Policy analysis

Spatial population genetics reveals competitive imbalances threatening local apex predator persistence

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ABSTRACT

The eastern wolf (*Canis lycaon*), a species of conservation concern in Canada, is currently restricted to small fragmented populations in south-central Ontario and hybridizes with both encroaching gray wolves and coyote-like canids. We examined niche dynamics in canids undergoing hybridization to determine whether competition among individuals with coyote (*Canis latrans*) or gray wolf (*Canis lupus*) ancestry, or their related hybrids, could threaten persistence of the eastern wolf in south-central Canada. Our integrative approach combined extensive genotyping and comparative niche analyses across the hybrid zone to assess available and utilized niche space across all three parental species and their hybrids within the zone of admixture. We focused on detecting niche imbalances within the canid clade that indicate competitive threats, and used these data to identify specific geographic regions that disproportionately favor eastern wolves and might confer natural exclusion of competing canids. We detected low genetic and ecological differentiation among groups across the region. Niche dynamics in the admixture zone were dominated by gray wolf and coyote-like canids, with coyote-like canids in particular exhibiting niche space that overlapped entirely with eastern wolves. Conservation action for eastern wolves must either exploit the narrow niche space that differentiates them from other canids, representing ~5% of their currently occupied space, or accept whichever group dominates the landscapes regardless of genetic makeup. This study suggests that competitive disadvantage can limit species' recovery efforts, and thereby potentially warrant management that targets factors promoting ecological differentiation between groups.

1. Introduction

Humans drive biodiversity loss through land use modification (Newbold et al., 2015) and direct persecution. For example, removal of large predators from the landscape can promote establishment and persistence of mesopredators that thrive in human-modified landscapes and that can alter the structure and function of ecosystems (Newsome et al., 2015; Newsome et al., 2017). Specifically, dissolution of allopatric boundaries between range-expanding and resident fauna can aggravate antagonistic or exploitative competition, resulting in range loss and niche space overlap and constriction (Peers et al., 2013). Furthermore, range expansions can have compounding effects on resident species if hybridization is possible (Grabenstein and Taylor, 2018). Hybridization with extensive backcrossing can be especially deleterious because high levels of introgression promote genetic and ecological homogenization and thereby further aggravate niche overlap and competition (Hata

et al., 2019; Rhymer and Simberloff, 1996). Over time, hybridization between encroaching and resident species could lead to loss of at-risk species with restricted niche breadth or limited geographical distribution, making contemporary range expansions a difficult conservation challenge (Meilink et al., 2015; van Wyk et al., 2016). A thorough understanding of niche dynamics between expanding and resident species in admixture zones is necessary to provide explicit direction for conservation efforts promoting persistence and recovery of at-risk species (Scott et al., 2010).

The taxonomic status of eastern wolves (*Canis lycaon*) is controversial (Heppenheimer et al., 2018; Hohenlohe et al., 2017; von Holdt et al., 2016), but their conservation status remains a priority as evidenced by their listing as threatened in Canada (COSEWIC, 2015) and Ontario (COSSARO, 2016). During the last century, coyotes (*C. latrans*) expanded from the midwestern United States into the Great Lakes region, likely due to the combined effects of wolf extirpation and
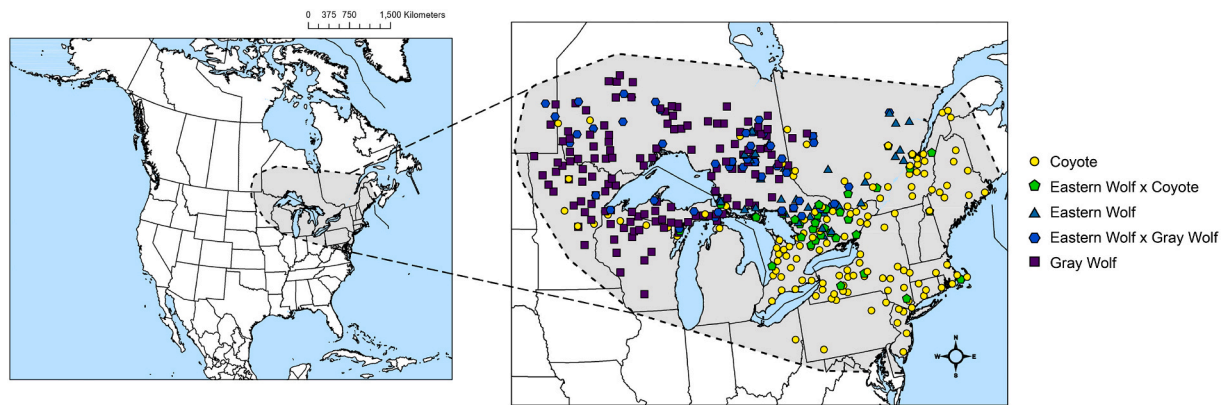

Fig. 1. Study region and sample distribution for 5 wild canid groups in eastern North America, genotyped at 12 microsatellite loci to determine ancestry. Samples depicted represent those retained after spatial thinning ($n = 408$). Canid groups are delineated by color, with the study extent outlined in gray. Coyote – *Canis latrans*; Eastern wolf – *C. lycaon*; Gray wolf – *C. lupus*.

widespread forest fragmentation and loss (Atwood and Weeks, 2003; Fener et al., 2005; Hody and Kays, 2018). Within the contact zone, eastern wolves exhibit extensive admixture because they hybridize and produce fertile offspring with both coyotes and gray wolves (*C. lupus*; Rutledge et al., 2015). In contrast, there is no known hybridization between coyotes and gray wolves within the historical range of coyotes (Pilgrim et al., 1998), making eastern wolves the conduit for current patterns of admixture across the zone (Rutledge et al., 2010). Indeed, genetic analysis reveals low genetic differentiation between canid groups in this region (von Holdt et al., 2016), although genomic markers detect clusters corresponding to each parental group (Heppenheimer et al., 2018). Hereafter, we refer to canids in the admixture zone according to parental species, which share a majority of ancestry with either coyotes, eastern wolves, or gray wolves. For example, "coyotes" refer to canids that share the most genetic ancestry with unadmixed coyotes from western North America. We recognize that species delineations are problematic in areas of intense hybridization but our discrete designations are necessary for robust analysis and ease of interpretation.

Coyotes and wolves have disparate natural history, with coyotes primarily consuming small prey and juvenile ungulates (Kilgo et al., 2010), including even juvenile moose within the admixture zone (Benson and Patterson, 2013a). Eastern wolves depredate on beavers (*Castor canadensis*), white-tailed deer (*Odocoileus virginianus*), and moose (*Alces alces*) (Benson et al., 2013) and gray wolves prefer large ungulates (Messier, 1994). All wolves generally occupy more extensive tracts of forested habitats and tend to avoid modified landscapes compared to coyotes (Benson et al., 2012). Gray wolves are found in more northerly boreal areas having moose and caribou (*Rangifer tarandus*) as primary ungulate prey (Benson et al., 2012). In contrast, eastern wolves are currently restricted to small parks and protected areas in south-central Ontario and Québec (Rutledge et al., 2017). In general, hybrid canids might exhibit niche dimensions that are intermediate to parentals (Otis et al., 2017), although this perception is based on a limited analysis excluding gray wolves. Accordingly, the current understanding of *Canis* niche dynamics is insufficient to identify the full extent of eastern wolf niche breadth and overlap with other *Canis* species.

The contemporary eastern wolf distribution is substantially smaller than its historical range (Kyle et al., 2006; Rutledge et al., 2017), and introgression is especially pervasive at the edge of the current distribution (Heppenheimer et al., 2018; Wheeldon et al., 2010). This dynamic could drive eastern wolf decline and even extinction, as reported in other cases of competitive displacement (Anttila et al., 1998; Johnson et al., 2016) or hybrid swamping (Childs et al., 1996). In theory, eastern wolves could go extinct if their niche space overlaps with that of competing *Canis* and hybridization continues unabated.

Here, we investigated niche space determinants across a wild canid admixture zone in North America, to determine niche space overlap of at-risk eastern wolves, encroaching coyote-like canids, resident gray wolves and their hybrids. We predicted that eastern wolves exhibit unique niche space compared to gray wolves and coyotes, and that hybrids exhibit intermediate niche space to their respective parentals. We also predicted, despite the potential overlap with hybrids, that competitive habitats exist for the eastern wolf (i.e., geographic space that disproportionately supports eastern wolf niche space).

2. Methods

2.1. Study extent & sampling

We examined contemporary *Canis* hybridization and niche dynamics in the Great Lakes region in North America, encompassing 2,268,350 km^2 (Stronen et al., 2012). Climatic conditions varied from −19.3 to 3.2 °C in winter, with snow precipitation ranging from 22 to 434 mm (Adaptwest, 2015; Fick and Hijmans, 2017). The region is occupied by other large predators including Canada lynx (*Lynx canadensis*), bobcat (*Lynx rufus*) and black bear (*Ursus americanus*). Potential prey species include moose and caribou in central and northern areas, white-tailed deer in central and southern areas, and beaver and smaller mammals throughout the region (Gallina and Lopez Arevalo, 2016; Gunn, 2016; Hundertmark, 2016).

Contemporary (1995–2017) *Canis* pelt samples ($n = 1366$; Fig. 1) were collected from fur auction houses in Ontario and from independent trappers with a locational certainty of within 1 km for most samples (approximately 85%). Our analysis is an extension of a previous assessment of canid niche overlap (Otis et al., 2017) and employs the same 12 autosomal microsatellite loci (including the same primers and standards) commonly used to infer population structure in this system (Rutledge et al., 2010; Wheeldon et al., 2010) (Table S1). Our total genotypic dataset ultimately included 136 individuals of a priori known ancestry that were used to evaluate genetic clustering, and 1230 unassigned individuals. Our individuals of known ancestry included 47 coyotes from Saskatchewan, 41 Gy wolves from the Northwest Territories, and 48 of the highest assigned (Q-value) eastern wolves from Ontario, openly available on DRYAD (Rutledge et al., 2010). Gray wolves and coyotes from these areas lie outside the putative admixture zone and are more likely to be unadmixed (Mech, 2011). Prey spatial data were obtained from the Global Biodiversity Information Facility (GBIF, 2019). Occurrence locations for prey and *Canis* groups were thinned at 20 km using *spThin* (Aiello-Lammens et al., 2015) to reduce spatial autocorrelation and sampling bias (Aiello-Lammens et al., 2015; Boria et al., 2014) (Table S2). After spatial thinning, our final canid
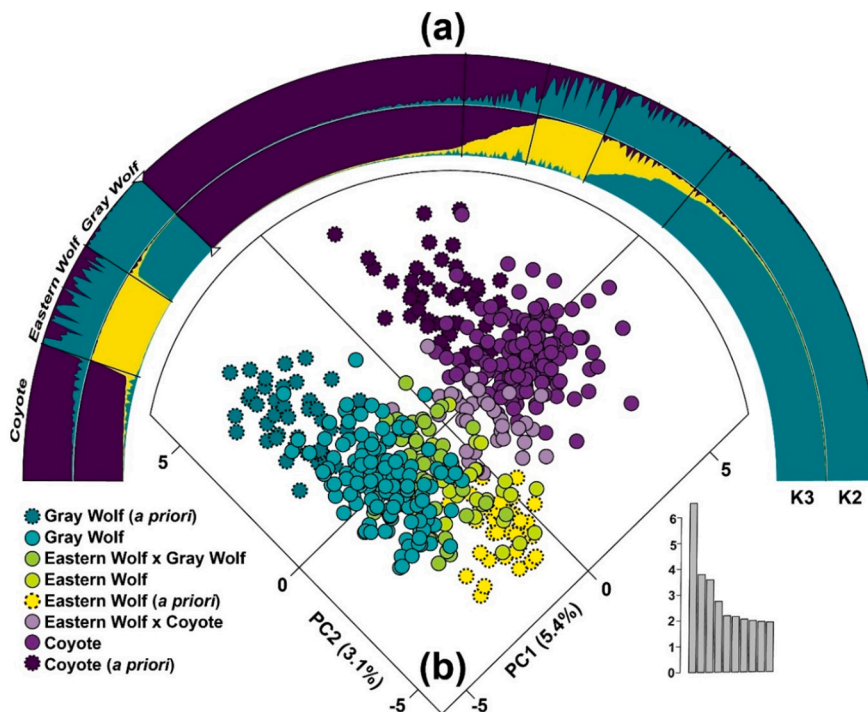
dataset included 408 samples, providing sufficient sampling across groups to examine niche characteristics (van Proosdij et al., 2016).

2.2. Population genetic analyses

We identified canid groups and putative hybrids from the 12 microsatellite dataset using an ancestry coefficient (*Q*-value) matrix generated from *Structure v2.3.4* (Falush et al., 2003). The optimal value of genetic clusters (K) was determined using *Structure Harvester* (Earl and von Holdt, 2012) based on the highest ΔK relative to MeanLnP(K). Unadmixed individuals were designated as having a Q-value ≥0.80 (i.e., ancestry coefficient greater than 80%) for any of the three genetic clusters (*K*), while remaining individuals were assigned respective hybrid designations based on proportionate assignments (Otis et al., 2017; Rutledge et al., 2010; Wheeldon and Patterson, 2012). We also examined population structure with a principal components analysis implemented in *adegenet v2.1.1* (Jombart, 2008), which does not rely on underlying population genetic assumptions about Hardy-Weinberg equilibrium. We calculated boot-strapped F_{ST} using *hierfstat* v0.5–7 (Goudet, 2005) and visualized structure plots with *strataG* (Archer et al., 2017). We examined the sensitivity of *Q*-value thresholds on niche overlap metrics by repeating all analyses at *Q* of 0.7 and 0.9, and also examined genetic population structure of the full dataset (*n* = 1366), with additional details found in Appendix A.1.

2.3. Ecological niche models

Ensemble ecological niche models were developed for prey species (e.g*.,* moose, white-tailed deer, caribou) using the package *sdm* (Naimi and Araújo, 2016) in R (R Core Team, 2017) using 11 biotic and abiotic variables predicted to be relevant for each species, including human population density, tree cover, and snow precipitation (see Appendix A.2 for full details). Variables for *Canis* models included prey ensemble niche models for (i) moose, (ii) white-tailed deer, (iii) caribou, and environmental data for (iv) snow precipitation, (v) vegetation index, (vi) percentage tree cover, (vii) mean diurnal temperature range (bio_2), (viii) mean temperature of the wettest quarter (bio_8), (ix) annual precipitation (bio_12), and (x) human population density. Further details

for all these covariates are found in Appendix A.2. In brief, these covariates are justified for our purposes because of the importance of prey in shaping predator distributions (variables i, ii, and iii), the previous use of similar variables in other macro-scale ecological studies of canids (variables iv and x; Otis et al., 2017), and the support of significant evolutionary correlations between several of these variables and genomic signatures of selection in gray wolf populations (variables v, vi, vii, and ix; Schweizer et al., 2016). A niche model for beaver was not included due to high collinearity with white-tailed deer (Fig. S1). Pearson's correlation coefficient (*r* > 0.7) was used to exclude correlated variables. Niche models were created using statistical learning, regression, and classification techniques, and were assessed by subsampling with 70% of the dataset allocated for training and evaluations reported with sensitivity (i.e., true positive rate) due to potential over-inflation of true negatives with pseudo-absences. Ensemble niche models for each group were then created by averaging the resultant grids across all replicate models. Further details of ecological niche modeling, including all computational code, can be found within Appendices A.3 and B.

2.4. Canis niche overlap

We quantified niche characteristics across *Canis* groups by (i) qualitatively comparing ensemble grids across the clade; (ii) assessing model covariate importance by conducting a permutation test to assess contribution to model fit (e.g., AUC); (iii) measuring *Canis* dyad overlap between niche variables and occurrence with response curves; (iv) measuring the degree of niche overlap (e.g., Schoener's D and Spearman's Rank Correlation Coefficient); and (v) directly assessing overlap in niche space without algorithmic modeling by comparing the first two principal components of available and occupied niche space for each *Canis* species (Cola et al., 2015; Fernández-López, 2017). We then identified regions with competitive habitat for eastern wolves by subtracting the realized niche of each parental and hybrid (i.e., predicted niche model grid bounded by observed occurrences) from the realized niche of eastern wolves (Fig. S2). As each canid group poses unique unknown existential threats (e.g., varying levels of direct competition, introgression), we subtracted the cumulative habitat suitability of all canids within the eastern wolf realized niche to identify where eastern
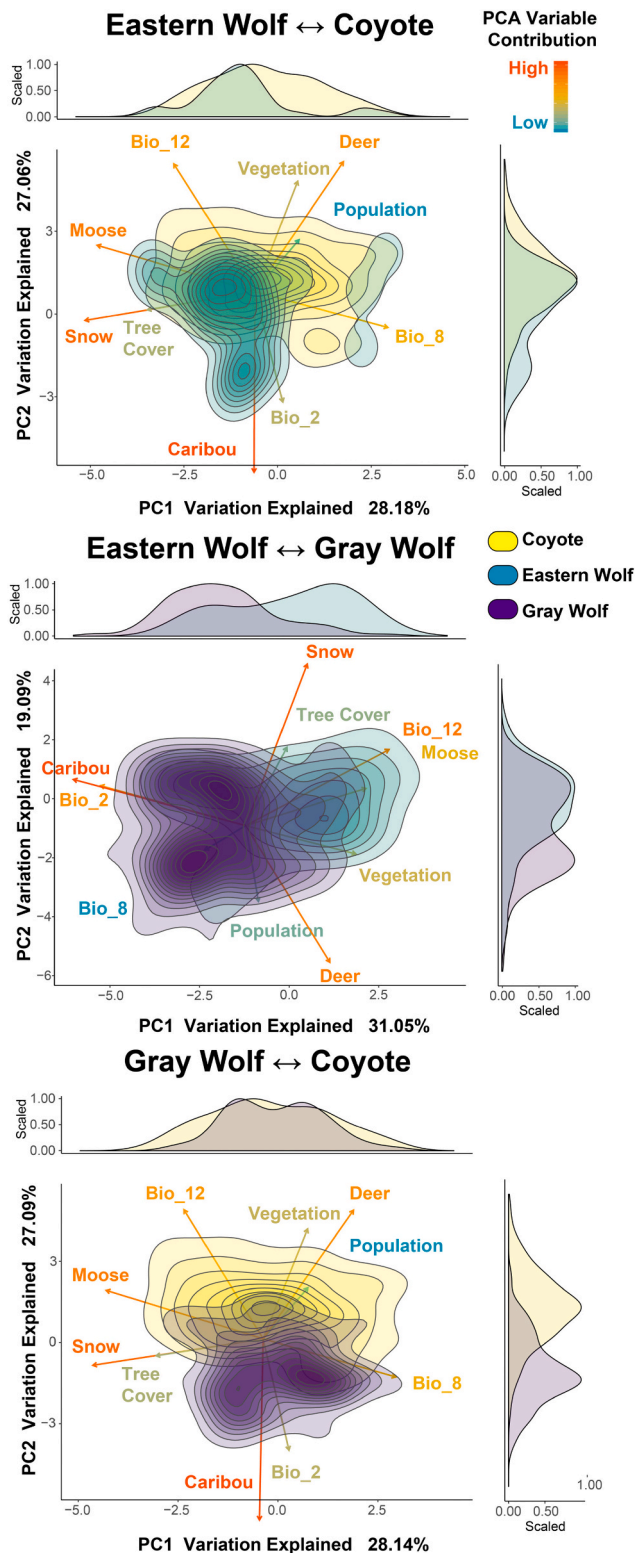
Fig. 3. Pairwise comparisons of niche space between canids without traditional algorithmic modeling, using Ecospat (Cola et al., 2015). Each plot shows a comparison of total utilized niche space for two canids along the first two principal components, indicated by color, based on their relative background environments. The inside contour plot shows relative density of occurrence in relation to environmental space, with the PCA of environmental determinants superimposed underneath is color-coded by relative importance. The density plots above and to the right show relative occurrence along each unique principal component axis in niche space.

wolves may outcompete all other *Canis* within their current distribution.

3. Results

3.1. Low genetic differentiation within admixture zone

Based on our analysis of microsatellite loci, *Canis* groups were weakly differentiated across the study region, with our selected $K = 3$ clusters capturing the substructure that exists between eastern wolves and coyotes and correctly identifying 91.9% ($n = 125$) of animals with known ancestry ($n = 136$; Figs. 2a; S3). In contrast, $K = 2$ left eastern wolves and gray wolves largely undifferentiated. Further analysis showed low differentiation between these genetic clusters within the admixture zone, with the highest differentiation between gray wolves and coyotes ($F_{ST} = 0.05$–0.08, 95% CI; Table S3). A consistent gradient in differentiation is observed across the clade, with negligible differentiation between eastern wolf and eastern wolf x gray wolf hybrids ($F_{ST} < 0.01$–0.01, 95% CI; Table S3) and intermediate differentiation between eastern wolves and coyotes ($F_{ST} = 0.04$–0.05, 95% CI; Table S3). A PCA identified a near complete continuum of genotypes within the hybrid zone, particularly in contrast to a reference sample of unadmixed individuals from outside the contact zone (Figs. 2b; S4; Table S3).

3.2. Niche divergence reflects a genetic continuum

Niche models fit the data well across the clade, with highest fit for eastern wolves (sensitivity = 0.96 ± 0.01, mean $\pm$ SEM; Table S4) and lowest fit for eastern wolf x gray wolf hybrids (0.81 ± 0.02). Qualitatively, niche models showed a continuum of distributions ranging from eastern wolves in the mid-eastern range, coyotes in the south-eastern range, and gray wolves in the north and western range, with eastern wolf and coyote distributions largely overlapping. Hybrids exhibited intermediate niche space compared to each parental across the landscape and for many specific environmental predictors (Figs. S5; S6; S7), and quantitatively fell intermediate in magnitude and directionality for niche overlap analyses (Spearman's rank coefficient, Fig. S8).

Moose niche most strongly predicted eastern wolf niche (permutation importance based on AUC = 0.41 ± 0.04, mean $\pm$ SEM), and Pearson correlation coefficients for both prey (0.23 ± 0.02, absolute value of mean $\pm$ SEM) and canid (0.24 ± 0.03) covariates showed minimal collinearity (Fig. S6; Table S5). Eastern wolf response curves were intermediate to those of both gray wolves and coyotes for nearly all niche variables (Fig. S7). Niche overlap reflected a genetic continuum, with the most dissimilar niche space being between gray wolves and coyotes (Schoener's D; Spearman's Rank Coefficient = 0.65, -0.55) and eastern wolves being intermediate to all groups (Fig. S8). Coyote and eastern wolf niche overlap was positively correlated (0.69, 0.23), whereas niche overlap was only negatively correlated between gray wolves and coyotes (0.65, -0.55), gray wolves and eastern wolf x coyote hybrids (0.71, -0.36), and coyotes and gray wolf x eastern wolf hybrids (0.70, -0.20; Fig. S8).

3.3. Coyotes overlap eastern wolf niche space

Eastern wolves and coyotes shared peaks of niche occupancy and exhibited extensive niche overlap. Our pairwise analyses of niche space determinants indicated that most parentals occupied unique niche space either along both principal component axes (eastern wolf ↔ gray wolf; PC1 = 31.05%, variation explained; PC2 = 19.09%) or only along the second principal component axis (gray wolf ↔ coyote; PC2 = 27.09%; Fig. 3). Between eastern wolves and coyotes, the first PC axis (eastern wolf ↔ coyote; 28.18%) indicated near complete overlap of eastern wolf niche space by coyotes. The second PC axis (eastern wolf ↔ coyote; 27.06%) showed a shared peak exhibited between both species of the same magnitude, with only a small secondary peak exhibited by eastern wolves that is not shared by coyotes. This difference appeared to be due

to a stronger relationship with caribou niche, although this likely is a statistical artifact given that eastern wolves and caribou are not known to currently co-occur in the region. An additional portion of unique niche space for eastern wolves is driven by moose niche, snowfall, and tree cover along the first principal component axis (Fig. 3). Similar patterns of overlap were observed between eastern wolves and eastern wolf x coyote hybrids (Fig. S9).

Additional analyses of total and occupied available niche space (i.e., fundamental and realized niches) indicated that all *Canis* groups had access to the same core environmental conditions (Fig. S10). Eastern wolves occupied niche space that was similar to both gray wolves and coyotes, although their core occupancy was completely subsumed by coyotes (Figs. 3; S10). Nonetheless, we observed near complete exclusion of occupied environmental space between gray wolves and coyotes.

4. Discussion

4.1. Limited genetic differentiation indicates minimal barriers to gene flow

Extensive admixture and low genetic differentiation indicate minimal genetic structure among *Canis* groups within the core hybrid zone, highlighting the precarious genetic integrity of the contemporary eastern wolf population (Heppenheimer et al., 2018; Rutledge et al., 2017). Within the admixture zone eastern wolves exhibit low differentiation with gray wolves and coyotes compared to unadmixed gray wolves and coyotes from outside the zone, supporting previous findings of widespread gene flow (Heppenheimer et al., 2018; von Holdt et al., 2016).

Genetic structuring in wild canids across eastern North America appears to be largely consistent with their geographic distributions across the landscape, suggesting that without strong ecological differentiation between canid groups to reinforce reproductive isolation, hybridization will continue. Observed levels of genetic homogenization indicate that introgressive hybridization has already affected much of the range of eastern wolves around the Great Lakes region, reiterating concerns over the erosion for this species of conservation concern (Heppenheimer et al., 2018). While our use of microsatellites precludes us from examining potential adaptive introgression or fine-scale gene x environment associations (e.g., Schweizer et al., 2016), our results are consistent with population structuring seen with genome-wide markers within the admixture zone (Heppenheimer et al., 2018).

4.2. Extensive niche overlap threatens eastern wolf persistence

Niche determinants were relatively consistent across the clade of canids and reflected a genetic continuum ranging from gray wolves to coyotes. Consistent with our prediction, we found evidence that hybrid individuals exhibit intermediate niche space compared to parentals (see also (Otis et al., 2017), suggesting that genetic differentiation reflects the degree of niche divergence observed in canids within this hybrid zone. Despite the observed continuum of divergence, total occupied niche space varied considerably, with coyotes favoring a wide range of environmental conditions compared to eastern wolves or gray wolves. While coyotes *are known* to use a broad spectrum of environmental resources, they may be excluded to some degree from remaining eastern wolf strongholds, such as Algonquin Provincial Park in Ontario, by interspecific aggression (Benson and Patterson, 2013b). However, eastern wolves facilitate introgression by leaving core protected areas like Algonquin Provincial Park and thereby eroding spatial segregation between groups and increasing the likelihood of further hybridization (Merkle et al., 2009). Coyotes and hybrids have a propensity for dispersing into and colonizing unoccupied wolf habitat (Gese et al., 2015), thereby causing significant conservation challenges.

Given the overlap in genetic and environmental space across the clade, continued eastern wolf hybridization will either result in complete introgression from other canids or extinction of the eastern wolf

lineage (Grabenstein and Taylor, 2018). Introgression could provide eastern wolves with functional genetic diversity (e.g., coyotes have increased MHC diversity), see (Hedrick, 2013; Hedrick et al., 2002) that could further increase their ability to occupy niche space in, for example, modified eastern forests. However, because reproductive barriers are low outside of eastern wolf population strongholds (Rutledge et al., 2010), and coyotes may receive functional benefits from wolf introgression in the region (Kays et al., 2010), the advantage of introgression for eastern wolves are likely dwarfed by the possibility of genetic homogenization. Therefore, with unabated hybridization a likely trajectory for eastern wolves within the admixture zone is full lineage extinction via genetic swamping (Rhymer and Simberloff, 1996; Todesco et al., 2016). This inference is problematic for eastern wolf conservation as they have a smaller population size and similar ecological niche compared to the coyotes and gray wolves that geographically surround them, providing multiple pathways for erosion of eastern wolf genes from encroaching species; this phenomenon is also seen in other systems where encroaching species have displaced local congeners (Childs et al., 1996; Gibson et al., 2019; Hata et al., 2019).

Contrary to our prediction that eastern wolves exhibit unique niche space compared to all other canid groups, we observed no unique peaks in niche space according to PC axes between eastern wolves and coyotes (Fig. 3). This highlights that coyotes and hybrids currently occupy virtually all core eastern wolf niche space. While we expected that a strong association between eastern wolves and moose would increase niche exclusivity for wolves, this environmental space still appears similarly suitable for coyotes, perhaps due to their generalist and flexible diets across a range of habitat types (Dumond et al., 2001). While a preference for larger prey (i.e., moose and caribou) could theoretically differentiate resource-use between eastern wolves and coyotes (but see Benson and Patterson, 2013a), large prey are similarly utilized by gray wolves and their hybrids (Benson et al., 2012), thereby reinforcing evidence in support of competitive challenges for eastern wolves. Further investigations that examine fine-scale diet preference in overlapping populations, such as via space use patterns (i.e., radio-telemetry) or dietary comparison (i.e., prey selection, scat or stable isotope analysis; Leighton et al., 2020; McAlpine et al., 2016), could elucidate patterns of overlap and differentiation across the clade beyond what was possible in our large-scale investigation. Nonetheless, for the variety of environmental variables examined eastern wolves appear to be at a distinct disadvantage compared to other canid groups, and this could translate to further genetic homogenization and ultimately, functional extinction. Notably, this process is also at the core of other conservation challenges in cases of range-expanding competitors, and exemplifies the profound problem caused by synergistic effects of hybridization and competition in prompting at-risk species displacement (Childs et al., 1996; Hata et al., 2019).

4.3. Policy and management recommendations

Establishing viable eastern wolf populations may be a conservation priority in eastern Canada, but a number of challenges have arisen that so far preclude successful recovery. First, a limited number of conservation actions are available when dealing with species having such large spatial requirements and faced with the relatively intractable challenges of competition and hybridization. These challenges are compounded given the many stakeholders involved in any management decision involving wolves, their ungulate prey, or the forests that they occupy. One possible approach, establishing stronghold eastern wolf populations in suitable habitat to allow for persistence of breeding populations and natural exclusion of coyotes (see Gese et al., 2015), seems destined to fail without a level of intensive and perpetual human intervention. Indeed, for red wolves (*Canis rufus*) in North Carolina this approach did not result in successful establishment of a viable population despite that the wolf population was relatively isolated from immigration by coyotes and coyote management through removal and sterilization was successful
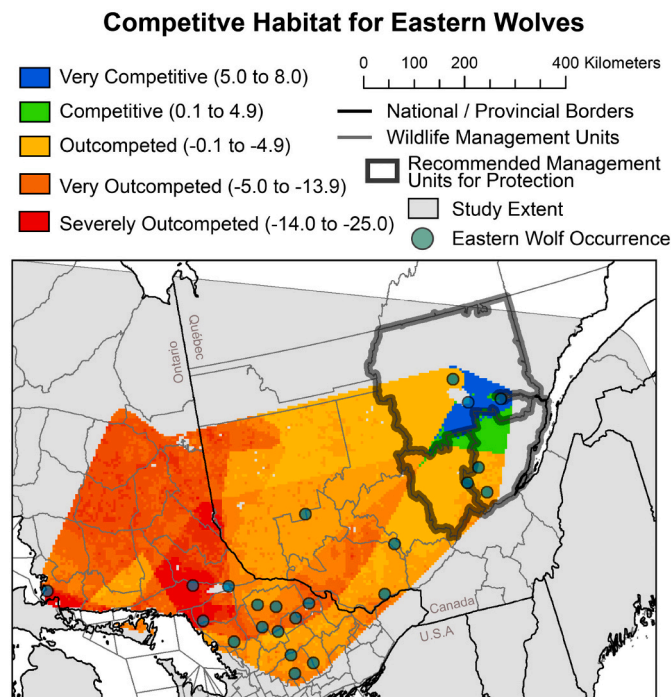
Competitve Habitat for Eastern Wolves

Fig. 4. Competitive habitats for eastern wolves (*Canis lycaon*) across their geographic distribution. Competitive habitat was identified by subtracting the cumulative realized niche of all observed canids (gray wolves, coyotes, and hybrids) from the realized niche of eastern wolves (see Supporting Information for further details). A value of 10.0 would be ideal habitat for eastern wolves with no competition, while lower values indicate regions of either poor habitat or high competition. The highlighted hunting zones in Québec (zones 26, 27, and 28) provide the highest priority conservation targets, indicating regions of realized niche in which suitable habitats favor eastern wolves over coyotes and hybrids.

([Murray et al., 2015](#)). In contrast, in the vast and remote landscapes of Ontario and Quebec it is unlikely that coyote management could be effected to the extent necessary for successful eastern wolf population establishment or restoration. This is confirmed by our detection of only a narrow region of competitive habitats within the eastern wolf realized niche where eastern wolves were more suited to the landscape than the cumulative threat of other *Canis* groups. Specifically, our analysis revealed only 5.3% of the current eastern wolf range harbors competitive habitats for eastern wolves compared to hybridizing congeners (see Fig. 4; Table S6). Because this region is at the eastern wolf range margin and outside the current species' stronghold in south-central Ontario, it highlights the likely disequilibrium in wolf-coyote dynamics and likely futility of conservation efforts targeted at wolf conservation in the core range, over the longer term. In contrast, wolf habitat protection should be prioritized strategically in south-eastern Québec where other *Canis* groups appear to have a weaker footing (i.e., hunting zones 26, 27, and 28; see Fig. 4). Although it is possible that no intervention will ensure the long-term viability of eastern wolves, if establishment of viable populations remains a priority, we advise that conservation efforts (e.g., wolf harvest ban, habitat protection, see COSSARO, 2016) should be appropriately directed where chances of success are highest.

Alternatively, it may be necessary to accept that eastern wolf populations cannot be successfully re-established in contemporary landscapes and that a laissez-faire approach will lead to establishment of the most competitive canids, irrespective of their genetic makeup, across the region (see Kyle et al., 2006). This could result in a gradient of canids in eastern North America, with more coyote-dominated hybrids in southern and rural areas and more wolf-dominated animals in more remote northern regions. These animals would evolve to best reflect local

conditions and thereby fill relevant ecological roles in particular ecosystems.

5. Conclusions

Our analyses support the hypothesis that eastern wolf hybridization with gray wolves and coyotes is widespread and that the intermediate niche space occupied by eastern wolves promotes introgressive hybridization and competition with other canids. Eastern wolves are unlikely to maintain stronghold populations over the longer term even with intensive and extensive management efforts, thereby suggesting the need to refocus conservation efforts towards supporting animals that will play a functionally relevant role in local ecosystems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Further details on genotyping, ecological niche modeling, and supplementary tables and figures (Appendix A), and all computation code used within this project (Appendix B), are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.Supplementary data to this article can be found online at doi:10.1016/j.biocon.2021.109062.

References

Adaptwest, 2015. In: Wang, T., et al. (Eds.), Gridded Current and Projected Climate Data for North America at 1km Resolution, Interpolated Using the ClimateNA v5.10 Software, 2015. Available at. adaptwest.databasin.org.

Aiello-Lammens, M.E., Boria, R.A., Radosavljevic, A., Vilela, B., Anderson, R.P., 2015. spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. Ecography 38, 541–545. https://doi.org/10.1111/ecog.01132.

Anttila, C.K., Daehler, C.C., Rank, N.E., Strong, D.R., 1998. Greater male fitness of a rare invader (*Spartina alterniflora*, Poaceae) threatens a common native (*Spartina foliosa*) with hybridization. Am. J. Bot. 85, 1597–1601. https://doi.org/10.2307/2446487.

Archer, F.I., Adams, P.E., Schneiders, B.B., 2017. STRATAG: an r package for manipulating, summarizing and analysing population genetic data. Mol. Ecol. Resour. 17, 5–11. https://doi.org/10.1111/1755-0998.12559.

Atwood, T.C., Weeks, H.P., 2003. Spatial home-range overlap and temporal interaction in eastern coyotes: the influence of pair types and fragmentation. Can. J. Zool. 81, 1589–1597. https://doi.org/10.1139/z03-144.

Benson, J.F., Patterson, B.R., 2013a. Moose (*Alces alces*) predation by eastern coyotes (*Canis latrans*) and eastern coyote × eastern wolf (*Canis latrans* × *Canis lycaon*) hybrids. Can. J. Zool. 91, 837–841. https://doi.org/10.1139/cjz-2013-0160.

Benson, J.F., Patterson, B.R., 2013b. Inter-specific territoriality in a *Canis* hybrid zone: spatial segregation between wolves, coyotes, and hybrids. Oecologia 173, 1539–1550. https://doi.org/10.1007/s00442-013-2730-8.

Benson, J.F., Patterson, B.R., Wheeldon, T.J., 2012. Spatial genetic and morphologic structure of wolves and coyotes in relation to environmental heterogeneity in a *Canis* hybrid zone. Mol. Ecol. 21, 5934–5954. https://doi.org/10.1111/mec.12045.

Benson, J.F., Mills, K.J., Loveless, K.M., Patterson, B.R., 2013. Genetic and environmental influences on pup mortality risk for wolves and coyotes within a *Canis* hybrid zone. Biol. Conserv. 166, 133–141. https://doi.org/10.1016/j.biocon.2013.06.018.

Boria, R.A., Olson, L.E., Goodman, S.M., Anderson, R.P., 2014. Spatial filtering to reduce sampling bias can improve the performance of ecological niche models. Ecol. Model. 275, 73–77. https://doi.org/10.1016/j.ecolmodel.2013.12.012.

Childs, M.R., Echelle, A.A., Dowling, T.E., 1996. Development of the hybrid swarm between Pecos pupfish (Cyprinodontidae: *Cyprinodon pecosensis*) AND sheepshead minnow (*Cyprinodon variegatus*): a perspecive from allozymes and mtDNA. Evolution 50, 2014–2022. https://doi.org/10.1111/j.1558-5646.1996.tb03588.x.

Cola, V.D., Broennimann, O., Petitpierre, B., Breiner, F.T., D'Amen, M., Randin, C., Engler, R., Pottier, J., Pio, D., Dubuis, A., Pellissier, L., Mateo, R.G., Hordijk, W., Salamin, N., Guisan, A., 2015. ecospat: An R Package to Support Spatial Analyses and Modeling of Species Niches and Distributions. https://doi.org/10.1111/oik.02629.

COSEWIC, 2015. COSEWIC Assessment and Status Report on the Eastern Wolf Canis spp. cf. lycaon in Canada. Committee on the Status of Endangered Wildlife in Canada.

COSSARO, 2016. Ontario Species at Risk Evaluation Report for Algonquin Wolf (Canis sp.), an Evolutionary Significant and Distinct Hybrid With Canis lycaon, C. latrans, and C. lupus Ancestry. Committee on the Status of Species at Risk in Ontario.

Dumond, M., Villard, M.A., Tremblay, É., 2001. Does coyote diet vary seasonally between a protected and an unprotected forest landscape? Ecoscience 8, 301–310. https://doi.org/10.1080/11956860.2001.11682657.

Earl, D.A., von Holdt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361. https://doi.org/10.1007/s12686-011-9548-7.

Falush, D., Stephens, M., Pritchard, J.K., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 1587, 1567–1587. https://doi.org/10.3410/f.1015548.197423.

Fener, H.M., Ginsberg, J.R., Sanderson, E.W., Gompper, M.E., 2005. Chronology of range expansion of the coyote, Canis latrans, in New York. Can. Field-Nat. 119 (1) https://doi.org/10.22621/cfn.v119i1.74.

Fernández-López, J., 2017. niceOverPlot, or When the Number of Dimensions Does Matter.

Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. Int. J. Climatol. 37, 4302–4315. https://doi.org/10.1002/joc.5086.

Gallina, S., Lopez Arevalo, H., 2016. Odocoileus virginianus. The IUCN Red List of Threatened Species 2016: e.T42394A22162580 [WWW Document]. URL https://doi.org/10.2305/IUCN.UK.2016-2.RLTS.T42394A22162580.en (accessed 11.9.20).

GBIF, 2019. GBIF Occurrence Download. White-tailed Deer. https://doi.org/10.15468/dl.s2xinb. Caribou: doi:10.15468/dl.cjdh0g; Moose: doi:10.15468/dl.exbtq0 [WWW Document]. URL GBIF.org. (accessed 6.14.19).

Gese, E., Knowlton, F., Adams, J., Beck, K., 2015. Managing hybridization of a recovering endangered species: the red wolf *Canis rufus* as a case study. Curr. Zool. 61, 191–205. https://doi.org/10.1093/czoolo/61.1.191.

Gibson, I., Welsh, A.B., Welsh, S.A., Cincotta, D.A., 2019. Genetic swamping and possible species collapse: tracking introgression between the native candy darter and introduced variegate darter. Conserv. Genet. 20, 287–298. https://doi.org/10.1007/s10592-018-1131-2.

Goudet, J., 2005. hierfstat, a package for r to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5, 184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x.

Grabenstein, K.C., Taylor, S.A., 2018. Breaking barriers: causes, consequences, and experimental utility of human-mediated hybridization. Trends Ecol. Evol. 33, 198–212. https://doi.org/10.1016/j.tree.2017.12.008.

Gunn, A., 2016. Rangifer tarandus. The IUCN Red List of Threatened Species 2016: e.T29742A22167140 [WWW Document]. URL https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T29742A22167140.en (accessed 11.9.20).

Hata, H., Uemura, Y., Ouchi, K., Matsuba, H., 2019. Hybridization between an endangered freshwater fish and an introduced congeneric species and consequent genetic introgression. PLoS One 14, e0212452. https://doi.org/10.1371/journal.pone.0212452.

Hedrick, P.W., 2013. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Mol. Ecol. 22, 4606–4618. https://doi.org/10.1111/mec.12415.

Hedrick, P.W., Lee, R.N., Garrigan, D., 2002. Major histocompatibility complex variation in red wolves: evidence for common ancestry with coyotes and balancing selection. Mol. Ecol. 11, 1905–1913. https://doi.org/10.1046/j.1365-294X.2002.01579.x.

Heppenheimer, E., Harrigan, R.J., Rutledge, L.Y., Koepfli, K.-P., DeCandia, A.L., Brzeski, K.E., Benson, J.F., Wheeldon, T., Patterson, B.R., Kays, R., Hohenlohe, P.A., von Holdt, B.M., 2018. Population genomic analysis of North American eastern wolves (Canis lycaon) supports their conservation priority status. Genes 9, 606. https://doi.org/10.3390/genes9120606.

Hody, J.W., Kays, R., 2018. Mapping the expansion of coyotes (Canis latrans) across north and Central America. ZooKeys 759, 81–97. https://doi.org/10.3897/zookeys.759.15149.

Hohenlohe, P.A., Rutledge, L.Y., Waits, L.P., Andrews, K.R., Adams, J.R., Hinton, J.W., Nowak, R.M., Patterson, B.R., Wydeven, A.P., Wilson, P.A., White, B.N., 2017. Comment on "Whole-genome sequence analysis shows two endemic species of North American wolf are admixtures of the coyote and gray wolf". Sci. Adv. 3, e1602250 https://doi.org/10.1126/sciadv.1602250.

von Holdt, B.M., Cahill, J.A., Fan, Z., Gronau, I., Robinson, J., Pollinger, J.P., Shapiro, B., Wall, J., Wayne, R.K., 2016. Whole-genome sequence analysis shows that two endemic species of North American wolf are admixtures of the coyote and gray wolf. Sci. Adv. 1–14 https://doi.org/10.1126/sciadv.1501714.

Hundertmark, K., 2016. Alces alces. The IUCN Red List of Threatened Species 2016: e.T56003281A22157381 [WWW Document]. URL https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T56003281A22157381.en (accessed 11.9.20).

Johnson, M.G., Lang, K., Manos, P., Golet, G.H., Schierenbeck, K.A., 2016. Evidence for genetic erosion of a California native tree, Platanus racemosa, via recent, ongoing introgressive hybridization with an introduced ornamental species. Conserv. Genet. 17, 593–602. https://doi.org/10.1007/s10592-015-0808-z.

Jombart, T., 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405. https://doi.org/10.1093/bioinformatics/btn129.

Kays, R., Curtis, A., Kirchman, J.J., 2010. Rapid adaptive evolution of northeastern coyotes via hybridization with wolves. Biol. Lett. 6, 89–93. https://doi.org/10.1098/rsbl.2009.0575.

Kilgo, J.C., Ray, H.S., Ruth, C., Miller, K.V., 2010. Can coyotes affect deer populations in southeastern North America? J. Wildl. Manag. 74, 929–933. https://doi.org/10.2193/2009-263.

Kyle, C.J., Johnson, A.R., Patterson, B.R., Wilson, P.J., Shami, K., Grewal, S.K., White, B.N., 2006. Genetic nature of eastern wolves: past, present and future. Conserv. Genet. 7, 273–287. https://doi.org/10.1007/s10592-006-9130-0.

Leighton, G.R.M., Bishop, J.M., O'Riain, M.J., Broadfield, J., Meröndun, J., Avery, G., Avery, D.M., Serieys, L.E.K., 2020. An integrated dietary assessment increases feeding event detection in an urban carnivore. Urban Ecosyst. https://doi.org/10.1007/s11252-020-00946-y.

McAlpine, D.F., Soto, D.X., Rutledge, L.Y., Wheeldon, T.J., White, B.N., Goltz, J.P., Kennedy, J., 2016. Recent occurrences of wild-origin wolves (Canis spp.) in Canada south of the St. Lawrence River revealed by stable isotope and genetic analysis. Can. Field-Nat. 129, 386. https://doi.org/10.22621/cfn.v129i4.1761.

Mech, L.D., 2011. Non-genetic data supporting genetic evidence for the eastern wolf. Northeast. Nat. 18, 521–526. https://doi.org/10.2307/41429237.

Meilink, W.R.M., Arntzen, J.W., van Delft, J.J.C.W., Wielstra, B., 2015. Genetic pollution of a threatened native crested newt species through hybridization with an invasive congener in the Netherlands. Biol. Conserv. 184, 145–153. https://doi.org/10.1016/j.biocon.2015.01.022.

Merkle, J.A., Stahler, D.R., Smith, D.W., 2009. Interference competition between gray wolves and coyotes in Yellowstone National Park. Can. J. Zool. 87, 56–63. https://doi.org/10.1139/Z08-136.

Messier, F., 1994. Ungulate population models with predation: a case study with the North American moose. Bull. Ecol. Soc. Am. 75, 478–488. https://doi.org/10.2307/1939551.

Murray, D.L., Bastille-Rousseau, G., Adams, J.R., Waits, L.P., 2015. The challenges of red wolf conservation and the fate of an endangered species recovery program. Conserv. Lett. 8, 338–344. https://doi.org/10.1111/conl.12157.

Naimi, B., Araújo, M.B., 2016. sdm: a reproducible and extensible R platform for species distribution modelling. Ecography 39, 368–375. https://doi.org/10.1111/ecog.01881.

Newbold, T., Hudson, L.N., Hill, S.L.L., Contu, S., Lysenko, I., Senior, R.A., Börger, L., Bennett, D.J., Choimes, A., Collen, B., Day, J., De Palma, A., Díaz, S., Echeverria-Londoño, S., Edgar, M.J., Feldman, A., Garon, M., Harrison, M.L.K., Alhusseini, T., Ingram, D.J., Itescu, Y., Kattge, J., Kemp, V., Kirkpatrick, L., Kleyer, M., Correia, D.L.P., Martin, C.D., Meiri, S., Novosolov, M., Pan, Y., Phillips, H.R.P., Purves, D.W., Robinson, A., Simpson, J., Tuck, S.L., Weiher, E., White, H.J., Ewers, R.M., Mace, G.M., Scharlemann, J.P.W., Purvis, A., 2015. Global effects of land use on local terrestrial biodiversity. Nature 520, 45–50. https://doi.org/10.1038/nature14324.

Newsome, S.D., Garbe, H.M., Wilson, E.C., Gehrt, S.D., 2015. Individual variation in anthropogenic resource use in an urban carnivore. Oecologia 178, 115–128. https://doi.org/10.1007/s00442-014-3205-2.

Newsome, T.M., Greenville, A.C., Ćirović, D., Dickman, C.R., Johnson, C.N., Krofel, M., Letnic, M., Ripple, W.J., Ritchie, E.G., Stoyanov, S., Wirsing, A.J., 2017. Top predators constrain mesopredator distributions. Nat. Commun. 8 (May), 1–7. https://doi.org/10.1038/ncomms15469.

Otis, J., Thornton, D., Rutledge, L., Murray, D.L., 2017. Ecological niche differentiation across a wolf-coyote hybrid zone in eastern North America. Divers. Distrib. 23, 529–539. https://doi.org/10.1111/ddi.12543.

Peers, M.J.L., Thornton, D.H., Murray, D.L., 2013. Evidence for large-scale effects of competition: niche displacement in Canada lynx and bobcat. Proc. R. Soc. 280, 20132495. https://doi.org/10.1098/rspb.2013.2495.

Pilgrim, K.L., Boyd, D.K., Forbes, S.H., 1998. Testing for wolf-coyote hybridization in the rocky mountains using mitochondrial DNA. J. Wildl. Manag. 62, 683. https://doi.org/10.2307/3802344.

van Proosdij, A.S.J., Sosef, M.S.M., Wieringa, J.J., Raes, N., 2016. Minimum required number of specimen records to develop accurate species distribution models. Ecography 39, 542–552. https://doi.org/10.1111/ecog.01509.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rhymer, J.M., Simberloff, D., 1996. Extinction by hybridization and introgression. Annu. Rev. Ecol. Syst. 27, 83–109. https://doi.org/10.1146/annurev.ecolsys.27.1.83.

Rutledge, L.Y., Garroway, C.J., Loveless, K.M., Patterson, B.R., 2010. Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. Heredity 105, 520–531. https://doi.org/10.1038/hdy.2010.6.

Rutledge, L.Y., Devillard, S., Boone, J.Q., Hohenlohe, P.A., White, B.N., 2015. RAD sequencing and genomic simulations resolve hybrid origins within North American *Canis*. Biol. Lett. 11, 1–4. https://doi.org/10.1098/rsbl.2015.0303, 20150303.

Rutledge, L.Y., Desy, G., Fryxell, J.M., Middel, K., White, B.N., Patterson, B.R., 2017. Patchy distribution and low effective population size raise concern for an at-risk top preadtor. Divers. Distrib. 27, 79–89. https://doi.org/10.1111/ddi.12496.

Schweizer, R.M., Robinson, J., Harrigan, R., Silva, P., Galverni, M., Musiani, M., Green, R.E., Novembre, J., Wayne, R.K., 2016. Targeted capture and resequencing of 1040 genes reveal environmentally driven functional variation in grey wolves. Mol. Ecol. 25, 357–379. https://doi.org/10.1111/mec.13467.

Scott, J.M., Goble, D.D., Haines, A.M., Wiens, J.A., Neel, M.C., 2010. Conservation-reliant species and the future of conservation. Conserv. Lett. 3, 91–97. https://doi.org/10.1111/j.1755-263X.2010.00096.x.

Stronen, A.V., Tessier, N., Jolicoeur, H., Paquet, P.C., Hénault, M., Villemure, M., Patterson, B.R., Sallows, T., Goulet, G., Lapointe, F.-J., 2012. Canid hybridization: contemporary evolution in human-modified landscapes. Ecol. Evol. 2, 2128–2140. https://doi.org/10.1002/ece3.335.

Todesco, M., Pascual, M.A., Owens, G.L., Ostevik, K.L., Moyers, B.T., Hübner, S., Heredia, S.M., Hahn, M.A., Caseys, C., Bock, D.G., Rieseberg, L.H., 2016. Hybridization and extinction. Evol. Appl. 9, 892–908. https://doi.org/10.1111/eva.12367.

Wheeldon, T.J., Patterson, B.R., 2012. Genetic and morphological differentiation of wolves (*Canis lupus*) and coyotes (*Canis latrans*) in northeastern Ontario. Can. J. Zool. 90, 1221–1230. https://doi.org/10.1139/z2012-090.

Wheeldon, T.J., Patterson, B.R., White, B.N., 2010. Sympatric wolf and coyote populations of the western Great Lakes region are reproductively isolated. Mol. Ecol. 19, 4428–4440. https://doi.org/10.1111/j.1365-294X.2010.04818.x.

van Wyk, A.M., Dalton, D.L., Hoban, S., Bruford, M.W., Russo, I.-R.M., Birss, C., Grobler, P., van Vuuren, B.J., Kotzé, A., 2016. Quantitative evaluation of hybridization and the impact on biodiversity conservation. Ecol. Evol. 1–11 https://doi.org/10.1002/ece3.2595.