatial genetic structure among New York, USA, coyotes sampled 2006–2007 identified with MEMGENE. Colors of sample points correspond to entity scores, which range from −1 (black) to +1 (white) and reflect the position of sample points along a principal components axis of genetic variation (i.e., contrasting shades have greater differentiation). Natural breaks in the entity scores were used to apply 5 shades to the sample points.

apparent along axis 3 and in a PCA with only New York samples (Fig. 3). Pairwise $F_{ST}$ indicated the greatest difference between NEHL and AP ($F_{ST}=0.010$, $P=0.08$), with the NEHL and EGLL ($F_{ST}=0.001$, $P=0.35$) and EGLL and AP ($F_{ST}=0.001$, $P=0.38$) being indistinguishable (Fig. 2b).

### Landscape-Scale Genetic Structure Within New York Coyotes

Spatial clustering using GENELAND supported one group, with $K=1$ achieving the maximum *a posteriori* estimate of $K$ in all 20 runs (Table S2, available online in Supporting Information). MEMGENE produced ≥1 significant eigenvector ($P<0.05$) in every run and the pattern from the first principal component was consistent across 10 runs. We mapped Memgene variables (similar to principal component scores) for sample locations using results from the run with the best fit ($R^2=0.022$), which indicated a random pattern that did not bear resemblance to ecoregions of New York (Fig. 4). Likewise, the pattern from sPCA analysis was not statistically significant (max. $R^2=0.011$, $P=0.16$). However, a state-wide pattern of isolation-by-distance was indicated by a Mantel test ($r_{Mantel}=0.033$, $P=0.016$).

### Fine-Scale Genetic Structure Within New York Coyotes

Significant positive autocorrelation was observed at the first lag distance (0–1 km) within the AP and EGLL (Fig. 5), with the magnitude of correlation being greatest (albeit most variable and not statistically significant) for samples within the NEHL. We failed to detect significant autocorrelation at the second or larger lag distances within or between ecoregions. Moreover, we did not find any further evidence using dbRDA that individual genetic distance among coyotes was influenced by ecoregion, either as the only predictor (pseudo-$F=0.76$, $P=0.79$) or with latitude and longitude as additional covariates (pseudo-$F=0.66$, $P=0.89$). $F_{IS}$ within ecoregions was low, which indicates little inbreeding (Table 2). Heterozygosities were similar among ecoregions (Table 2).

## DISCUSSION

We assessed genetic structure of coyotes with microsatellites because they provide an advantage over other markers when evaluating genetic structure in that they can be used to identify recent divergence in canids and exhibit high variability within and among populations (Rutledge et al. 2012, Monzón et al. 2014). In combination with spatial information, microsatellite analyses may have sufficient power to resolve distinct groups of coyotes over relatively small regions, such as within a given state, which is important both due to the spatial dependence of landscape genetic data and the low genetic differentiation that we expected (Sacks et al. 2004, Guillot et al. 2005*a*, Meirmans 2012). Despite this relatively powerful technique, genetic differentiation in New York coyotes was not statistically significant; what genetic discontinuities we observed did not coincide with ecoregion boundaries or other obvious landscape forms. Similar to a study of coyotes in the Appalachian Mountains region of the eastern United States, which used 17 microsatellites with spatial data (Bohling et al. 2017), the lack of concordance with geographical forms we observed in New York suggests one homogenous coyote population in the larger region. As a result, our study corroborates previous work indicating that coyotes in the eastern United States exhibit high gene flow, experience few if any barriers to dispersal, and lack clear genetic discontinuities in the northeastern region (Monzón et al. 2014, Bohling et al. 2017).

Although the historical contact zone between the eastern and western coyotes may have eroded due to dispersal and interbreeding, exactly where the range of the eastern coyote lineage extends remains of interest, as does how admixture with wolves may vary spatially on a broader regional scale.

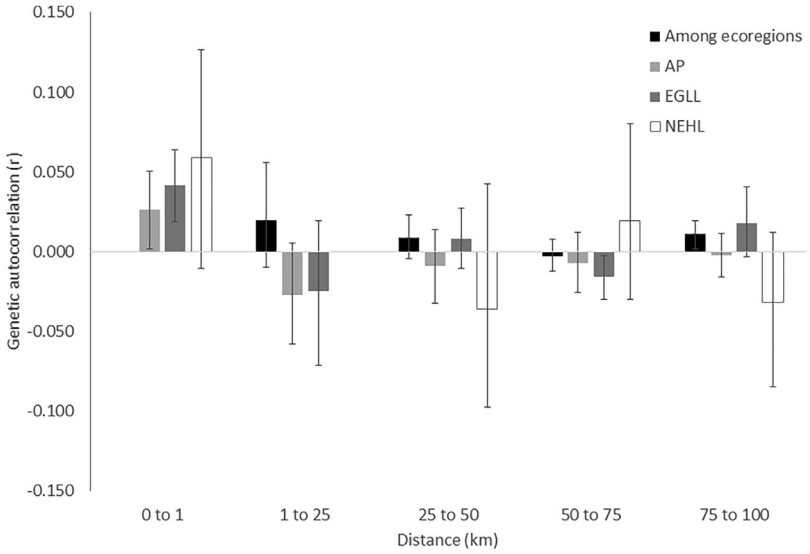


**Figure 5.** Genetic autocorrelation (*r*) values among pairs of coyotes at increasing lag distances across New York, USA, plotted at the midpoint of each lag. Pairs were sampled 2006–2007 and located within the Appalachian Plateau (AP; dark gray), the Eastern Great Lakes Lowlands (EGLL; medium gray), the New England Highlands (NEHL; light gray), as well as among ecoregions (white). Error bars represent bootstrapped 95% confidence intervals about the mean. No pairs of samples occurred at the second lag distance for the NEHL so no point was plotted.

Monzón et al. (2014) hypothesized that the genetic lineage of the eastern coyote has expanded further south and west of New York, beyond the original contact zone based on the degree of wolf ancestry genes in coyotes from the Midwestern states. Bohling et al. (2017) found that coyotes in West Virginia and Virginia, USA, just south of the original contact zone, largely grouped with western coyotes. They suggested that those individuals may contain less admixture from wolves than areas like New York on the Canadian border. Though fine-scale analyses in the eastern United States, such as within a state, have failed to detect genetic structure and resolve the range of the eastern coyote lineage, broader scale regional analyses, such as the one we conducted between New York and South Carolina, consistently find differences among states spanning the putative contact zone (e.g., Way et al. 2010, vonHoldt et al. 2011). However, genetic structure from individual-based analyses may arise due to isolation-by-distance and clustered sampling, so our results and these other regional analyses do not directly confirm the presence of genetic discontinuity in the region (Schwartz and McKelvey 2009). We were unable to resolve the relative similarity of coyotes in South Carolina and New York to a representative sample of western coyotes from Wyoming. We acknowledge that our small sample ($n = 8$) from the latter group may have precluded a clearer PCA result (Novembre and Stephens 2008). Consequently, it remains to be resolved whether broader regional genetic differences among coyotes in New York and South Carolina are solely the result of an equilibrium condition of isolation-by-distance, such as we observed on a finer scale within New York, or a legacy of the colonization pattern of the 2 coyote lineages (Parker 1995).

**Table 2.** Mean heterozygosities for 10 microsatellite markers genotyped in coyotes from New York, USA, sampled 2006–2007. Observed ($H_O$), unbiased expected ($H_E$) and the fixation index ($F_{IS}$) are given with standard errors are given in parentheses.

| Ecoregion | $H_O$ | $H_E$ | $F_{IS}$ |
|---|---|---|---|
| Appalachian Plateau | 0.792 (0.017) | 0.799 (0.015) | 0.0001 (0.018) |
| Eastern Great Lakes Lowland | 0.804 (0.015) | 0.813 (0.017) | 0.0003 (0.023) |
| New England Highlands | 0.837 (0.032) | 0.811 (0.025) | −0.070 (0.047) |

The fine-scale genetic structure we observed within New York coyotes was not consistent with the hypothesis of dispersal biased toward natal habitat at the scale of ecoregions (Sacks et al. 2008). Nevertheless, some inferences can be made about the dispersal processes that generated the spatial genetic patterns we observed (Epperson 2005). Positive genetic autocorrelation at the first lag distance (i.e., individuals sampled from the same location) may reflect delayed dispersal leading to the formation of coyote "packs" (Bekoff and Wells 1980, Messier and Barrette 1982). An alternative explanation that samples came from a "predispersal" window is unlikely because animals were trapped during the autumn season, which coincides with the onset of coyote dispersal (Harrison 1992). Genetic autocorrelation (*r*; Smouse and Peakall 1999) is inversely related to Wright's neighborhood size ($NS = 4\pi d\sigma^2$, where $d$ represents coyote density and $\sigma$ is the average parent–offspring distance; Wright 1946, Epperson et al. 1999). Thus, we expected the larger density of coyotes in the forest-dominated NEHL (~10–50% greater than the rest of the state; Hansen 2013) to result in a lower spatial autocorrelation than the other ecoregions, but we did not find this result. Subtle spatial autocorrelation differences may be biologically important because a small increase in *r*-value can convey considerable

spatial genetic structure (Epperson and Li 1996). Thus, the fine-scale structure among the rugged forest region and largely agricultural regions of New York may be worth exploring with more detailed genetic analysis and greater sample sizes in the future.

Though we did not detect barriers to gene flow within New York, some coyotes from the NEHL and EGLL appeared to separate genetically from other New York coyotes in our PCA analyses. This pattern may be a snapshot of the dynamic and ongoing process of gene flow among coyote populations from New York and those from more northerly regions in the eastern United States. The sporadic influx of more northerly coyotes across or around water barriers (e.g., Lake Ontario, the St. Lawrence Seaway, and Lake Champlain) could result in a persistent mixing zone in New York as has been observed with other vagile carnivores in the region including raccoons (*Procyon lotor*; Cullingham et al. 2009, Rees et al. 2009) and fishers (*Pekania pennanti*; Hapeman et al. 2011). More extensive sampling across the NEHL, across major water barriers, and into areas adjacent to New York in northeastern most United States and Canada would be needed to validate this possibility.

Despite considerable gene flow, observed differences in coyote density and diet across New York indicate that the ecological effect of coyotes may differ regionally (Boser 2009, Warsen 2012, Hansen 2013). Whereas we expected selection for wolf ancestry genes (vonHoldt et al. 2016) or habitat-biased dispersal (Sacks et al. 2008) to result in spatial genetic structure in coyotes, and by extension relate to differing ecological impacts by different genetic groups, the lack of genetic differentiation at neutral markers we observed across space indicates that local ecological impacts of coyotes across New York today are likely due more to local abundance and ecological context than genetic legacies.

In conclusion, though we found a lack of genetic differentiation among coyotes from different New York ecoregions, we corroborated distinctions previously observed among coyotes at a regional scale, providing further evidence of differentiation between coyotes in the northeastern United States and those further south along the Atlantic Coast (Way et al. 2010, vonHoldt et al. 2011, Rutledge et al. 2012). We observed a slight distinction of some individual New York coyotes from the NEHL and EGLL, which may stem from the same ecological processes that formed the original contact zone (i.e., isolation of coyote populations by the Great Lakes and limited gene flow across water barriers), and also provided evidence of fine-scale genetic structure that may be of interest for future research.

## MANAGEMENT IMPLICATIONS

Eastern and western lineage coyotes may yet exist as disparate types throughout the eastern United States; and over large spatial scales, sufficient differences in genome, morphology, and niche may justify a distinct classification for the eastern coyote (Way and Lynn 2016). However, our results indicate that either the contact zone between these lineages is located further south, between New York and South Carolina, or that gene flow has eroded the historical legacy of the eastern–western coyote contact zone altogether (Bozarth et al. 2011). As a result, at a scale akin to the size of New York, coyote types are likely not sufficiently distinct or spatially aggregated to consider management prescriptions based solely on genetic heritage.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

Figure S1. Locations of coyotes from South Carolina, USA, sampled in 2010.

Figure S2. Summary of evidence for genetically differentiated coyote groups in the United States from recent research.

Table S1 Microsatellite loci scored for a population genetic analysis of coyotes from Wyoming (sampled 2006–2007) and South Carolina (sampled 2010), USA.

Table S2 Average posterior probabilities for GENELAND runs for a genetic analysis of coyotes sampled 2006–2007.