UNIVERSITY OF CALIFORNIA, MERCED

Resilience and Sensitivity to Changing Environments in North American river otters (*Lontra canadensis*)

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in

Quantitative & Systems Biology

by

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Table of Contents

List of Tables iii

List of Figures iv

Acknowledgements v

Curriculum Vitae vi

Abstract of the Dissertation ix

Chapter 1: Ecological consequences of habitat change: how the past informs the present in two otter lineages 1

- 1.1 Introduction 1
  - 1.1.1 Background 1
  - 1.1.2 River otters as a representative case study 2
- 1.2 Biogeographic history of two major semi-aquatic New World otter clades 3
  - 1.2.1 Background 3
  - 1.2.2 *Pteronura* 4
  - 1.2.3 *Lontra* 6
- 1.3 Evolution & ecology of modern species 8
  - 1.3.1 *Pteronura brasiliensis* 8
  - 1.3.2 *Lontra canadensis* 9
  - 1.3.3 *Lontra longicaudis* 10
  - 1.3.4 *Lontra provocax* 11
  - 1.3.5 *Lontra felina* 12
- 1.4 Synthesis & Future Directions 14
- 1.5 References 19
- 1.6 Figures 30

Chapter 2: Maximum entropy species distribution modeling of the North American river otter (*Lontra canadensis*) 32

- 2.1 Introduction 32
- 2.2 Methods 33
  - 2.2.1 Model Inputs 33
  - 2.2.2 Modeling 35
  - 2.2.3 Model evaluation 36
- 2.3 Results 37
  - 2.3.1 Model Performance 37
  - 2.3.2 Predictor Variables 38

2.3.3 Predicted Range 39
2.4 Discussion 39
2.4.1 Challenges 39
2.4.2 Correspondence between predicted suitability and previous range maps 40
2.4.3 What factors are associated with the internal structure of otter distributions?. 41
2.4.4 Challenges for modeling otter distributions 43
2.4.5 Overall conclusions and prospects for future river otter models 45
2.5 References 47
2.6 Tables 52
2.7 Figures 57
2.8 Appendix 62
Chapter 3: Patterns of morphological variation in a broadly distributed semiaquatic carnivore, the North America river otter (*Lontra canadensis*) 73
3.1 Introduction 73
3.2 Methods 75
3.2.1 Specimen Selection & Acquisition 75
3.2.2 Data Collection & Processing 75
3.2.3 Aim 1 Analyses 76
3.2.4 Aim 2 Analyses 76
3.3 Results 77
3.3.1 Aim 1 Results 77
3.3.2 Aim 2 Results 78
3.4 Discussion 78
3.5 References 81
3.6 Tables 85
3.7 Figures 96

List of Tables

Table 2.1: Sources and uses of distribution data.. 52
Table 2.2: WorldClim variables. These variables are used in the Climate and Combined models... 53
Table 2.3: Landcover variables. These variables are used in the *Landcover* and *Combined* models... 54
Table 2.4: Summary of all models. Final models discussed in the manuscript were based on the 80km spatial resolution thin with the default number of background points... 55
Table 2.5: 3-way ANOVA results comparing influence of dataset type, background points, and spatial resolution on model performance as defined by AUC values. Dataset type and spatial resolution are significant at a 95% confidence interval. DF num and DF denom are degrees of freedom of the numerator and denominator, respectively. F is the f-ratio, and p is the p-value. A post-hoc Tukey HSD test indicated that 80km spatial resolution had significantly better AUC values than 150km spatial resolution ($p < 0.0001$). Combined dataset models had significantly better AUC values than both climate ($p=0.0016$) and landcover ($p=0.0009$) model types... 56
Table 3.1: Specimens sampled.. 85
Table 3.2: WorldClim variables (www.worldclim.org) used to calculate climatic distance. 92
Table 3.3: Landcover variables used to calculate environmental distance, from the Land Process Distributed Active Archive Center... 93
Table 3.4: Number of individuals scanned per location-based subspecies...................... 94
Table 3.5: Procrustes ANOVA results of museum-assigned subspecies, location-based subspecies, and sex. The asterisks after the p-value indicate the level of significance: $*p \leq 0.05$ and $**p \leq 0.001$. 95

List of Figures

Figure 1.1: Evolutionary relationships among extant Lutrinae, reproduced from the chronogram of the Mustelidae based on Bayesian analysis by Koepfli et al 2008 (Figure 2), with *Lontra provocax* included as sister to *L. felina* based on Vianna et al 2011 (Figure 3). 30
Figure 1.2: Distributions of *Lontra* and *Pteronura*. These distribution maps were produced using shapefiles of each species' distribution provided by the IUCN Red List. More information about how distributions were surveyed is available at the IUCN Red List (www.iucnredlist.org).......... 31
Figure 2.1: Occurrences of *Lontra canadensis*. Grey points are the cleaned unthinned points obtained from BISON, GBIF, and various Fish & Wildlife agencies. Red points are those obtained from an 80km spatial thinning and used in the 80km resolution models. A similar map showing the points used for the 150km resolution models can be found in Supplemental Figure 2. Inset is the distribution of river otters from NatureServe for comparison (NatureServe 2020, https://explorer.natureserve.org/). 57
Figure 2.2: AUC plots. A: AUC values of models in each dataset type by number of background points, across both the 80km and 150km resolutions. B: AUC values of models in each dataset type by spatial resolution. C: AUC values of 80 & 150km spatial resolutions by dataset type.......... 58
Figure 2.3: Comparison of variable contribution and permutation importance across model types. 59
Figure 2.4: Variable response curves for the six most predictive variables (mean contributions over 10%) of the combined dataset, 80km, default background point models. Within a response curve, the X axis is the value of the variable in question, and the Y axis is probability of presence. Each row (1-6) is a different feature class of model. Feature class of each model is listed in Appendix 1. 60
Figure 2.5: The average habitat suitability prediction for the combined dataset model (80km resolution, default background points). Green indicates most suitable habitats, pink to white indicates least suitable. Outline is the IUCN Otter Specialist Group distribution map. 61
Figure 3.1: Specimens sampled by location-based subspecies. 96
Figure 3.2: PCA plot of GPA-aligned coordinates of location-based subspecies 97
Figure 3.3: Mantel test of geographic distance, climate distance, and ecological distance vs morphological distance. Independent variable distance is on the X axis. The Y axis is the Mantel r value. Shaded circles indicate a significant relationship between geographic and morphological distance. Positive values indicate positive associations, and negative values indicate negative associations. 98
Figure 3.4: Multiple Matrix Randomized Regression geographic, climatic, and ecological distances vs morphological distance. 99

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Abstract of the Dissertation

Resilience and sensitivity to changing environments in North American river otters
(*Lontra canadensis*)

by

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Doctor of Philosophy, Quantitative & Systems Biology
University of California, Merced 2020
Dr. Jessica L. Blois, Graduate Advisor
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The North American river otter is a semiaquatic carnivore that occupies freshwater habitats across most of North America, and is a conservation symbol across most of its range. It is used as an indicator species, a keystone species, an umbrella species, and an example of conservation success. The broad distribution of river otters suggests a broad range of habitat tolerances, but they appear to be highly sensitive to anthropogenic habitat alterations. This apparently conflicting robustness across habitat types and sensitivity to habitat change has baffled researchers for decades. Using morphometric, modeling, and synthetic approaches I explored how otters relate to their environment on a broad scale to gain a better understanding of the conditions to which they are robust, the conditions to which they are sensitive, and the mechanisms by which they adapt to varying environments. I determined that river otter distributions are not strongly affected by climate or macro-environmental variables. Further, morphological variation which may affect feeding and locomotion is more strongly related to geography at local than broad scales, indicating local morphological adaptation is not strong in this species. Finally, a review of the phylogeography and ecology of river otters and related species indicated that this species evolved from a low-speciation lineage that tends to produce species that can occupy a wide variety of environments without undergoing evolutionary change. Overall, I determined that river otters do indeed have a broad ecological niche, and do not respond strongly to climatic or environmental differences or changes across their habitats through altering their distributions or locally adapting. Evidence suggests that river otters may respond strongly to anthropogenic alterations of their habitats because anthropogenically-induced habitat alterations tend to have strong consequences for aquatic food chains, and otters may be more reliant on robust food webs than they are on other aspects of their habitats. These findings have implications for how we think of otter conservation and the conservation of species and ecosystems that are strongly affected by otter presence, as well as what otters indicate about their habitat quality. Additionally, these results may shed light on the ecologies of other mustelid carnivores.

In the first chapter of my dissertation I conducted a review of the phylogenetics and biogeography of North and South American river otters to investigate how geography and environmental change have driven river otter evolution in the Americas, and how this informs the ecology of the modern species. I focused first on reviewing the biogeography and evolutionary history of *Lontra* and *Pteronura*, and then on the modern ecology, threats, and conservations status of each of the four modern species of *Lontra* and the single extant species of *Pteronura.* I chose to review these species because they overlap in geographic and environmental space, and because their shared history provides means for an evolutionarily-grounded examination of relative rarity, specialization, and level of conservation concern. I found that speciation between American otters primarily occurs allopatrically, and there is little functional differentiation in response to allopatric speciation, though they can and have adapted in response to extreme conditions when necessary, as seen with *L. felina*. Additionally, allopatric speciation primarily occurs in response to changes in waterway connectivity, which is also responsible for changes in population connectivity within the modern species. Most otters have less specific habitat requirements than previously thought, as all species of *Lontra* persist across a wide variety of climates and semi-aquatic environments, and most of them do so without exhibiting a strong evolutionary response. All American river otters exhibit a strong sensitivity to anthropogenic habitat destruction, though several of them have also shown some ability to coexist with humans. Evidence from this study provides strong indication that this has to do with how human activities near waterways affect food webs, and more generally that on closer examination many habitat components previously thought to be requirements for otters may be better interpreted as indicators of food availability.

In the second chapter of my dissertation I use species distribution modeling to examine river otter associations with climate and environment across their range to explore their apparently conflicting robustness to habitat change and sensitivity to human presence. Specifically, I examined the climate and landcover variables that constrain the geographic distribution of otters. I obtained otter occurrences from GBIF and BISON biodiversity databases, climate variables from BIOCLIM, and environmental layers from NASA's SEDAC program. I built Maximum Entropy species distribution models at 80 and 150 km spatial thinning and varying numbers of background points. The combined model at 80 km spatial thinning and default number of background points produced the highest quality models. Six climatic and landcover variables explained over 10% of otter distributions each: open shrubland, net primary productivity, urban/built, water, annual mean temperature, and precipitation of coldest quarter. Of these variables I determined that water, annual mean temperature, and precipitation of coldest quarter likely have biological significance. However, the predicted range map generated by these models do not match river otter distributions generated by the IUCN and NatureServe. This is likely due to incomplete occurrence data because of low reporting in parts of the species range. I conclude based on these data that river otters have broad climatic and habitat tolerances (as there are six weakly predictive variables as opposed to 1-2 strongly controlling variables) and that local habitat factors, such as intact-ness of riverine food webs, may have greater impact on otter distributions than broad regional variables. Additionally, I

call for improved monitoring and reporting of this and other broadly-distributed species to ensure we can adequately track their habitat requirements and conservation status.

In the third chapter of my dissertation I use geometric morphometrics to explore the role of cranial morphological variation in otter persistence across the array of otter habitats. I address two research questions: 1. Is there morphological variation and structure in river otters across subspecies? 2. Is morphological variation in river otters best explained by a pattern of isolation by distance or isolation by ecology? I obtained 100 river otter crania from across the species range from museums. I 3-D scanned them using a Geomagic 3-D scanner and landmarked them using the IDAV Landmark program. To answer Q1 I conducted discriminant function analyses Procrustes ANOVAs and a Principal Components Analysis. None of these tests revealed strong morphological patterning, indicating there is not morphological differentiation across subspecies in cranial shape. To address Q2 I conducted Mantel tests and a Multiple Matrix Regression with Randomization (MMRR) on the relationship between morphological distance and geographic, climatic, and environmental distance. Both the Mantel test and the MMRR results indicated no significant relationship between morphological distance and climatic or environmental distance in otter crania. Both indicated a weak but negative relationship between morphological distance and geographic distance, indicating morphological variation is greater at short geographic distances and lower at broad geographic distances. I conclude that there is significant cranial variation between individuals, but little definable structure in this variation. The weak but significant (and potentially complex) relationship between geographic and morphological distance indicates the possibility that diversifying selection across smaller spatial scales may be more important than differentiation across broader populations, possibly indicating individual specialization within a generalist population.

Chapter 1: Ecological consequences of habitat change: how the past informs the present in two otter lineages

1.1 Introduction

1.1.1 Background

Many species are under threat of extinction today from a variety of different anthropogenic pressures, including habitat loss and transformation, invasive species, hunting and poaching, and climate change (Pimm et al 2014). While the impacts of many of these pressures (e.g., pollution, widespread habitat loss) are species-specific, region-specific, or emerged only recently, climate change is a global factor influencing species, now and throughout their evolutionary histories (e.g., Blois & Hadly 2009, Urban et al. 2015). Further, climate change does not just impact the ambient atmospheric conditions experienced by species, it affects their habitats and thus also the resources available to support species (e.g., Barnosky et al).

The capacity of species to respond to these climatic pressures has been strongly shaped by their evolutionary histories (Nadeau et al 2017, Brown et al 2016). Because both climates and habitats have become so transformed by human activities over the last several hundred years, studying species' histories and evolution outside of anthropogenically-modified conditions an important way to gain insights into their niches and capacity to respond to environmental change, outside of anthropogenically-altered conditions (Dietl et al 2015, Willis & Birks 2006). Indeed, patterns observed on the landscape today are at times more strongly correlated with past environments than present environments (Svenning & Skov 2007, Sandel et al 2011). Thus, fully understanding a species' past, and the past environments it occupied, is necessary for a complete understanding of the patterns and processes observable in the species in the present (Barnosky et al 2017, Dietl et al 2015, Schoonmaker & Foster 1991).

For example, examining whether a past climatic event caused a species of interest or its relatives to shift its range, evolve, speciate, go extinct, or remain unchanged, provides insights into the potential outcomes when that species is facing a similar climate event in the future (Dietl et al 2015, Lenoir & Svenning 2015). If a lineage shows consistent associations across space and time with a specific type of resource or environment, that resource will likely remain important for future persistence of that lineage (Soberon & Nakamura 2009, Ackerly 2003). On the other hand, some resources may seem necessary to a species' persistence based on contemporary associations, but if those resources or environments are not strongly associated with a species or lineage in the past, they may be less critical to species persistence than they seem (Rheingatz et al 2014, Sepulveda et al 2007, Veloz et al 2012, Blois et al. 2013). If a species is common or widespread, gaining a broader understanding of past environments it has occupied can provide context to determine whether it is common or widespread because it is a generalist species or because the conditions under which it flourishes are prevalent at the current time (Leon-Cortes et al 1999, Gaston 2010, Frimpong 2018). Species' dispersal abilities and the rate of species range shift are also sometimes evident in the fossil record or phylogenetic data, and understanding modes of and limitations on dispersal is particularly critical to predicting whether a species will be able to track suitable habitats at a rate commensurate with the rate of environmental change (Sandel et al 2011, Lenoir & Svenning 2015). Studying the past can also provide information on the relationships between species, potentially offering insight into shared characteristics that may influence persistence or extinction at the genus or family level (Ricklefs 2007, Hadly et al 2009). Since modern species share traits with their ancestors and relatives, we may then be able to extrapolate likely future responses to similar events (Peterson 2011, Wake et al 2009, Desantis 2012). Overall, understanding the mechanisms of evolution at play in a species or a lineage, and the range of environmental conditions a species has persisted through in the past, can enable us to better understand and predict the adaptive capacities and potential restrictions for species of great interest to conservation biologists as well as provide insight into the evolutionary pressures and potential responses to future changes of other species that are similar, rely on similar resources, or are affected by the species of interest.

Here we conduct a synthetic review of the phylogenetics and biogeography of New World otters in order to inform their future conservation. We bring together previously published fossil, genetic, biogeographic, and paleobiological information to investigate how geography and environmental changes have driven otter evolution in the Americas, and to explore the potential for these factors to impact modern species into the future. We trace their lineages from entry into North America, through several waves of environmental change and speciation, into the conditions in which the current species formed. We highlight paleoecological and biogeographical processes associated with speciation within the genus *Lontra*, compare these processes to those that have most strongly affected the distantly related giant otter (genus *Pteronura*), and ultimately tie these processes to the ecology of the extant species.

1.1.2 River otters as a representative case study

River otters provide an interesting case study that illustrates the potential importance of the past for making informed conservation decisions today. Only one of the thirteen species of otters is listed as least concern and has a stable population trend according to the IUCN Otter Specialist Group (IUCN 2015). All others are declining due to human-induced habitat fragmentation and alteration, and range from near threatened to endangered; the New World otters encompass all of these conservation trends. Among the New World otters, some species overlap in habitat and environmental conditions. The four species of *Lontra* provide an example of how habitat changes relate to trait and niche breadth changes between closely related species. Inclusion of the giant otter (genus *Pteronura),* whose range overlaps with that of one *Lontra* species, provides a vehicle for comparison of how related but distinct species adapt to similar environmental conditions. Their shared history, and potentially shared drivers of contemporary population declines, provide a means for an evolutionarily-grounded examination of relative rarity, specialization, and level of conservation concern between species. And as we will show, the evolutionary history of the five extant species of New World river otters is strongly affected by habitat change in a variety of ways - it is a story of new habitats becoming available and those habitats changing into the environments present on the contemporary landscape, of organisms developing unique adaptations to a variety of conditions, and of anthropogenic habitat alteration threatening even broad-ranging species.

1.2 Biogeographic history of two major semi-aquatic New World otter clades

1.2.1 Background

Otters belong to the subfamily *Lutrinae*, in the family Mustelidae, the weasel family. Mustelidae is one of the oldest and most diverse lineages of carnivorans, including species that range from fully aquatic, to semi-arboreal and fossorial. It includes the smallest carnivore (the least weasel), two of the most ferocious (wolverine and honey badger), and two species of marine mammal, one of which we will discuss in this review. Otters currently occupy all continents except Australia and Antarctica.

The subfamily *Lutrinae* originated in Eurasia in the late Miocene, approximately 9mya (Koepfli et al. 2008) and radiated into seven genera, which then radiated into numerous aquatic and semi-aquatic species across the globe. According to current understanding of otter evolution there are three major branches of the otter family tree (Fig. 1), two of which (*Lontra* & *Pteronura*) are endemic to the Americas. There were multiple dispersals of members of *Lutrinae* from Asia into North America, through Beringia. The first event occurred in the late Miocene (~8mya) before the first opening of the Bering Strait, during which members of the genus *Mionictis* crossed the Bering land bridge. This genus persisted in Eurasia and North America for some time, before disappearing in Eurasia around 7 mya (Koepfli & Wayne 1998, Willemsen 1992) and dropping out of the fossil record in North America 3-5mya; there are no living descendants of this lineage. Based on mitochondrial DNA evidence, Koepfli & Wayne (2008) believe the next major dispersal event occurred during the late Miocene about 5.4-5.5 mya, at which time they suggest that both the genus *Satherium*, probable ancestor to the giant otter, and *Lutra licenti*, possible ancestor to the genus *Lontra*, immigrated to North America from Asia. Both genera are well-documented in Asia and North America during the Miocene (Koepfli et al 2008). Fossil evidence supports an entry time into North America of around 5.2-6 mya for *Satherium* and ancestors of *Lontra* (Willemsen 1992, Prassack 2016).

Currently, there are six extant species of otters in the Americas, five of which are endemic to the Americas. The giant otter (*Pteronura brasiliensis*), native to South America, belongs to the oldest extant lineage of otters and is the only remaining member of its genus. The genus *Lontra* includes one member in North America and three members in Central and South America (Fig. 2). The the North American river otter (*Lontra canadensis*) is sister to all other species in this genus, and is the only freshwater otter in North America. *Lontra canadensis* also inhabits a much broader range of environments than other members of this genus. The (the Neotropical otter, *Lontra longicaudis*) occupies a wide range in Central and northern South America, and the two most closely realted species (the southern river otter, *Lontra provocax*, and the marine otter, *Lontra felina*) are both the furthest south and the most restricted in their habitats. This study does not include the sea otter (*Enhydra lutris*). Sea otters are evolutionarily, ecologically, and morphologically distinct from other extant otters, and the genus is not endemic to the Americas.

1.2.2 *Pteronura*

The earliest divergence within *Lutrinae* was the split between the lineage leading to *Pteronura* and the rest of the otters, supported by both phylogenetic divergence time estimates and fossil evidence (Koepfli et al 2008, Willemsen 1992). However, the origins of *Satherium*, the most likely ancestor of *Pteronura*, remain unclear. According to phylogenetic divergence time estimates, the predecessor of *Satherium* likely originated in the late Miocene (estimated 6-9mya) in Eurasia, shortly after the origination of *Lutrinae* around 9mya (Koepfli et al. 2008, Koepfli & Wayne 1998). Morphological evidence (Willemsen 1992, Van Zyll de Jong 1972) supports a relationship to *Lutrogale*, an early-diverging Asian lineage of otters, and based on this evidence Willemsen suggested a common ancestor of these two lineages in Asia. While there is currently no fossil evidence to support this hypothesis, it is the most parsimonious explanation of events. The earliest known otter fossils in North America, which were ambiguous but resemble *Satherium,* date to ~5.3mya (Koepfli et al 2008, Bjork 1970); thus, the *Satherium*/*Pteronura* lineage likely crossed Beringia into North America shortly after speciating, before the first opening of the Bering Strait roughly 5.4-5.5 mya (Gladenkov et al 2002, Koepfli et al 2008, Webb 2006). While this is the most parsimonious explanation of events, there is currently little fossil evidence to support the molecular-based estimates of event timing and thus considerable uncertainty. Koepfli et al (2008) note, however, that most of the species of mustelids occupying North America appear to have crossed Beringia from Asia around this time. The late Miocene and early Pliocene were very warm compared to today, particularly at northern latitudes, and this likely made Beringia quite hospitable to warmer-adapted organisms, as *Satherium* appears to have been (Webb 2006, Krylov et al 2008, Salzmann et al 2008).

Satherium is well-documented in Pliocene faunal remains across the United States from ~3.7-1.6 mya, with several sites in eastern Washington and Idaho at the far northwest, and sites in Florida at the far southeast, of their distribution cites. The best-known remains of this otter are from several deposits at Hagerman Fossil Beds in Idaho (Bjork 1970, Lindsay et al 1984).

There were at least two species of *Satherium*, *Satherium piscinarium* (Leidy, 1873) and *Satherium ingens* (Gazin, 1934). Little is known about *Satherium ingens*. The only specimens come from an upper Pleistocene site, the Snake River basin in Idaho. It appears to have been larger and more robust than *Satherium piscinarium* or *Pteronura*, and had some morphological differences in the dentition (Prassack 2016, Gazin 1934). *Satherium piscinarium* was a large otter, with a long, broad, flattened skull, a large, powerful tail, a robust mandible, and large teeth. It had a long, muscular tail, a short, robust femur, and a long tibia, indications that it was likely a powerful swimmer (Ruez 2009, Leidy 1873, Bjork 1973). It resembles *Pteronura* and some fossil otters from Eurasia. The breadth of its teeth and overall robustness of the mandible have led several to conclude that the diet of *Satherium piscinarium* likely included a large proportion of shellfish (Ruez 2009, Bjork 1973).

Environmental reconstructions at some fossil sites, such as the Glenns Ferry Formation at Hagerman Fossil Beds National Monument, suggest *Satherium* may have occupied relatively warm and open wetland habitats (Ruez 2006, Ruez 2009, Ruez 2016, Forester 1991, Emslie 2007, Morgan & Emslie 2010). Such sites likely would have been much more common in the Pliocene than the Miocene, as climate cooled and grasslands and open deciduous forests dominated much of what is now the United States (Ruez 2006, Stein et al 2016, Morgan & Emslie 2010).

While Pliocene North America was warmer and wetter than it is today, it was cooler and more arid than the Miocene, resulting in the expansion of grasslands and open forest with interspersed wetlands (Stein et al 2016, Morgan & Emslie 2010, Salzmann et al 2008). If *Satherium* preferred warm wetland habitats, this may be why it does not appear to have established much further north than Idaho and Washington (Salzmann et al 2008, Pound et al 2015, Koepfli et al 2008). Habitats may have been favorable for crossing the Bering Land Bridge in the late Miocene, but grown cold enough at more northerly latitudes to become unfavorable by the time *Satherium* was well established in North America in the Pliocene. Close relatives of many species that are now endemic to the tropics existed at Hagerman Fossil Beds in Idaho, the most prolific *Satherium* site, during the Pliocene (Prassack 2016, Bjork 1970), so Pliocene environments of Idaho may have been similar to the modern tropics. The last known fossils of *Satherium*, or anything resembling it, in North America are from around 1.6 mya (Morgan 2005). Climate continued to become cooler and more arid through the Pliocene into the Pleistocene, and with the cooling came expansion of glaciers (Clark & Bartlein 1995). These factors likely contributed to the extirpation of *Satherium* in North America (Gill et al 2009, Prevosti & Ferrero 2008). It seems plausible that as climate became less favorable in North America, *Satherium/Pteronura* shifted its range south, to the still warm and moist tropics.

Satherium piscinarium has been suggested as an ancestor of the Giant Otter *Pteronura* based on cranial, dental, and long bone features (Bjork 1970, Robertson 1976, Garcia et al 2007). No rigorous and detailed comparisons of *Satherium piscinarium* and *Pteronura brasiliensis* have been conducted as of yet (Prevosti & Ferrero 2008), though Prevosti & Ferrero (2008) performed an initial, inconclusive analysis. While this area requires further research. we will follow other authors and nearly all available evidence (Bjork 1970, Robertson 1976, Garcia et al 2007) and assume that *Satherium piscinarium* is ancestral to *Pteronura brasiliensis*.

Pickles et al (2011) suggest based on phylogenetic evidence that the ancestor of *Pteronura brasiliensis* may have crossed to South America via waif dispersal across the seaway (Flynn et al 2005) before the Great American Biotic Interchange (GABI) around 3.5 mya. Several other species, such as raccoons, are thought to have populated South America in this fashion (Fulton & Strobeck 2007, Coates et al 2004). It is possible that *Satherium piscinarium*, or an unknown closely related species, crossed into South America before or during GABI and began to speciate into *Pteronura* while maintaining a concurrent presence in North America, before climate change eventually led to its extinction in North America. However, there is very little fossil evidence of the assumed transition from *Satherium* to *Pteronura* or of early *Pteronura*. The most recent known fossils of *Satherium piscinarium* are from De Soto Shell Pit, Florida around 1.6 mya (Morgan 2005) and the first fossils of *Pteronura brasiliensis* in South America are from 130-120 kya (Prevosti & Ferrero 2008, Pickles et al 2011, Cartelle & Hirooka 2005), representing a million-plus year gap in the fossil record. Divergence time estimates indicate that the oldest phylogroup of giant otters in South America split from the other phylogroups 1.24-1.7 mya (Pickles et al 2011), meaning *Pteronura brasiliensis* may have diverged as a separate species from *Satherium piscinarium* and fully settled South America before *Satherium piscinarium* went extinct in North America. Overall, it is unclear when *Satherium/Pteronura* crossed the Isthmus of Panama into South America, what form it took when it did, or when it went extinct in North America.

Pteronura brasiliensis differs from *Satherium* in a few key ways (Prevosti & Ferrero 2008, Garcia et al 2007): its jaws and teeth are better adapted to eating fish than shellfish, and it is slightly smaller (though still much larger than other otters). This suggests that many of the morphological changes from *Satherium* to *Pteronura* may have been adaptations to a fish-eating diet in a more generally forested habitat where prioritization of speed and ease of movement may have selected for smaller body sizes. *Pteronura* may also experience less pressure to be large to discourage predation, as there are fewer extremely large carnivores co-occurring in the habitat of *Pteronura* then there were in that of *Satherium* (find better citations). Giant otters prefer slow-moving high-productivity creeks over fast-flowing rivers (Duplaix, 1980; Zambrana Rojas, 2007), and this may reflect a retained preference from *Satherium*, which seems to have had a similar preference for wetlands.

1.2.3 *Lontra*

The earliest diversification within *Lutrinae* led to the divergence of the *Satherium/Pteronura* lineage from all other lineages within *Lutrinae*. The phylogeny of Mustelidae constructed by Koepfli et al (2008) (Fig. 1) suggests this larger group of otters subsequently experienced a burst of diversification, leading to an early split (also in the late Miocene, around 6.5 mya) between the lineage leading to *Lontra* and the other otters in that group in Eurasia. However, *Lontra* itself did not diversify until the Pliocene and little is known about the events leading to the *Lontra* diversification event; as of yet, there are no clear Eurasian ancestors for *Lontra*.

Predecessors of the genus *Lontra* most likely entered North America through Beringia 4-7 mya (Gladenkov et al 2002, Koepfli et al 2008, Prassack 2016). Until the recent discovery of *Lontra weiri* (Prassack 2016) there was no clear New World ancestor either, and *Lontra* was thought to be descended from either *Mionicits* or *Lutra licenti*, two North American fossil otters that share some morphological features with modern *Lontra* otters (Van Zyll De Jong 1972, Koepfli et al 2008, Marmi et al 2004) but have since gone extinct.

A new fossil otter from the Hagerman Fossil Beds, *Lontra weiri* (described in 2016 by K. Prassack), is the oldest known member of the genus *Lontra*, the only known extinct member of the genus, and either the direct ancestor or a close relative of modern *Lontra* species. The single known specimen of *Lontra weiri* consists of a right lower jaw and a left humerus that date to ~3.8 mya (Prassack 2016, Hart and Brueseke 1999). Given this date, and divergence times between *Lontra* species (Koepfli et al 2008), it is likely that *Lontra weiri* or its ancestor crossed the Bering Land Bridge before the first opening of the Bering Strait around 5.5 mya in a similar time frame as *Satherium* and many other mustelid genera did (Kopefli et al 2008, Tedford et al 2004).

Lontra weiri was a small-bodied otter, roughly the size of *Lontra felina*, and had many dental and humeral features in common with modern *Lontra* species. Dental features suggest that *Lontra weiri* primarily ate small fish. Interestingly, it was concurrent at Hagerman Fossil Beds with *Satherium*, and Prassack (2016) suggests that *Satherium piscinarium* and *Lontra weiri* may have had a similar niche separation to what *Pteronura brasiliensis* and *Lontra longicaudis* have today (Silva et al 2014, Peterson 2011).

Definitive fossils of early *Lontra canadensis*, the oldest extant member of the genus, appear in the fossil record from 1.8 to 0.3 mya from across North America (Anderson 1984, Bentley et al 1994, Corner 1977, Kurten and Anderson 1980, McDonald et al 1996). At this time, there were several other proposed species of *Lontra* occupying much of North America, though it is unclear whether these morphologically similar organisms are different species or subspecies and it is also unclear when these fossils should be referred to as members of the modern species. As of yet no morphological comparison has been conducted between *Lontra weiri* and these specimens. Early *L. canadensis* appears to have occupied a variety of habitats across much of North America (Anderson 1984, Bentley et al 1994, Corner 1977, Kurten and Anderson 1980, McDonald et al 1996), much like modern *L. canadensis*.

In South America, the genus *Lontra* is known from the early Pleistocene (Marshall 1985). Given the poor fossilization across most of South America for this time period, this corresponds well to an entry time during or before the GABI. Genetic evidence also corroborates this story, suggesting a split of the rest of the genus from *Lontra canadensis* 2.8-3.4 mya (Koepfli & Wayne, 2008), almost certainly due to decreased gene flow during migration into South America. Genetic evidence also suggests rapid speciation as *Lontra* moved south, as *L. longicaudis* diverged from the other two species of the genus approximately 1.5 mya and *L. provocax* from *L. felina* around 883kya (Vianna et al 2011). It is common in South America for more basal species within lineages to be distributed further north, and more recent species to occur further south (Marshall et al 1982), and to an extent, this pattern holds true with the colonization of South America by members of *Lontra.*

The Pleistocene in the Americas was characterized by glacial/interglacial driven climate change and landscape transformation (Baker & Fritz 2015, Clark & Bartlein 1995). Glaciation in North America was far more extensive than it was in South America, but little is known about its effect on otter populations, as fossil occurrences are from limited sites and no in-depth molecular studies have been conducted on *Lontra canadensis* on a scale to provide insights into Pleistocene population structures. There is molecular evidence that in South America, glaciation cycles in the Andes contributed to population structuring, and perhaps in one case speciation, by acting as dispersal barriers between established populations (Vianna et al 2010, 2011, Hammen & Hooghiemstra 2000). Additionally, climatic and topographical effects associated with these cycles appear to have had profound effects on river hydrology in several parts of South America (Hewitt 1996, Correa-Metrio et al 2011, Pickles et al 2011). As most otters depend on river connectivity for dispersal, signs of these shifts in waterways are still evident in population structures of modern otter species (Pickles et al 2011, Vianna et al 2010, 2011).

1.3 Evolution & ecology of modern species

Currently, there are four extant members of *Lontra* and one of *Pteronura* distributed across the Americas (Fig. 2). *Lontra canadensis* occupies most of North America. *Lontra longicaudis*, *Lontra provocax,* and *Lontra felina* occupy different habitats in South America, and the only extant member of *Pteronura, Pteronura brasiliensis,* persists across much of north-central South America. Changes in climate and environment since the Pleistocene have influenced evolutionary processes and the modern ecology of each of these species in a variety of ways. In this section we examine the effects of both climate and environment on the evolution of each modern species, and how climate, environment, and anthropogenic impacts continue to affect them today.

1.3.1 *Pteronura brasiliensis*

Pteronura brasiliensis is a large otter distributed in north-central South America (Carter & Rosas 1997, Eisenberg 1989). The species is comprised of four distinct phylogroups that are highly structured geographically (Pickles et al. 2011). While no single historical process—paleogeography, hydrogeology, and glacial/climatic refugia—seems to completely explain divergence among the phylogroups (Pickles et al 2011, Ruiz-Garcia et al 2018), what seems most salient about each process was how it affected the waterways. *Pteronura brasiliensis* is highly affected by environmental changes that result in an alteration of water flow. Geologic processes and climatic changes that resulted in alterations of water flow or reduction in connectivity between rivers have had the greatest effect on the population structure and genetic diversity of *Pteronura brasiliensis* (Pickles et al 2011, deThoisy et al 2011, Ruiz-Garcia et al 2018). The divergence time between the two main clades, and their internal phylogroups, correspond well to the cycles of cooling and drying events that occurred with the glacial/interglacial cycles during the Pleistocene. Population re-dispersals may have occurred due to the expansion of forests in Western Amazonia during the Holocene, as some evidence suggests giant otters have a habitat preference for areas with at least moderate tree cover (Carter & Rosas 1997, Duplaix et al 2015).

Today, *Pteronura brasiliensis* are the largest extant otters, ranging in length from 1.5-2 m long, and in weight from 22-34 kg. They eat primarily fish, but also frequently consume crustaceans, snakes, small mammals, and other riverine organisms. Historically these otters inhabited many waterways in forested or wetland (including prairie wetlands in southern central) South America but their current distribution is confined to the rainforest waters of north-central South America (Carter & Rosas 1997, Eisenberg 1989). *Pteronura brasiliensis* prefer high-productivity, calm rivers and lakes with shallow banks and tree cover in tropical areas (deThoisy et al 2011, Duplaix 1980). Since *P. brasiliensis* hunt by sight (Carter & Rosas 1997, Duplaix 1980), they also tend to prefer rivers with relatively low quantities of suspended particulates (deThoisy et al 2011). They are among the most aquatically adapted of all lutrines and are not thought to travel large distances overland.

This species is currently listed as endangered and decreasing (IUCN Otter Specialist Group, Carter & Rosas 1997, deThoisy et al 2011). Populations were initially destabilized due to the fur trade in the 1800's and 1900's. In modern times, habitat destruction for farming & development, heavy metal pollution & water disturbance from mining, and illegal hunting all pose serious threats to *P. brasiliensis* (Carter & Rosas 1997). Like other otters, this species appears to be extremely sensitive to environmental changes that might result in a change in abundance to its food sources.

1.3.2 *Lontra canadensis*

There is some debate about when *L. canadensis* (Schreber 1777) *sensu stricto* appeared in the fossil record. What is clear, however, is that there have been organisms functionally and morphologically nearly identical to North American river otters for at least 1.8 my (Kurten & Anderson 1980). North American river otters *sensu lato* have been widespread in North America for much of this time. There are some fossils of several similar 'species' of this otter known from the early Pleistocene (van Zyll de Jong 1972), and fossils in the mid to late Pleistocene (300-11kya) are well known from localities spanning much of their current distribution (Lariviere and Walton 1998, Avina 1969; Alvarez 1969).

Contemporary *L. canadensis* is comprised of seven morphologically- and behaviorally-described subspecies distributed across virtually all of North America (Lariviere 1998), but a lack of phylogenetic synthesis hinders full understanding of this species' evolution and ecology. Several small-scale genetic diversity and habitat connectivity studies have been conducted on modern *L. canadensis* that identify isolation by distance (IBD) as the primary driver of genetic differentiation between populations (Brandt et al 2013, Latch et al 2008, Ben-David & Golden 2009, Blundell et al 2002). Seymour et al (2012) suggest that isolation between populations of *L. canadensis* that has caused subspeciation may be due in part to Pleistocene glaciation, though further research on populations outside of Alaska is needed.

L. canadensis are medium sized otters (5-15 kg) that occupy a diversity of habitats, from forests to grasslands, ponds and streams, to the ocean. They are strong swimmers, but they generally prefer calmer lakes and rivers as this is where prey species are more abundant and easier to catch (Lariviere 1998, Scognamillo 2005). Their primary requirements are access to a permanent source of clean, fresh water, and an abundant supply of food organisms, which may be anything from small terrestrial vertebrates to clams, though the bulk of most of this species' diet is fish. *L. canadensis* are extremely sensitive to pollution (Bowyer et al 2003, Ben-David et al 2002, Taylor et al 2001) and other human disturbances of their habitats (DePue 2007, Guertin et al 2012), despite being otherwise capable of persisting in a variety of semi-aquatic habitats (Ben-David et al 2002, Mowry et al 2015, Raesly 2001). They are highly mobile, and are capable of dispersing 60-90 km (Blundell et al 2002) if not impeded by anthropogenic disturbances (DePue 2007, Guertin et al 2012).

L. canadensis is the only species of otter currently listed as least concern with a stable population trend (IUCN Red List, 2015). Historically, the fur trade led to extirpation in many parts of their range (Scognamillo 2005), though reintroduction efforts across the United States and Canada in the 1970's through 1990's (Raesly 2001, Mowry et al 2015) were very successful at helping them re-establish stable population sizes across much, though not all, of their historical range. Current threats to *L. canadensis* include habitat degradation and loss of connectivity, pollution, and competition with humans for resources. Overall, however, the relative paucity of fossils despite its broad distribution, the apparent ecological similarity of early *L. canadensis* to modern *L. canadensis*, and the relative conservation stability and broad range of the species seem to have discouraged broader paleontological reviews or phylogeographic studies of this species.

1.3.3 *Lontra longicaudis*

According to phylogenetic estimates, the neotropical otter *Lontra longicaudis* diverged from *L. canadensis* 2.8-3.4 mya (Koepfli et al 2008). There are fossils of *L. longicaudis* in Argentina and Uruguay from ~1.8-0.8 mya (Prevosti & Ferrero 2008, Rusconi 1932, Berta & Marshall 1978, Soibelzon et al 2005, Berman 1994), and from several sites in Mexico dated to around 300-10.5 kya (Arroyo-Cabrales, 2013). Evolutionary data suggest speciation was parapatric and due to a decrease in gene flow while colonizing the South American continent (Trinca et al 2012). There is now a wide arid band across north-central Mexico (Sonoran and Chihuahuan deserts) that restricts contact between these two species (Gallo 1991, Zink et al 2000). *Lontra longicaudis* appears functionally similar to *L. canadensis*, and it is possible little functional differentiation has taken place throughout the evolution of these two groups; the primary functional difference between *L. longicaudis* and *L. canadensis* appears to be their thermal preferences (Rheingantz et al 2014).

There are at least 4 geographically structured phylogroups in *L. longicaudis*: Columbia, Bolivia, Amazonia and French Guyana, and eastern South America (Trinca et al 2012). Genetic evidence suggests the Columbian phylogroup diverged from the other members of *L. longicaudis* around 575 kya, which is interesting because it indicates a similarly deep split between these two phylogroups of *L. longicaudis* as exists between two other species within the genus, *L. provocax* and *L. felina* (Trinca et al 2012). Trinca et al (2012) suggest that the genetic structure within *L. longicaudis* exists because geographic dispersal barriers (mountains and lack of water connectivity) prevent gene flow, and suggest further analyses to determine whether evolutionarily significant differentiation has taken place between populations. They draw parallels between the phylogeographic structure of *L. longicaudis* and the structure Garcia et al (2007) found between populations of *Pteronura brasiliensis*, and it is likely this strong but idiosyncratic geographic structuring is due, as it is in *P. brasiliensis*, to the courses of waterways changing over time with cycles of cooling and drying events altering the localities otters can access and strength of connections between populations (Trinca et al 2012, Garcia et al 2007, Ruiz-Garcia et al 2018).

L. longicaudis are similar in size, appearance, behavior, and ecology to *L. canadensis* (5-15 kg). This otter occupies a variety of semiaquatic habitats from central Mexico down to northern Argentina (Lariviere 1999, Chehebar 1990), but tends to prefer faster-flowing streams with riparian tree cover (Lariviere 1999). They tend to eat slow-moving fish, but are carnivorous opportunists and will also eat mollusks, crustaceans, birds, or small mammals when convenient (Silva et al 2014, Juarez-Sanchez et al 2019). *L. longicaudis* are currently listed as near threatened and decreasing (IUCN Red List, 2015). Like other species of otter, they are very sensitive to habitat disturbances (Foster-Turley et al 1990), and habitat loss and fragmentation are one of the biggest threats currently facing this species. Other threats to *L. longicaudis* include illegal hunting (though most of this stopped in the 1960's), pollution, and competition with humans for resources (Macdonald & Mason 1990, Lariviere 1999).

1.3.4 *Lontra provocax*

Lontra provocax, or the southern river otter, diverged from *L. longicaudis* ~1.57 mya (Vianna et al 2010) and occurs today in southern Chile and Argentina. It appears to have speciated allopatrically due to isolation from *L. longicaudis* by the Andes mountains and the Atacama Desert (Vianna et al 2010), but there is currently very little information on the specifics of the divergence between *L. longicaudis* and *L. provocax*, partly because there is no fossil evidence of *L. provocax*. It is likely, though, that fluctuations in water connectivity and habitat coverage by ice during the Pleistocene glacial/interglacial cycles (Rabassa & Coronato 2009) separated these populations and eventually trapped *L.*

provocax between the Andes and the Pacific Ocean (Vianna et al 2011). Its highly restricted range as compared to *L. longicaudis* is almost certainly due in part to the topography of this area making dispersal extremely difficult (Vianna et al 2011).

Genetic evidence (Vianna et al 2011, Centron et al 2008) suggests that during the last glacial maximum when much of their habitat was covered by ice sheets, populations of *L.* provocax persisted in the fjords and marine channels of southern Chile as well as to the north of the ice sheets that covered the southern Andes. Glacial cycles generally cause species ranges to contract and interglacials tend to cause them to expand (Hewitt 2000, 2004), and the patterns of differentiation Vianna et al (2011) identified are consistent with this. Conditions similar to these during a prior series of glaciations are likely what led to the divergence of the marine otter (*L. felina*) and its adaptations to marine life (Vianna et al 2010, 2011). Availability of appropriate water sources, and the effects of topography on overland travel and waterway connectivity appear to have strongly affected this otter's distribution throughout its history (Vianna et al 2011).

L. provocax are small otters (5-10 kg) that occupy fresh and saltwater habitats. They have the smallest geographical range of all otters (Kruuk 2006), as opposed to *L. canadensis* and *L. longicaudis*, which have some of the largest geographic ranges. Despite their extremely narrow geographic distribution, *L. provocax* have a broad climatic range (Medina-Vogel et al 2008, Sepulveda et al 2007). In the northern end of their distribution, they occupy a variety of freshwater habitats, from wetlands to rivers and streams. In the southern end of their range, they occupy marine and freshwater habitats and their distribution is concurrent with sister species *L. felina* (Lariviere 1999b). Coastal *L. provocax* occupy fjords and marine channels with protection from strong waves and currents, and reliable access to fresh water, and thus their populations remain distinct from *L. felina* that occupy the outer coastline (Chehebar, 1990; Sielfeld, 1990, Ebensperger and Botto-Mahan, 1997). *L. provocax* primarily eat crustaceans and shellfish, as these are the most abundant prey in their habitat, but like most otters they are opportunistic hunters and will consume whatever they can catch (Vianna et al 2011, Van Zyll de Jong 1972).

L. provocax are listed as endangered and decreasing (IUCN Red List, 2015). They are very sensitive to anthropogenic alterations of their habitats, particularly those that interfere with productivity and connectivity of waterways (Vianna et al 2011, Aued et al 2003). Several studies have suggested that their site preferences may be more strongly influenced by presence of forests and ability to get cover than by low-level human impacts (Lariviere 1999b, Chehebar et al 1986) though other studies have concluded that *L. provocax* can persist in a variety of environmental conditions provided it has a reliable food source (Medina-Vogel et al 2003, Aued et al 2003, Sepulveda et al 2007).

1.3.5 *Lontra felina*

L. felina (Molina, 1782) diverged from *L. provocax* ~883 kya (Vianna et al 2010) and are found today along the coastline of Peru, Chile, and Argentina (Apaza et al 2003, Olrog &

Lucero 1981). There is no fossil evidence of *L. felina*, so all evidence of its history is phylogeographic and historical. Vianna et al (2010) hypothesize that *L. felina* diverged from *L. provocax* parapatrically in the marine channels of Patagonia to take advantage of abundant marine resources, and so it could depend less on fresh water (Kruuk 2006) as rainfall and terrestrial sources of fresh water are both rare where *L. felina* occurs. *L. felina* and *L. provocax* co-occur in southern Chile but utilize different habitats (Sielfield 1990). There is a strong ecological difference between the sheltered fjords and channels, and the coastline in this area which makes sympatric speciation seem quite probable (Vianna et al 2010, Ebensberger & Botto-Mahan 1997). In cases like this, it is expected that "divergence in ecological traits should be greatest between… sister species occupying the same area" (Koepfli et al 2008 p. 15), and in this case differential habitat use keeps these two species from overlapping in resource use or interbreeding, though they are physically and genetically close enough that they could theoretically hybridize in the wild in parts of their ranges (Vianna et al 2010, Valqui 2012, Trigo et al 2008). It is thought that the mid-Pleistocene glacial-interglacial cycles caused habitat shrinkage (as former habitats were covered by ice) and increasing aridity in the middle latitudes of South America, forcing coastal populations of *L. provocax* to occupy every possible habitat physically available to them, including the rocky intertidal zone (Vianna et al 2010). These populations in the rocky intertidal had little to no access to fresh water, and speciated parapatrically into *L. felina*. Vianna et al (2011) found evidence of genetic divergence between coastal and inland populations of *L. provocax* due to separation by glacial events that support this hypothesis of divergence of *L. felina* from *L. provocax* as a consequence of glacial activity. More work on contact and differential resource use between these two otters where they currently co-occur might provide more insights. It is currently unclear whether or how glaciation has impacted *L. felina* since it diverged from *L. provocax*. Genetic data (Vianna et al 2010) suggests that the southernmost population of *L. felina* has been isolated from other populations for 35 ky, but it is unclear whether it colonized its current locations before or after the last glacial maximum 23-17 kya.

L. felina occupy rocky seashores in the intertidal zone (Apaza et al 2003, Olrog & Lucero 1981). They are dependent on rocky coastlines for dens and for protection, and thus long sandy beaches and habitat fragmentation caused by humans are major barriers to dispersal (Vianna et al 2010, Thiel et al 2007, Medina-Vogel et al 2008). There is strong genetic differentiation among populations separated by long sandy beaches, and the deepest differentiation between haplogroups in this species is a split that occurred ~490.5 kya and is geographically concordant with the longest stretch of sandy beach without rocky outcroppings in their range (Vianna et al 2010, Sielfield 1983). There is low connectivity between relatively recently diverging populations, indicating high susceptibility to local extirpations (Lariviere 1998).

L. felina is the most recent mammal to adapt to marine conditions, and also the smallest (3.2-5.8 kg) (Estes 1989, Medina-Vogel et al 2007). All otters except *L. felina* and *Enhydra lutris* require reliable access to clean fresh water to drink, and to clean their fur so it remains insulative (Kruuk 2006, Lariviere 1998). *Enhydra lutris* developed the densest fur of any mammal and elaborate grooming behaviors to stay warm, and have adapted to marine environments so completely that they rarely come to shore (Williams et al 1992). In contrast, *L. felina* keep themselves warm by spending 80% of their time on land, the most of any otter (Medina-Vogel et al 2007, 2008).

Since *L. felina* are dependent on abundant marine resources, the northern edge of their distribution is marked by the end of the Humboldt Current System, where water becomes warmer and less productive (Vianna et al 2010, Gutierrez et al 2016). They seem to be less affected by terrestrial climate, as their distribution ranges from tropical to sub-arctic habitats (Vianna et al 2010). Vianna et al (2010) point out that there is an interesting contrast between *L. felina*'s ability to colonize a variety of coastal habitats and its limited adaptation to cold water.

L. felina are endangered and their populations are decreasing (IUCN red list). They were hunted for the fur trade until around the 1960's. Now they are in constant competition with humans for use of marine resources, and are often killed in crab pots. They are also still actively hunted in parts of their range, and face similar threats of habitat destruction and fragmentation, which they may be even more sensitive to than most otters because of their very low dispersal capabilities (Chehebar 1990, Medina-Vogel et al 2006, 2008).

1.4 Synthesis & Future Directions

In this review we examined the phylogenetics and biogeography of New World otters in conjunction with their modern ecologies to gain insight into the mechanisms of evolution and adaptive capacities of these five species. We found that New World otters are capable of persisting across a variety of conditions without demonstrating a strong evolutionary response (i.e., allopatric speciation without strong functional differentiation), but also demonstrate the ability to adapt in response to extreme conditions when necessary. We demonstrated that integrating evolutionary with contemporary data provides insights that contemporary data alone cannot, simply because the range of environments and climates experienced throughout a species' evolutionary history far exceeds those observable on historical timescales. Thus, understanding the mechanisms of evolution at play in a species or a lineage by incorporating recent historical, fossil, phylogenetic, and paleoenvironmental data enables us to better understand and predict species potential responses to future changes.

This perspective is illustrated well through the river otters case study. For example, *P. brasiliensis* is thought to have very specific ecological requirements that include intact rainforest habitats with dense tree cover and small, slow-moving waterways (Carter & Rosas 1997, Eisenberg 1989). These assertions, however, appear to be based on conclusions reached studying the modern, anthropogenically altered, distributions of the species only. Examination of the historical distribution of this species suggests that it is robust to a variety of habitat conditions, but likely requires relatively warm water with extremely abundant sources of slow-moving prey (Pickles et al 2011, Leuchtenberger et al 2020). It is currently unclear whether the requirement for tree cover is an ecological requirement of *P. brasiliensis* or simply an indicator of a healthy and productive riverine ecosystem, as healthy riparian zones even in prairie areas tend to have moderate tree cover (Tucker & Leninger 1990, Macfarlane et al 2017, Holland et al 2009).

Comparison of the habitat requirements of *Pteronura* to what we know about its probable ancestor, it seems plausible that there is a degree of habitat or climatic niche conservatism between *P. brasiliensis* and *Satherium* (Peterson 2011, Peterson et al 1999). Both species seem to occupy or have occupied warm, high-productivity wetland habitats and prefer broad/slow-moving waterways. Both species seem also to have relatively good dispersal ability across connected waterways. However, interpreting niche conservatism in this lineage is limited because there are only two known species of *Satherium*, and one of *Pteronura.* Whether this is because of taphonomic issues (i.e., more species were present but did not fossilize well or their fossils have not yet been discovered or described), or if this is a relatively low-speciation lineage (Upham et al 2020), is unknown.

An integrated evolutionary/contemporary approach similarly shows that members of *Lontra* demonstrate an ability to persist across a variety of conditions without strong evolutionary response (*L. canadensis, L. longicaudis*), as well as adapt in response to extreme conditions through natural selection and subsequent speciation (*L. felina*). It is generally thought that *L. canadensis* and *L. longicaudis* are relatively generalist species, whereas *L. provocax* and *L. felina* are much more specialized, and have more specific habitat requirements. For *L. provocax*, and to a lesser extent *L. felina*, there appears to be less evidence to support these statements than originally thought (Medina-Vogel et al 2003, Medina-Vogel et al 2008, Aued et al 2003, Sepulveda et al 2007, Vianna et al 2010), and it seems that the apparent sensitivity of *L. felina* may simply be a factor of its occupation of a more extreme environment than other otters, and not due to any particular specializations. Generally, *Lontra* species are amenable to a wide variety of climatic conditions, habitats, and prey types. In Chapter 3 of this dissertation (DeNeve Weeks 2020) I find that morphological variation in cranial shape in *Lontra canadensis* is higher within populations than between populations, supporting the evidence here that river otters may not experience functional differentiation across their habitats.

The *Lontra* lineage appears to speciate slowly, and primarily allopatrically, in response to shifting connectivity of waterways. This hypothesis is supported by the fact that most known phylogeographic splits within *Lontra* species as well as between species seem to be due to changes in waterway connectivity. Continued functional similarity between *L. canadensis* and *L. longicaudis* (and possibly *L. provocax*) support this hypothesis as well by suggesting that these phylogeographic splits do not have strong functional and thus evolutionary implications (i.e., populations are being separated and speciating without experiencing a great deal of functional change, rather than ecological speciation to take advantage of different resources). The primary exception is the event that led to the divergence of *L. felina* from *L. provocax*. *Lontra provocax* and *L. felina* both evolved out of bottleneck situations in extreme environments. *Lontra provocax* is smaller than, and definitely differentiated from, *L. longicaudis*, but appears to retain the broad climatic niche and diet preferences of other otters in this lineage. If dispersal ability is heavily tied to waterways, and strong ocean currents (Vianna et al 2010, Valqui et al 2010, Seymour et al 2012) and the extremely cold water temperature in western South America keep *L. felina* from being in the water for an extended period of time, this may explain the low dispersal ability of this species. Alterations to waterways appeared to be important in the evolution of *Pteronura brasiliensis* as well, and habitat connectivity by waterway is likely a key feature affecting otter dispersal, gene flow between populations, and speciation (Trinca et al 2012, Garcia et al 2007). As such, when assessing habitat viability, population viability, or otter ability to escape from poor-quality to high-quality habitats without human interference, connectivity and health of waterways should likely be the primary concern.

Several researchers identified what appeared to be key resources or strong habitat preferences of otters that subsequent studies determined were of less consequence than they originally appeared, such as tree cover for *P. brasiliensis, L. longicaudis,* and *L. provocax* (Lariviere 1999, Aued et al 2003, Sepulveda et al 2007), or low human population density for *L. canadensis* and *L. longicaudis* (Stearns & Serfass 2011, Rheingatz et al 2014, DeNeve Weeks Chapter 2). Since tree cover is often indicative of ecosystem health in riparian zones, even in relatively arid climates (Tucker & Leninger 1990, Macfarlane et al 2017, Holland et al 2009), it is possible that what was actually being indicated was a need for intact ecosystems. However, it is known that some otters re-colonize former habitats years after major disturbances and some populations persist in areas with high human population densities (Stearns & Serfass 2011, Dickins et al 1999). Thus, strictly whether or not a habitat is intact or close to its natural state may not be an entirely appropriate interpretation of the data. The most parsimonious explanation of this apparent conflict – the need for intact habitats yet presence of some otters in highly modified environments – is that the primary requirements of otters have little to do with dependence on any *specific* resource or habitat feature other than intact waterways, and more to do with robustness of the overall food web. Other factors that are often associated with otter presence, such as woody debris near rivers or specific structures for denning, on closer inspection also seem more likely to be potential indicators of food web stability, prey habitat, or categories of resources needed than otter reliance on a specific resource or habitat feature. None of these species rely on singular or obscure resources (with the possible exception of *L. felina*, which may rely on specific habitats, but it is unclear because this species may physically not be capable of reaching other suitable habitats) (Rheingatz et al 2014, Vianna et al 2010, 2011, Scognamillo 2005). My findings in Chapter 3 of this dissertation (DeNeve Weeks 2020) introduce evidence to support the hypothesis that river otters differentiate little across their ranges, and a possible mechanism for otters' ability to take thorough advantage of a semiaquatic food web: there is greater cranial morphological variation within populations than between populations, indicating possible diversifying selection and/or the possibility that individual otters are dietary or microhabitat specialists within an overall generalist population (see Newsome et al 2009, Ferry-Graham et al 2002). Dispersal is mediated almost entirely by waterway connectivity, and while populations of otters seem to be resilient to being disconnected from others populations for extended periods of time (Vianna et al 2010, 2011, Pickles et al 2011, Seymour et al 2012), maintenance of populations at times of extreme environmental change (such as now for many otters) require traversable waterways. Further research is required to determine precisely what features make a waterway traversable to otters, as well as what measures of ecosystem productivity are most suitable for otter persistence.

Otters are top predators, and often function as keystone species as well as indicators of habitat quality or ecosystem health (Ben-David & Golden 2009, Ben-David et al 2005, Foster-Turley et al 1990, Scognamillo 2005). Despite their ecological significance, many species of otters are under-studied because aspects of their ecology and natural history make data collection a challenge, especially at the broad spatial scales of their geographic distributions. It has puzzled researchers for some time that many otter species are apparently contradictorily adaptable to a variety of environmental conditions and extremely sensitive to habitat disturbances. Recently some (e.g., Medina-Vogel et al 2003) have suggested that various species of otters may in fact be excellent indicators of ecosystem health regardless of the ecosystem type because otter presence is a blunt measure of food web connectivity and productivity: if an ecosystem can support a population of medium-sized semi-aquatic carnivores that can consume 20% of their body weight a day (Dekar et al 2010, Munoz-Garcia & Williams 2005) in fish and aquatic invertebrates, that ecosystem must support a robust food web regardless of its structure. In Chapter 2 of this dissertation (DeNeve Weeks 2020) I constructed species distribution models to explore the climatic and environmental niche of *Lontra canadensis* across its range. I found support for only a weak influence of by climate and environment on river otter distributions, and my results show that otters likely occupy many habitats that have been anthropogenically modified. Their overall pattern of broad resource use supports otters as indicators of overall ecosystem health and food web productivity rather than indicating a specific, unaltered ecosystem: if a habitat has a food web that is productive enough to support semiaquatic carnivores with high caloric needs, and is relatively unpolluted, evidence suggests otters will likely find a way to persist.

It is likely that the greater importance of productive ecosystems, rather than the specific ecosystem type, is generalizable to most species of otters, if not much of Mustelidae, but further work will also need to be done to explore applicability to other species. Mustelidae as a family is known for having species with fast metabolisms and large appetites—the wolverine earned its Latin name, *Gulo gulo,* the glutton, for its voracious appetite (Dalerum et al 2009, Kollias & Fernandez-Moran 2015). There is reason to suspect that many species of mustelids have adaptations to a broad ecosystem type (like otters to rivers, or wolverines to cold, mountainous environments, or black-footed ferrets to prairies) and are adaptable to a variety of conditions within this range. We may be able to use mustelids generally as barometers-not always of how “intact” an ecosystem is, but how productive its food web is (and usually these things are linked). If an ecosystem can support its metabolically-intensive carnivores, it is likely still highly productive (Jorge et al 2013, Dirzo et al 2014).

The broad range of conditions occupied by otters, today and throughout their past, does not, however, mean that otters are not of conservation concern. (IUCN Red List). Their dietary requirements and reliance on intact waterways make them particularly vulnerable to anthropogenic habitat alterations, which are happening across aquatic ecosystems worldwide (Grill et al 2019, Pickles et al 2011, Vianna et al 2012). Rivers and lakes are foci for human settlement and utilized and altered heavily for food, water, agriculture, power, and transportation. 63% of the world's largest rivers are impeded by dams and reservoirs (Grill et al 2019). Around 70% of the human population lives within 5km of the closest body of water (Kummu et al 2011). There are relatively few activities that humans currently engage in related to waterways that aren't exploitative. Given our findings that waterways have been critical to the ecology and evolution of all New World otters, and that they are otherwise quite capable of occupying a variety of ecosystems, direct human impacts on riverine food chain stability and waterway connectivity are much more immediate threats to otters than most other potential threats. Further research is necessary to explicitly examine this hypothesis and its applicability to other otter species.

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1.6 Figures

Figure 1.1: Evolutionary relationships among extant Lutrinae, reproduced from the chronogram of the Mustelidae based on Bayesian analysis by Koepfli et al 2008 (Figure 2), with *Lontra provocax* included as sister to *L. felina* based on Vianna et al 2011 (Figure 3).

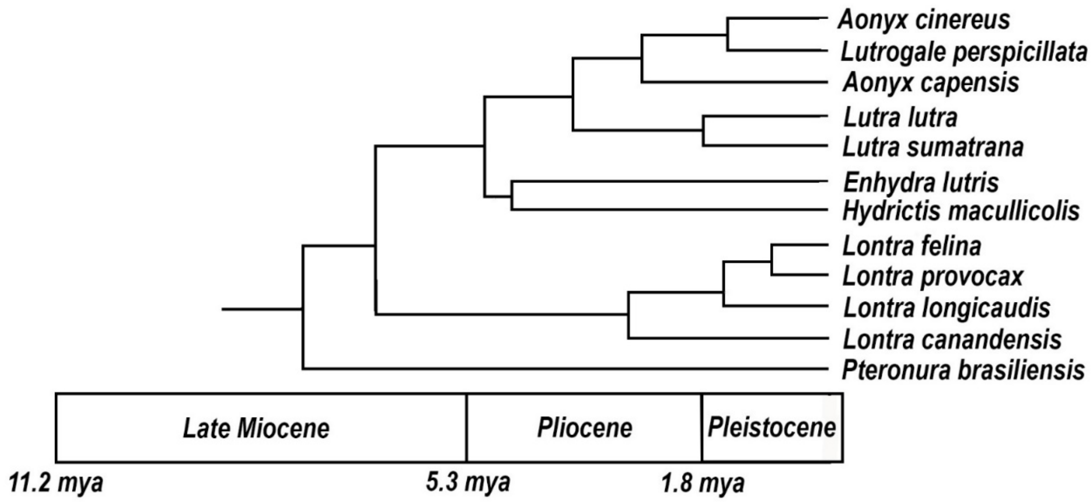
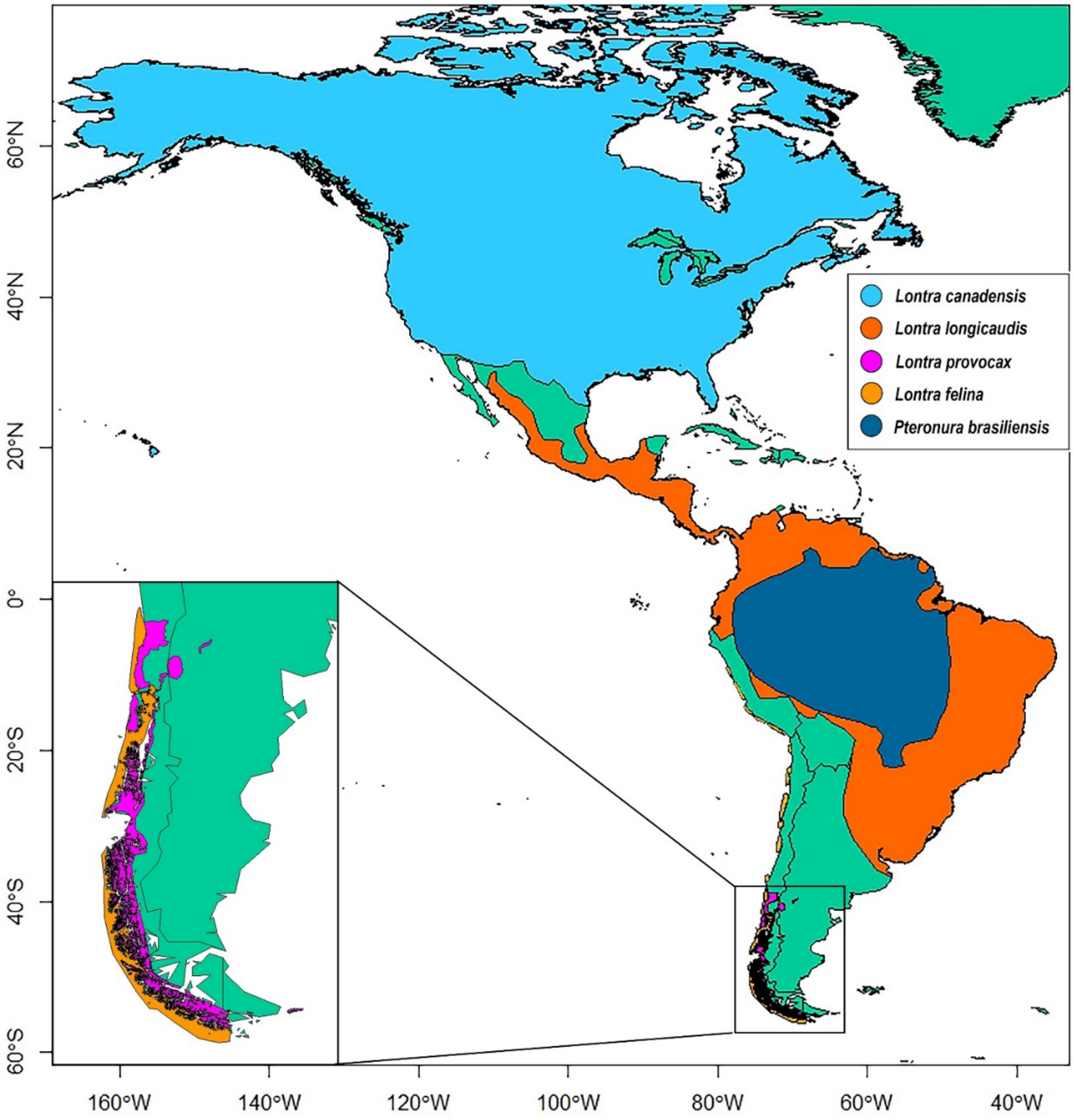


Figure 1.2: Distributions of *Lontra* and *Pteronura*. These distribution maps were produced using shapefiles of each species' distribution provided by the IUCN Red List. More information about how distributions were surveyed is available at the IUCN Red List (www.iucnredlist.org).



Chapter 2: Maximum entropy species distribution modeling of the North American river otter (*Lontra canadensis*)

2.1 Introduction

One of the most important pieces of information we can have about a species is quite simple but can be deceptively challenging to determine: where it lives. Knowing where a species lives can provide a variety of insights into its ecology, evolution, and natural history such as how it has been affected by climate and habitat (Brown et al 1996, Atkins & Travis 2010, Gaston 2003), how its evolutionary history or the history of the region has shaped contemporary distributions (Sexton et al 2009, Luna-Aranguré et al 2019), how stable the species has been throughout its past (Graham et al. 1996), and what factors determine its interactions with co-occurring members of its ecological community (Soberon 2019, Warton et al 2015). In a world threatened by climate change, habitat fragmentation, and habitat destruction (IPCC 2013, Pereira et al. 2012), understanding how all these factors interact to affect species' distributions can help us protect species by predicting how vulnerable they are to natural and anthropogenic changes, identifying and protecting critical habitats and resources, areas where they may do well in the future, and whether or not they are capable of moving to more suitable locations on their own (Atkins & Travis 2010, Briscoe et al 2019, Lawler et al 2010).

Species distribution modeling is an invaluable tool for inferring how distributions are shaped (Elith & Leathwick 2009). SDMs provide an overarching look at how species interact with their habitats by taking into account multiple potential predictor variables such as climate and landcover, and interactions among them, to create a more complete overall picture of a species' niche (Peterson et al 2016). This understanding of the variables affecting distributions can be combined with complementary information that together provide insights that support effective conservation strategies, such as about processes underlying local variation in genetics, morphology, and diet (Lee-Yaw et al 2016, Jezkova et al 2015), and what locations might be most suitable in the future, or questions about species interactions (Lawler et al 2010, Marcelli & Fucillo 2009).

One species whose range is relatively well known but perplexing is the North American river otter (*Lontra canadensis*; hereafter "river otter"). The river otter is a medium-sized semiaquatic carnivore that occupies freshwater habitats across most of North America, and is a conservation symbol across most of its range. It is used as an indicator species, a keystone species, an umbrella species, and an example of conservation success (Lariviere & Walton 1998, Gallant et al 2009, Zalewski 2011, Crait & Ben-David 2007). The river otter historically occupied much of North America, extending to the easternmost and westernmost edges of the continent, and to the southernmost edge in many areas. The remaining boundary to the south appears to be defined by the absence of clean fresh water in some areas (Raesly 2001), and inadequate conservation measures in others

(Serfass et al 1998, Raesly 2001). The latitude of the northernmost boundary line is unclear. Despite knowing the geographic dimensions of the river otter range in a broad sense, we do not understand well the factors that historically controlled the boundaries or accounted for patchiness within the overall range (Brandt et al 2014).

The broad distribution of river otters suggests a broad range of habitat tolerances, but they appear to be highly sensitive to anthropogenic alterations (Scognamillo 2005, Lariviere & Walton 1998, Gallant et al 2009). The contemporary distribution of river otters is patchy due to fur trade, pollution, and habitat destruction that occurred from ~1800-1950 (IUCN Otter Specialist Group 2015, Serfass et al 1998, Raesly 2001, Lariviere & Walton 1998). After extirpation in 11 US states and one Canadian province, and declines in many other states and provinces, extensive reintroduction efforts in the 1980's and 1990's brought river otters back to a stable population trend as a species, and they are still recovering. The river otter is now the only species of otter that has a secure conservations status (is both listed as Least Concern and not currently in decline) (IUCN Otter Specialist Group, 2015, www.otterspecialistgroup.org/).

Regardless of these conservation successes, relatively little is known about the habitat requirements contributing to the persistence of these common, charismatic, yet cryptic animals (Latch et al 2008). This is problematic since otters are frequently used as bioindicators: it is not entirely clear what features of the landscape they are indicating (Latch et al 2008, Crowley et al 2018, Gallant et al 2009). Examining river otter associations with climate and environment across their range may provide insight into their apparently conflicting robustness and sensitivity, and provide tools that can be applied to conservation of this and related species. Additionally, their frequent use as both a bioindicator and an umbrella species necessitates improved comprehension of river otter habitat requirements.

In this study, we examine the climatic and environmental niche limits of North American river otter to answer the question: What climatic and landcover variables constrain the geographic distributions of river otters? Based on the species' range limits, we expected broad features of climate to be largely unimportant in predicting river otter distributions. We expected that habitat factors, primarily those that indicate food supply, availability of fresh water, or impacts from humans, would be more relevant to explaining distributions.

2.2 Methods

2.2.1 Model Inputs

Occurrence Data: We used the spocc R package (v1.1.0, Chamberlain et al 2014) to query the BISON (https://bison.usgs.gov/#home) and GBIF (Global Biodiversity Information Facility, www.gbif.org) databases for reported occurrences of *L. canadensis* and downloaded 3,000 occurrence records with coordinate data from each database. Due to low reporting of otter occurrences in Canada in these databases, we obtained information on otter distributions from several Canadian provinces by contacting provincial Fish & Wildlife offices directly (Table 1). Most provinces provided data that could not be directly included in the models (such as trapper counts per broad wildlife management zone) but were useful in qualitatively assessing model accuracy at a broad scale. The British Columbia Conservation Data Centre provided 662 occurrences with coordinate data, and the Manitoba Dept. of Sustainable Development provided 34 occurrence points. We merged the cleaned occurrence data from GBIF, BISON, British Columbia, and Manitoba into a single file. We removed duplicates, occurrences without numeric coordinates, and occurrences with coordinates outside of North America for a total of 5,133 occurrences.

The occurrence data are heavily biased toward certain regions, and locality-specific information is absent in some areas where otters are known to occur (based on local reports and range maps that lack associated coordinate data) (Fig. 1). This is not an uncommon phenomenon in modeling poorly reported taxa (Bilney 2014, Gaston 2011, Leon-Cortes et al 1999), particularly in regions where reporting species occurrences is not yet a common practice. The high densities of occurrences on the west coast of North America and east of the Mississippi River relative to other parts of the continent are likely at least partially due to bias in sampling effort and reporting, as otters are known to occur in the Midwest but coordinate-based reporting is very low. We used the R package SPthin (version 0.2.0; Muscarella et al 2014, Aiello-Lammens et al 2015) to balance the density of occurrences across space and minimize spatial autocorrelation among occurrences. Spatially thinning occurrences decreases the effect of reporting bias by eliminating points in a randomized fashion at a pre-determined spatial interval (Boria et al 2014).

We initially explored multiple different resolutions for spatial thinning to determine the level of spatial thinning that would best balance preserving as many occurrences as possible with reducing spatial bias. We ran all final models using spatial thins of 1 occurrence/80km and 1 occurrence/150km. Ultimately, we chose these two resolutions for thinning because they produced the best performing models in preliminary tests; other resolutions performed significantly worse and were not considered further. The 80 km spatial thinning output yielded 583 useable occurrences, and the 150 km thin yielded 269 occurrences (Fig. 1, Supplemental Fig. 1).

Predictor variables: In order to fully examine the influence of different variables on the river otter niche, we created three separate datasets of predictor variables: a climate dataset, a landcover dataset, and a climate-landcover dataset.

For the climate dataset, we downloaded the WorldClim 2 (http://www.worldclim.org/, Fick, S.E. and R.J. Hijmans, 2017) bioclimatic dataset in 2.5 arcminute grid cells in August 2018. The WorldClim 2 dataset is a standard set of 19 historical climatic variables that capture different aspects of the climate system (Table 2). We stacked the 19 bioclimatic variables, then projected them to a WGS84 map projection and clipped them to a bounding box around the occurrence points that includes most of North America.

For the landcover dataset, we downloaded two different landcover data layers from the Land Process Distributed Active Archive Center (LPDAAC) in 2018: Landcover and Net Primary Productivity (NPP).

We relied on the MODIS Land Cover Dynamics Product MCD12Q1.006 for our estimate of landcover. This assigns fine-scale grid cells to one of 17 different land cover type variables (Table 3). We decreased the resolution of the landcover layers from 500m to 2.5 arcminutes to match the resolution of the climate layer by aggregating each land cover variable to a percent cover of the variable across the new grid cell. We thus converted the single landcover product into 17 different variables (one raster for each of the land cover types), representing percent cover of each variable. We stacked these new layers and projected them to a WGS84 map projection, then clipped them to a bounding box around the occurrence points that includes most of North America.

For NPP, we used the Daily GPP and Annual NPP (MOD17A3) Products, NASA Earth Observing System MODIS Land Algorithm (https://earthobservatory.nasa.gov/). We did not use the Daily GPP layer in this dataset. We decreased the resolution of the Annual NPP layer from 500m to 2.5 arcminutes to match the resolution of Worldclim by calculating the mean NPP across all grid cells contained within the larger grid cell. We projected this layer to a WGS84 map projection and clipped it to a bounding box around the occurrence points that includes most of North America, and stacked it with the landcover layers.

The combined dataset included the full set of climate and landcover data described above, for a total of 37 different predictor variables (Tables 2, 3).

2.2.2 Modeling

We used the R package ENMeval (version 0.3.0, Muscarella, et al 2014) to create Maximum Entropy (MAXENT) models for each of the three different predictor variable datasets. We chose MAXENT modeling because it is a machine learning approach to species distribution modeling that is robust to dealing with presence-only data. MAXENT looks for the distribution of values in predictor variables that is closest to uniform (Thuiller et al 2009, Elith & Leathwick 2009, Merow et al 2013, Phillips et al 2006). The predictions from MAXENT models are relatively straightforward to interpret in an ecological context because they include response curves that demonstrate the probability of species presence across the values of each predictor variable (Merow et al 2013).

We created separate sets of MAXENT models for climate predictors and landcover predictors to explore how variables within each category behaved independently, as including fewer variables potentially allows relationships to be identified more clearly. We then created another set of MAXENT models with all predictors combined so we could explore how different predictor types influence the species distribution relative to each other, and how their combined effects might influence distributions.

Spatial thinning reduced bias towards the locations with the highest numbers of occurrences, but substantially decreased the number of occurrence points available to model. We tested whether altering the number of background points to be more similar to the number of occurrences had an effect on model predictions. Selection of background ponts can influence response curves (Merow et al 2013), and we wanted to explore whether altering the density of background points influences outcomes. We created MAXENT models using 500 (comparable to the maximum number of occurrences used in the models), 1000, and default background points (flexible but usually around 1000). We used a 70%/30% training/testing dataset split for all models.

We used the ENMeval R package to determine optimal parameters for MAXENT models. We used the function 'ENMevaluate' to compare outputs from a variety of model feature classes that describe how predictor response curves are allowed to vary: linear, quadratic, hinge, threshold. These preliminary models did not clearly indicate the best settings for the full models, so we produced full MaxEnt models for the preliminary models with the three lowest AIC values and the three highest AUC values (see *Model evaluation* below). Thus, each unique dataset (for example Climate dataset, 80 km spatial thin, 500 background points; Table 4) was used to infer 6 different final models. In this way, we were able to explore variability among different final models resulting from uncertainty in the preliminary models. A full list of final models with all parameter settings can be found in Appendix 1. All analyses were done in R (R Core Team, 2020).

2.2.3 Model evaluation

We evaluated the final models using a variety of metrics. AUC is the standard model evaluation statistic for presence-only models. It is defined as the probability that the habitat suitability value of a randomly selected presence point will be higher than that of a randomly selected background point. We also report training omission rates & fractional predicted area at equal training sensitivity and specificity, as these are other commonly used model evaluation statistics. Sensitivity (omission rate) is the proportion of correctly predicted presences, and specificity (fractional predicted area) is the proportion of correctly predicted absences (Allouche et al 2006). We calculated mean, standard deviation, and range of each statistic across the 6 final models for each unique dataset (Table 4). When evaluating the final models, we primarily focused on AUC (Appendix 1). While it penalizes predictions that occur far beyond presence locations, a potential drawback is that AUC is based straightforwardly on range predictions. This may be particularly problematic in contexts such as this where occurrences are thought to only partially reflect the species range (e.g., occurrences appear to substantially underestimate occupied area based on the NatureServe map, Fig. 1). We did not emphasize training omission & fractional predicted area for model evaluation because determining biologically relevant thresholds for these values can be challenging even under ideal modeling conditions and with real absence data (Merow et al 2013). Additionally, MAXENT models utilize background points that are not synonymous with pseudoabsences, making measures of specificity inappropriate.

We determined the influence of dataset type, spatial resolution, and background points (Table 4) on model performance (AUC) through ANOVA. We then conducted a post-hoc Tukey HSD test to examine the relationships between variables within categories.

The uneven density and patchy coverage of point data across regions made use of expert-produced maps essential to assessment of biological relevance of model predictions (see inset Fig. 1). For this purpose we used range maps obtained from IUCN Otter Specialist Group and NatureServe (NatureServe 2020, https://explorer.natureserve.org/). We did not explicitly calculate the quantitative difference in area of suitable habitat based on the Maxent models to these expert generated range maps for two reasons: First, determining an "appropriate" cutoff value of habitat suitability based on the Maxent models seems biologically questionable under these modeling conditions. Second, both expert-created maps appear to have been generated using less rigorous means of establishing presence, and both describe presence of the species across nearly all of North America north of Mexico without providing raw data or quantifiable probability or density estimates. Thus, the Maxent models are predicting quantitative attributes of both the boundaries as well as the internal structure of suitability across the range, while the expert range maps primarily assess presence-absence within broad provincial boundaries and historical trends within these bounds.

We initially examined the predictor variables contributions using 80km spatial resolution and the default number of background points across all three dataset types to verify whether they indicate similar relationships between occurrences and predictors, and subsequently focused on the combined dataset models at 80km spatial and default background points, in order to examine fitted relationships among the 6 top model types. We examined the individual variable contributions to determine which variables most influenced the predictions of overall suitability. We focused on variables with a mean contribution over 10% for at least one model type.

2.3 Results

2.3.1 Model Performance

We found no significant difference in model performance for any dataset type, regardless of whether 500, 1000, or default background points were used for modeling; no single choice of background points influenced which models were consistently among the top models (Fig. 2, Table 5). This is the expected outcome for MAXENT models, as altering density of background points should not alter performance statistics (Merow et al 2013). In contrast, spatial resolution significantly impacted model performance as measured by the 80km spatial resolution models significantly outperformed the 150km models (Fig. 2, Table 5). AUC values for the 80km resolution models were all over 0.75. A raw AUC value of 0.70 or over is considered acceptable, and a value of 0.75 or over is considered good, which indicates that not only do the 80km resolution models perform significantly better than 150km resolution models, they perform objectively well. Combined dataset models that incorporate climate and landcover performed significantly better than climate or landcover models on their own, and the combined dataset models at 80km thinning all have AUC values between 0.75 and 0.8, indicating that the models are accurately predicting the occurrences provided, but are likely not overfit (Radosavljevic & Anderson 2014).

2.3.2 Predictor Variables

Overall, the most influential predictive variables were similar across dataset types (Fig. 3). Four variables out of 37 were consistently important regardless of dataset type: percent cover urban, percent cover water, annual mean temperature, and precipitation of the coldest quarter. Two additional predictor variables -- NPP and percent cover open shrubland -- had high mean contributions in the landcover dataset models, but mean contributions under 10% in the combined models.

Variable contribution was typically higher for the landcover and climate models than for the combined models (Fig. 3). This is expected since the combined models include twice the number of variables and contributions are calculated as a percentage of total. Given this, there doesn't appear to be an unusually large discrepancy between variable contribution in combined and landcover/climate models, with the exception of NPP and open shrubland.

Most models provided relatively consistent measures of variable importance, though the combined models had very large standard deviations among the 6 final models for some variable contributions (percent cover urban, annual mean temperature, and precipitation of the coldest quarter). Of these, annual mean temperature was the most variable between feature classes, and had the lowest contribution overall.

Within the set of final models, the top four variables yielded relatively consistent response curves across model types. Open shrubland and NPP both varied widely across feature classes, sometimes showing strong positive relationships between probability of otter presence and the value of the variable, and at other times strong negative relationships (Fig. 4). These variables with the highest variability in the shape of the response curve (NPP & percent open shrubland) are also those with the highest permutation importance (a measure of how strongly variable importance is influenced by stochastic modeling processes) (Fig. 3).

Across all final models there were some consistencies about the relationship between variables and suitability. The probability of presence changed from 0 to 0.1 as percent urban/built environment increase, then plateaus. If we take this relationship at face value it would indicate that river otters only inhabit urban spaces. The probability of presence generally increases as the percent cover of water increases, with some interesting fluctuations. The likelihood of otter presence is zero where there is no water, and shows a strong positive correlation with percent cover water, up to a point (different in each feature class). In some models, likelihood of presence dips at very high percent cover of water, indicating either that there are no otters, or no humans to report them. Otter presence appears to have a nonlinear relationship with annual mean temperature, with most models indicating that river otters should prefer an annual mean of ~10-30°C. Response curves indicate that the relationship between otter presence and precipitation of the coldest quarter is not well inferred by our models. Most models indicate that high winter precipitation is good, though two models (feature class models 1 & 2) indicate no relationship. In most models, otter presence is contraindicated with open shrubland, though there are deviations from this pattern in feature class models 4 & 5 (Fig. 4). NPP shows a great deal of variation, with half of the models indicating a strong positive relationship, and the other half indicating a strong negative relationship between probability of presence and NPP (Fig. 4).

2.3.3 Predicted Range

Regardless of which feature class was used to infer suitability, the predicted river otter habitat suitability maps for the combined dataset, 80km, default background points models are reasonably similar to one another, showing high suitability in the west and northwest of North America, across southern Canada, the Midwest and the eastern parts of North America, with low to unsuitable habitat across a broad region of North America spanning from the Great Basin and Great Plains, and into northern Canada (Fig. 5). However, in all cases the MAXENT-generated suitability predictions differed substantially from the NatureServe and IUCN range maps, which show otters occupying nearly the entirety of North America (except Greenland) north of Mexico (Fig. 5).

2.4 Discussion

2.4.1 Challenges

The persistence of river otters is a paradox: they are very sensitive to habitat disturbances through time, but also apparently robust to habitat variation across their range (Lariviere & Walton 1998, Gallant et al 2009, Scognamillo 2005). While the factors that contributed to river otter declines through time are relatively well known, the factors that facilitate their broad geographic distributions are comparatively less known (Raesly 2001). The fact that the river otter is frequently used as both an indicator and umbrella species makes understanding its habitat requirements even more important, as conservation actions focused on otters potentially affect many other species (Frimpong 2018, Stevens et al 2011). Thus, the aim of this study was to examine the climatic and landcover variables that best describe controls on the geographic distribution of river otters.

This study proved challenging for reasons that have implications for how and why we do distribution modeling. It is perhaps a truism that species are challenging to map with incomplete occurrence data. This is not an uncommon problem in distribution modeling, and there are a variety of means to mitigate or correct for this issue (Hernandez et al 2006). Most research on the challenges of incomplete distribution data, however, has focused on rare species with very few occurrences rather than common ones (Lindenmayer et al 2011, Bilney 2014). Common and widespread species are as important to study as uncommon species, because the state of being common or widespread is itself rare, and not yet well understood (Frimpong 2018, Lindenmayer et al 2010, Gaston 2011). However, in some cases, accurate and unbiased distribution data is not well collected even for common species because they are thought to be unimportant or not at risk, leading in some cases, as with the river otters, to large biases or skew in the available distribution data suitable for modeling (Isaac & Pocock 2015). For example, one of the authors (DDW) contacted wildlife managers across the US and Canada (Table 1) to request river otter occurrence data. Communications with those wildlife managers revealed that in many places river otters were quite common but lacked precise coordinate data (DDW pers. comm.). Additionally, casual searches for otter sightings in parts of the American Midwest indicate they are present in many locations that do not show up in the scientific databases (for example, occurrences in Kansas are sparse, but according to the Kansas Wildlife, Parks, & Tourism website, otters appear to occur at low density across much of the state https://ksoutdoors.com/Hunting/Furharvesting/Furbearers/River-Otter). This is a place where community science efforts focused on capturing coordinate data for sightings of common species could be particularly valuable.

Given the discrepancy between informal and scientifically-reported occurrences, exploring the habitat requirements of common and widespread species becomes challenging. This is troubling because common or widely-spread species may not appear to be at risk when they are in fact experiencing severe population declines (Gaston & Fuller 2007). Some examples of recently common species that have since become endangered or extinct are passenger pigeons, North American bison, greater prairie chicken, green sea turtles, and African elephants (Halliday 1980, Isenberg 2000, IUCN Red List), and the river otter itself experienced substantial past declines (Raesly 2001). Thus, failing to monitor common in addition to rare species can also lead to poor conservation outcomes.

Despite these challenges, we were able to identify and examine broad-scale variables that may affect otter presence. Here, we discuss the factors most strongly associated with otter distributions, discrepancies in different inferences of otter distribution, and challenges associated with modeling an extremely widespread species.

2.4.2 Correspondence between predicted suitability and previous range maps

The MAXENT-generated habitat suitability predictions differ substantially from expert-generated range maps by NatureServe and the IUCN Red List (Fig. 5, Fig. 1). There are several possible explanations for these differences. First, the expert-generated maps do not provide information on the status of river otters within the overall boundaries of their distributions, and are also clearly pegged to broad geopolitical units rather than natural boundaries (Fig. 1, Fig. 5). So, by using these maps as our expectation of truth, we may be underestimating the power of the MAXENT-generated maps. However, despite this, there are some clear discrepancies. For example, large parts of Canada are predicted to have low suitability, yet we know from wildlife management zone-level data (provincial trapper data, DDW, pers. comm.) that otters are quite abundant (Table 1). In this case, poor fit between the models and the range maps is likely due to the lack of georeferenced occurrence points to include in the training data.

The departures from expectation across the American Midwest are a little more challenging to quantify – is low predicted habitat suitability due to statistical (a lack of occurrence data) or to ecological factors? Otters do occur in at least low densities across this region based on local reports (Raesly 2001, Green et al 2015, Jeffress et al 2011), but there is little information on otter densities to determine the model's accuracy. The NatureServe range map provides basic information about conservation status by state/province. River otters are listed as "vulnerable" across a similar portion of the American Midwest that is predicted to have low habitat suitability by the MAXENT models. Additionally, apart from potential statistical issues, it is difficult to determine whether likely low otter densities are due to low suitability of the region for otter habitation at this time or whether they have not fully recovered from historical extirpations in these locations. In many of the states in this region, otters are listed as "Vulnerable" or "Imperiled", which may indicate that historical extirpations are a stronger factor. These two factors also likely interact and both may be true: that is, otters have been under pressure due to anthropogenic factors in many places, and more resilient to anthropogenic pressure in locations with higher baseline habitat suitability (Cianfrani et al 2010, D'Elia et al 2015, Ruckelshaus et al 2002, Newbold et al 2010, Crawford & Hoagland 2010). Overall, intensive efforts to identify otter presences within the predicted low suitability regions of the range would be extremely useful for improving estimates of river otter range and, more importantly, internal structure.

2.4.3 What factors are associated with the internal structure of otter distributions?

Given the model underprediction of otter presence in key parts of the species range (Fig. 5), the modeled relationships between suitability and the predictor variables may not capture the true biological relationships, but can help illuminate relative importance and coherence across final models. Additionally, our approach of capturing variation between different model specifications, rather than choosing a single "best" model, helps illuminate those variables in which we have higher confidence vs those whose effect changes across models. Overall, we identified six variables (urban/built landcover, water, annual mean temperature, precipitation of the coldest quarter, NPP, and open shrubland landcover) that are strongly correlated with otter presence. Equally important, we have gained a better understanding of the environmental and climate variables that are not likely to strongly influence river otter presence. Five of the six main variables important in the final models are likely to have biological significance, whereas one variable (urban/built) is likely an artefact of reporting bias in occurrence data (Kramer-Schadt 2013, McPherson et al 2004).

NPP: Half of the models (with the notable exception of several models in the combined, 80km, default bg category (Fig. 4)) predict a positive relationship between likelihood of otter presence and NPP, and further indicate that otters are not found in zero

NPP environments. This result seems reasonable, as environments with extremely low to zero NPP may be less able to support large, stable food chains (Santini et al 2017), and could be used to identify less suitable habitats.

Urban/built: We expected this variable to have a slight negative influence on otter distributions, following the rationale that otters are relatively elusive and prefer less disturbed habitats (Gallant et al 2009, Zalewski 2011). On close inspection of the fitted trends in the final models, however, the trend indicated in most models is positive, not negative. While we know that otters occur in urban areas, it is extremely unlikely they have an active affinity for them (Lariviere & Walton 1998). Thus, this positive relationship between percent cover of urban or built environments and otter habitat suitability more likely represents reporting bias than biological reality, as seen in many other studies (e.g., Kramer-Schadt 2013, McPherson et al 2004). The occurrence data are skewed towards areas of high population density, and our efforts to obtain occurrence data from areas of low population density revealed that otter occurrences may not be recorded in some Canadian provinces because otters are such a common species that there is no need to keep track of localities or abundances. Additionally, since occurrence data require an observer, it is possible that observations are skewed towards places where there are more people to report observations, and reporters believe the information is worth reporting (such as in urban areas where wildlife is relatively more rare (Henckel et al 2020, Callaghan et al 2020)). This actually suggests a situation where if otters are extremely common in an area, observers may not consider the information to be worth reporting.

Water: The likelihood of otter presence is zero where there is no water, increases with increased water availability, and decreases as presence of water nears one (indicating that 100% of the grid cell is covered by water). As otters are semiaquatic, they clearly depend on water. However, they also do not tend to venture too far from land (Serfass et al 1990). Thus, the predicted relationship between water and otter presence is consistent with what we expect from otter natural history, and also supports the importance of water and waterway connectivity identified in Chapter 1 (DeNeve Weeks 2020).

Open shrubland: The predicted relationship between likelihood of otter presence and open shrubland for most models shows that otters are more likely to be present in regions with very little open shrubland. Open shrublands tend to be dry, have little cover, low NPP, and relatively few resources to sustain river otter populations (Sulla-Menashe & Friedl 2018, NASA Earth Observatory https://earthobservatory.nasa.gov/). Thus, this variable should show the opposite effect as NPP and water, which is generally what we see.

Annual mean temperature: There is a huge amount of model-to-model variation in the fitted relationship between temperature and otter presence, which makes this variable difficult to interpret. Most models indicate increased likelihood of otters above 10°C. This makes sense for an animal that relies on liquid fresh water, but we also know from local trapper counts in several Canadian provinces that parts of the species' known range are in much colder areas than are included in the occurrence data. Given this, more accurate occurrence records from the full range of environments that the otters inhabit would be useful. Alternatively, mean annual temperature may not be a suitable surrogate for the temperatures that otters actually experience, which is what ultimately may control their distribution (Suggit et al 2018). This disconnect between the microclimate experienced by an organism and the inferred climate as sensed through weather stations or related equipment has been seen in a variety of other systems (Lembrechts et al 2019, Potter et al 2013, Bennie et al 2014), and may be particularly important for a semi-aquatic animal where water temperature may be as or more important than air temperature. Future work focused on river otter microclimates would be extremely valuable.

Precipitation of coldest quarter: This variable generally indicates higher likelihood of otter presence in locations with at least some winter precipitation, because places with zero winter precipitation are not likely to have enough running or standing water to support a semi-aquatic animal. Some models indicate that very high winter precipitation has a slight negative effect on likelihood of otter presence. The point at which winter precipitation becomes "too much" may have to do with temperature or terrain.

Overall, these six variables provide initial hypotheses about controls on otter climate and habitat preferences, but they need to be followed up with field validation and refinement of occurrence data. Our work also highlights why it is important when modeling species with broad and/or incompletely captured ranges to consider the potential biological significance of model predictions for whether they make sense, and to consider the variables that do not predict distributions as much as variables that may. These results also lend support to the hypothesis that otters are robust to a variety of climates and habitats. There is not one or two master controlling variables; instead, there are at least six variables that each appear to incompletely influence distributions, or perhaps influence distributions in different parts of the range.

2.4.4 Challenges for modeling otter distributions

We began this endeavor with a simple question: what climate and habitat variables predict the distributions of otters? Given the simplicity of the question, the relative ubiquity of the species of interest, and the extent to which otters are used to motivate conservation actions, we assumed modeling their distribution would be a relatively straightforward process. However, in the course of addressing this question, we ran into four major problems:

1. *Reporting (occurrence data) of otters is surprisingly patchy and skewed*. In some parts of the range otters are reported very consistently, and in other parts (even where otters are known to occur at relatively high densities) reporting is very poor. This makes creating good models difficult ('garbage in, garbage out'). In effort to obtain more representative

occurrence data, we found that in many places those data just don't exist. Thus, the key way forward for improving predictions of otter distributions is to obtain uniformly high quality presence (and absence) data across the range of the species. This has been found to be a problem with accurately modeling other common & widespread species, such as the common blue butterfly (*Polyommatus icayus*), where fine-scale population analysis revealed a 74% decline in the species in roughly 100 years that was invisible from assessing presence at larger scales (Leon-Cortes et al 1999). In India, several species of widespread macaques were thought to be so ubiquitous their populations weren't extensively surveyed for 40 years. When they were resurveyed, both population sizes and distributions of each species had radically changed due to anthropogenic factors (Kumara et al 2010).

2. *Regional variation in important variables.* For a broadly distributed species like otters, that are able to persist in most places, there is likely quite a bit of variability as to the factors that determine their presence and abundance in any given region. For example, for most modeling purposes precipitation is treated as having the same effect whether it is soft, constant rain, infrequent downpours, hail, or snow. For example, in one study conducted on tundra vegetation, the authors found that including more specific water variables and examining vegetation across finer spatial scales led to a very different understanding of how water influences vegetation in these habitats. Responses to water differed both within and between taxonomic groups depending on the state the water was in, and the importance of water variable exceeded the importance of temperature to explain vegetation distributions at fine scales (Kemppinen et al 2019). By trying to fit a single fitted relationship for each predictor variable across the full distribution, any sort of regional variability in important variables may blur the overall signal of factors influencing the full range. Determining whether some factors are more important than others at regional scales is an important next step for this work.

3. *Scalar mismatch between occurrence data, predictor data (climate/environment), and the questions being addressed*. Determining the factors influencing suitability for a broad-ranging species actually requires very fine grain data to drill down on differences between habitats and habitat qualities. While the climate and landcover layers we utilized are available at relatively fine resolutions, most of the specific variables available at the scale of the species range are relatively coarse, and thus affect distributions at broad rather than local scales. For example, at local scales the overarching percent cover of a particular habitat type might be less important than the specific microhabitat or microclimate experienced by an individual, or the land use history of the location may be more important. For example, river otters are sensitive to aquatic pollutants (particularly those that bioaccumulate, such as mercury) (Peterson & Schulte 2016, Crowley et al 2018), and to habitat disturbances (Gallant et al 2009). Thus, otters may be more sensitive to quality – such as whether or not there is unpolluted fresh water – than to percent cover of particular landcovers or types of environments. Additionally, some of the environmental variables that may most strongly affect otter distributions (Gallant et al 2009, Zalewski 2011) cannot currently be modeled at broad spatial scales, such as ecosystem health and stability, habitat disturbance, habitat connectivity, pollution, and

food web stability. We have reason to believe from related work (DeNeve Weeks 2020, Chapters 1, 3) that river otters may require stable and abundant sources of food to persist in an ecosystem but are capable of occupying a variety of semi-aquatic environments without undergoing strong morphological changes, but that local habitat differences might cause morphological differentiation that is not seen on a broad spatial scale. These outcomes support the hypothesis that variables at smaller spatial scales may be more important to understanding otter distributions.

4. *Historical variables*. The type and format of available data affects what types of conclusions can be reached; because the conservation history of otters is complicated (Serfass et al 1998, Raesly 2001, Lariviere & Walton 1998), with historical impacts light in some areas and very heavy in others, including predictor variables that capture this history would also help improve the models. For example, in one study that focused on historical distributions, Hendricks et al (2016) determined that the historical distribution of the Mexican wolf was far broader in the past than it was after anthropogenic impacts, and that parts of its former range are likely more suitable for reintroduction than many parts of its modern range. In a study that focused on human impacts, Fukasawa & Akasaka (2019) examined archaeological land-use patterns and found that specific historical land-use activities by humans in Japan had profound and lasting effects on modern mammal distributions and diversity.

Here, we used percent cover of urban/built environments as a surrogate for human impacts, but this variable simply describes the current status of one form of human impact and does not adequately capture the history of potential human impacts (Collins et al 2000, Gallant et al 2009). Further, it exacerbates bias due to its effect on reporting. Including variables that estimate length and intensity of human activities in a region in the past, particularly activity in farming or the fur trade, may shed light on this issue. Several recent studies have gone about including such variables into species range predictions in a variety of ways, though one challenge is that two types of information are required to do this accurately: historical distributions of the species of interest, and historical land use data. Both types of data can be challenging to procure. Historical land use data for regional areas, such as estimates of past land use in and around urban areas, is much more widely available (e.g., Angel et al 2016) than similar data at a continental, or even state, level.

2.4.5 Overall conclusions and prospects for future river otter models

Our work has brought to light a common problem that is particularly troubling in conservation-driven research: We know very little about broadly distributed species. Indeed, “many common species are as poorly studied as many rare ones” (Gaston, 2011). Rarer species tend to get more attention, and common species get little attention until they become rare. When they return to being common, or common enough, research focus again falls off.

There are several reasons why understanding and conserving common species is equally important to conserving rare species. From a conservation standpoint, common species are the main victims of overexploitation and habitat loss, degradation, and fragmentation (Lindenmayer et al 2011, Gaston 2010). Since initially substantive decreases in population size can lead to relatively small immediate impacts on population geographic range (Gaston 2011), it can be difficult to detect issues until there is a fairly substantial decline (Ceballos et al 2017, Ceballos & Ehrlich 2002). We should not rely strictly on rarity as an indicator of species in peril, as some species may be common and imperiled, and others might be naturally rare. Since common species may have important ecosystem roles, decreases in abundance may have large impacts on other species (Frimpong 2018, Lindenmayer et al 2011). If the goal of conservation is to prevent species decline or extinction, then we need to be proactive and implement conservation measures now rather than wait until there is a crisis. And to do this, we need to be proactive about collecting fundamental data on species occurrences across space and time, even for the most common of species.

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2.6 Tables

Table 2.1: Sources and uses of distribution data.

Data type	From	Data Provided	Use/Function
Range maps	IUCN	Range Map	Assess model accuracy
	NatureServe	Range Map	Assess model accuracy
Occurrence Points	GBIF	*Lontra canadensis* occurrence points	Build SDM
	BISON	*Lontra canadensis* occurrence points	Build SDM
Provincial Data			
Alberta	Alberta Environment & Parks Information Centre	Confirmation on population size	Assess model accuracy
British Columbia	British Columbia Conservation Data Centre	Map of occurrences/ sightings as points (662)	Build SDM
Manitoba	Manitoba Dept. of Sustainable Development	List of occurrence points (34)	Build SDM
New Brunswick	Fish & Wildlife Branch of Energy & Resources Dept.	Capture counts per wildlife management zone	Assess model accuracy
Northwest Territories	Environment & Natural Resources, Wildlife Division	Distribution map	Assess model accuracy
Ontario	Ontario Ministry of Natural Resources & Forestry	Shapefile of distributions	Assess model accuracy
Quebec	Ministry of Forests, Animals, & Parks	Capture counts per wildlife management zone	Assess model accuracy
Saskatchewan	Ministry of Environment: Fish, Wildlife, & Lands Branch	Capture counts per wildlife management zone	Assess model accuracy

Table 2.2: WorldClim variables. These variables are used in the Climate and Combined models.

WorldClim Variable	Bioclimatic Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Temperature Range
BIO3	Isothermality
BIO4	Temperature Seasonality
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Table 2.3: Landcover variables. These variables are used in the *Landcover* and *Combined* models.

Layer name	Landcover variable
evergreen_needleleaf	Evergreen needleleaf forest
evergreen_broadleaf	Evergreen broadleaf forest
deciduous_needleleaf	Deciduous needleleaf forest
deciduous_broadleaf	Deciduous broadleaf forest
mixed_forest	Mixed forest
closed_shrubland	Closed shrubland
open_shrubland	Open shrubland
woody_savanna	Woody savanna
savanna	Savanna
grassland	Grassland
permanent_wetland	Permanent wetland
cropland	Cropland
urban_built	Urban built
crop_natural_mosaic	Crop & natural mosaic
snow_ice	Snow & ice
barren	Barren
water	Water
NPP_mean	Net primary productivity

Table 2.4: Summary of all models. Final models discussed in the manuscript were based on the 80km spatial resolution thin with the default number of background points.

Predictor Variable	Spatial resolution	Background points
Climate	80	500
		1000
		default
	150	500
		1000
		default
Landcover	80	500
		1000
		default
	150	500
		1000
		default
Combined	80	500
		1000
		default
	150	500
		1000
		default

Table 2.5: 3-way ANOVA results comparing influence of dataset type, background points, and spatial resolution on model performance as defined by AUC values. Dataset type and spatial resolution are significant at a 95% confidence interval. DF num and DF denom are degrees of freedom of the numerator and denominator, respectively. F is the f-ratio, and p is the p-value. A post-hoc Tukey HSD test indicated that 80km spatial resolution had significantly better AUC values than 150km spatial resolution (p <0.0001). Combined dataset models had significantly better AUC values than both climate (p=0.0016) and landcover (p=0.0009) model types.

Effect	DF num	DF denom	F	p
dataset	2	90	11.783	<0.0000*
background	2	90	0.066	0.9360
resolution	1	90	35.517	<0.0000*
dataset:background	4	90	1.095	0.3640
dataset:resolution	2	90	1.587	0.2100
background:resolution	2	90	0.7	0.5000
dataset:background: resolution	4	90	1.303	0.2750

2.7 Figures

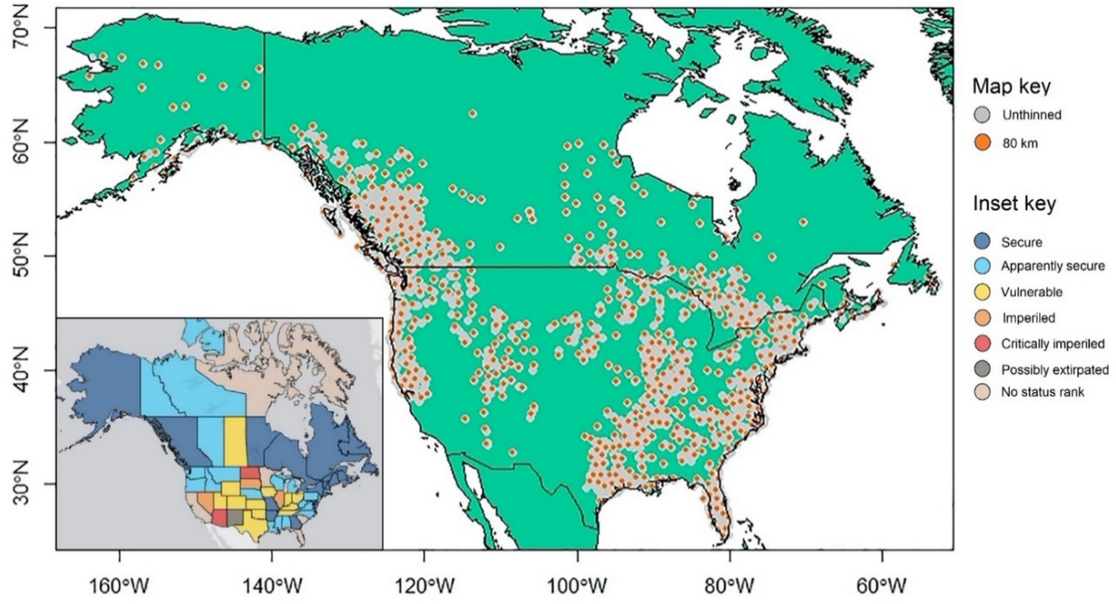

Figure 2.1: Occurrences of *Lontra canadensis*. Grey points are the cleaned unthinned points obtained from BISON, GBIF, and various Fish & Wildlife agencies. Red points are those obtained from an 80km spatial thinning and used in the 80km resolution models. A similar map showing the points used for the 150km resolution models can be found in Supplemental Figure 2. Inset is the distribution of river otters from NatureServe for comparison (NatureServe 2020, https://explorer.natureserve.org/).

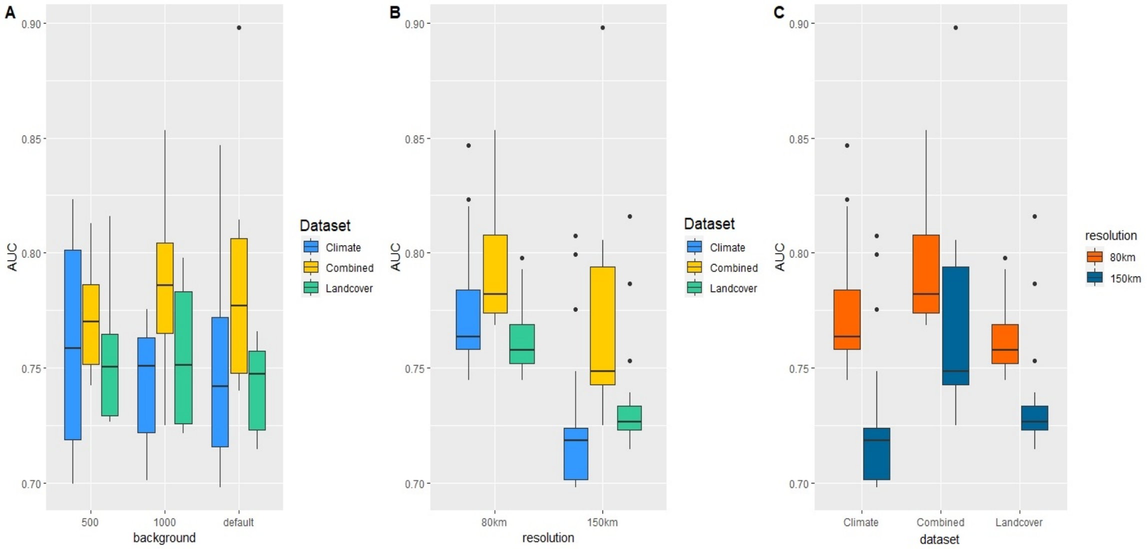

Figure 2.2: AUC plots. A: AUC values of models in each dataset type by number of background points, across both the 80km and 150km resolutions. B: AUC values of models in each dataset type by spatial resolution. C: AUC values of 80 & 150km spatial resolutions by dataset type.

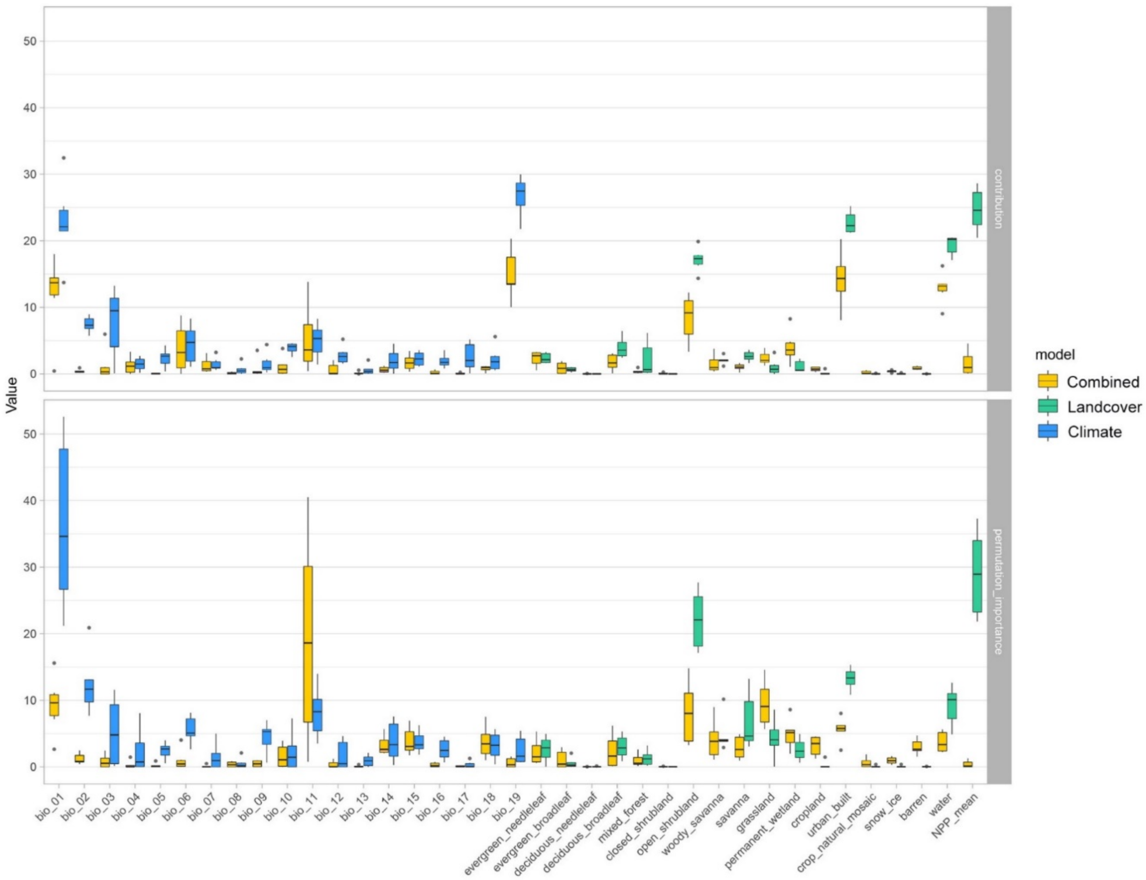

Figure 2.3: Comparison of variable contribution and permutation importance across model types.

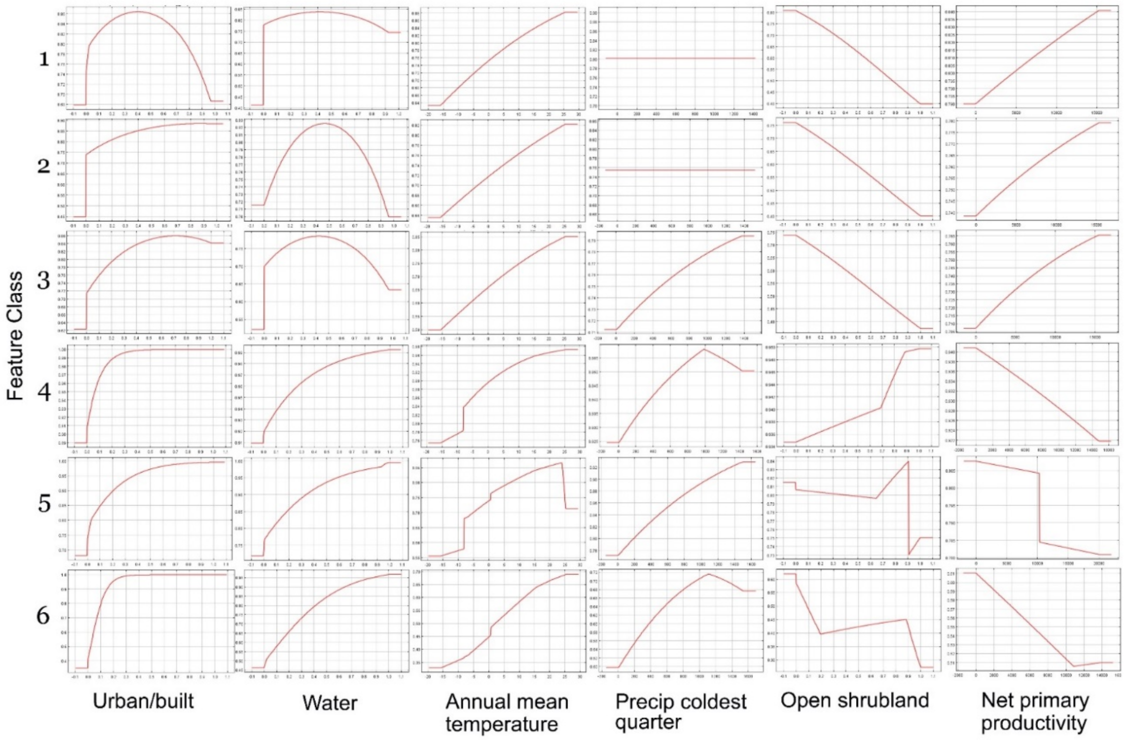

Figure 2.4: Variable response curves for the six most predictive variables (mean contributions over 10%) of the combined dataset, 80km, default background point models. Within a response curve, the X axis is the value of the variable in question, and the Y axis is probability of presence. Each row (1-6) is a different feature class of model. Feature class of each model is listed in Appendix 1.

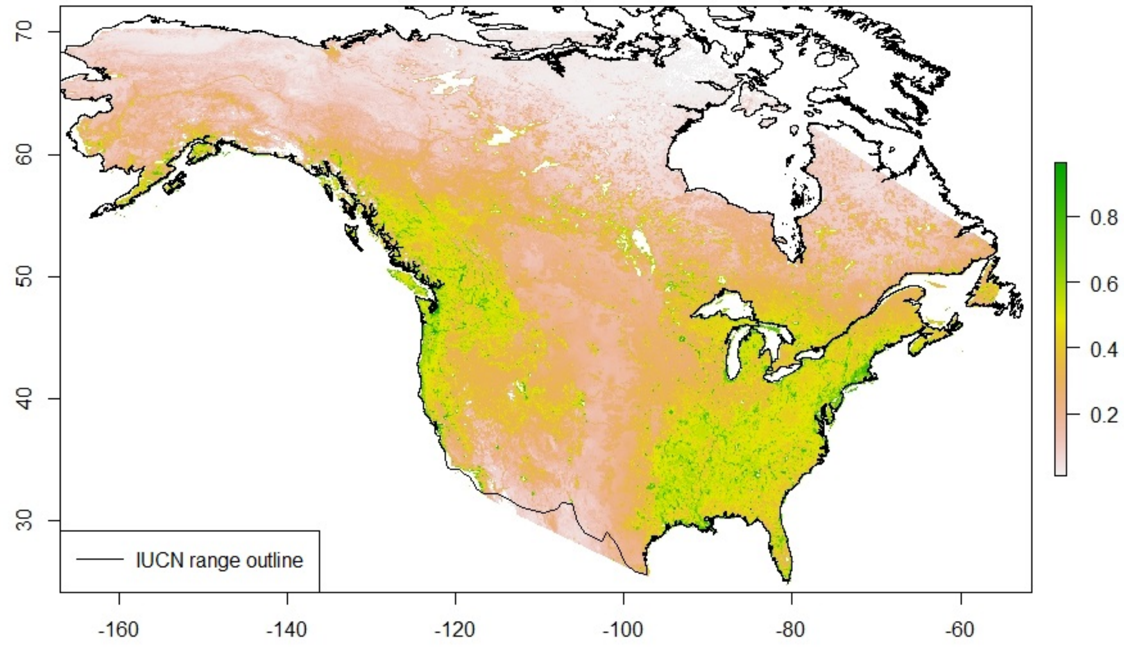

Figure 2.5: The average habitat suitability prediction for the combined dataset model (80km resolution, default background points). Green indicates most suitable habitats, pink to white indicates least suitable. Outline is the IUCN Otter Specialist Group distribution map.

2.8 Appendix

Table 2.8.1: The settings and model performance statistics for the 6 final models. The input settings are shown in the columns indicating the dataset type, the spatial resolution, number of background points (BG points), Maxent feature class settings, and selection criterion used to determine whether it was one of the six final models. Model performance statistics are shown in the Training AUC, Equal TSS thresh (Equal training sensitivity and specificity cumulative threshold), Equal TSS thresh2 (Equal training sensitivity and specificity logistic threshold), Equal TSS area (Equal training sensitivity and specificity area), and Equal TSS omission (Equal training sensitivity and specificity training omission) columns.

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Training AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Climate	80km	500bg	L_2	AICc	0.763	34.452	0.457	0.306	0.306
Climate	80km	500bg	L_2.5	AICc	0.759	34.695	0.458	0.306	0.306
Climate	80km	500bg	LQ_6	AICc	0.745	34.34	0.455	0.32	0.32
Climate	80km	500bg	LQH_2.5	seq	0.758	34.168	0.458	0.311	0.311
Climate	80km	500bg	H_1	seq	0.823	36.798	0.462	0.252	0.252
Climate	80km	500bg	LQH_1	seq	0.819	35.37	0.45	0.262	0.263
Mean					0.778	34.97	0.457	0.293	0.293
Climate	80km	1000bg	LQ_4	AICc	0.753	34.27	0.455	0.313	0.313
Climate	80km	1000bg	LQ_3.5	AICc	0.753	33.968	0.454	0.311	0.311
Climate	80km	1000bg	LQ_5	AICc	0.745	34.584	0.455	0.318	0.318
Climate	80km	1000bg	LQHP_4.5	seq	0.766	33.198	0.441	0.308	0.307
Climate	80km	1000bg	LQHP_3.5	seq	0.762	33.965	0.454	0.302	0.302
Climate	80km	1000bg	LQHP_4	seq	0.767	33.52	0.44	0.303	0.302
Mean					0.758	33.917	0.45	0.309	0.309

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Training AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Climate	80km	defaultbg	LQHP_5	AICc	0.76	34.21	0.455	0.302	0.302
Climate	80km	defaultbg	LQHP_6	AICc	0.764	33.986	0.446	0.302	0.302
Climate	80km	defaultbg	LQHP_4	AICc	0.766	34.025	0.446	0.299	0.299
Climate	80km	defaultbg	LQHP_0.5	seq	0.82	36.1	0.465	0.26	0.261
Climate	80km	defaultbg	LQH_1.5	seq	0.847	36.952	0.448	0.232	0.231
Climate	80km	defaultbg	H_1.5	seq	0.789	35.189	0.455	0.282	0.282
Mean					0.791	35.077	0.452	0.279	0.279
Climate	150km	500bg	L_2	AICc	0.719	37.733	0.468	0.342	0.343
Climate	150km	500bg	LQH_5	AICc	0.702	37.813	0.471	0.355	0.355
Climate	150km	500bg	LQ_6	AICc	0.699	39.283	0.477	0.347	0.347
Climate	150km	500bg	LQH_2	seq	0.717	37.626	0.478	0.343	0.343
Climate	150km	500bg	LQH_1	seq	0.799	41.579	0.482	0.283	0.283
Climate	150km	500bg	LQHP_1	seq	0.808	41.233	0.475	0.27	0.272
Mean					0.741	39.211	0.475	0.323	0.324

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Training AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Climate	150km	1000bg	LQ_2	AICc	0.717	37.292	0.471	0.339	0.34
Climate	150km	1000bg	LQHP_6	AICc	0.724	37.643	0.474	0.328	0.328
Climate	150km	1000bg	LQ_0.5	AICc	0.775	36.867	0.452	0.309	0.309
Climate	150km	1000bg	L_4	seq	0.702	39.799	0.485	0.346	0.347
Climate	150km	1000bg	L_4.5	seq	0.701	39.482	0.472	0.347	0.347
Climate	150km	1000bg	LQHP_1.5	seq	0.749	36.636	0.459	0.325	0.325
Mean					0.728	37.953	0.469	0.332	0.333
Climate	150km	defaultbg	LQ_2	AICc	0.698	38.968	0.469	0.355	0.355
Climate	150km	defaultbg	LQHP_5.5	AICc	0.704	38.774	0.471	0.348	0.347
Climate	150km	defaultbg	LQHP_6	AICc	0.702	39.764	0.474	0.344	0.343
Climate	150km	defaultbg	L_4	seq	0.72	37.945	0.473	0.336	0.336
Climate	150km	defaultbg	L_4.5	seq	0.721	36.938	0.465	0.332	0.332
Climate	150km	defaultbg	L_5	seq	0.724	37.685	0.468	0.328	0.328
Mean					0.711	38.345	0.47	0.34	0.34

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Trainin g AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Landcover	80km	500bg	H_6	AICc	0.749	41.032	0.475	0.327	0.327
Landcover	80km	500bg	H_5.5	AICc	0.752	41.244	0.473	0.32	0.32
Landcover	80km	500bg	H_5	AICc	0.745	33.914	0.427	0.334	0.334
Landcover	80km	500bg	LQH_2	seq	0.763	33.068	0.41	0.312	0.312
Landcover	80km	500bg	H_0.5	seq	0.793	32.687	0.386	0.289	0.289
Landcover	80km	500bg	LQH_0.5	seq	0.77	33.23	0.406	0.312	0.312
Mean					0.762	35.862	0.429	0.316	0.316
Landcover	80km	1000bg	H_6	AICc	0.751	41.423	0.475	0.319	0.32
Landcover	80km	1000bg	H_5.5	AICc	0.751	39.7	0.469	0.328	0.327
Landcover	80km	1000bg	H_5	AICc	0.752	38.983	0.459	0.321	0.321
Landcover	80km	1000bg	LQHP_0.5	seq	0.798	32.45	0.38	0.275	0.275
Landcover	80km	1000bg	LQHP_2.5	seq	0.782	32.386	0.397	0.302	0.302
Landcover	80km	1000bg	LQHP_1	seq	0.792	32.49	0.382	0.287	0.287
Mean					0.771	36.239	0.427	0.305	0.305

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Training AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Landcover	80km	defaultbg	LQH_3.5	AICc	0.757	34.248	0.426	0.318	0.318
Landcover	80km	defaultbg	H_3	AICc	0.757	36.048	0.44	0.319	0.32
Landcover	80km	defaultbg	H_4	AICc	0.755	37.701	0.447	0.316	0.316
Landcover	80km	defaultbg	H_2.5	seq	0.764	35.966	0.429	0.307	0.307
Landcover	80km	defaultbg	H_2	seq	0.766	35.263	0.428	0.307	0.307
Landcover	80km	defaultbg	H_3	seq	0.758	37.235	0.443	0.317	0.318
Mean					0.759	36.077	0.436	0.314	0.314
Landcover	150km	500bg	H_4	AICc	0.728	42.139	0.463	0.353	0.352
Landcover	150km	500bg	H_5	AICc	0.727	43.905	0.472	0.348	0.348
Landcover	150km	500bg	H_5.5	AICc	0.727	44.967	0.483	0.346	0.344
Landcover	150km	500bg	LQH_2.5	seq	0.73	36.402	0.411	0.34	0.34
Landcover	150km	500bg	LQH_0.5	seq	0.816	32.589	0.348	0.274	0.273
Landcover	150km	500bg	LQHP_5	seq	0.753	36.046	0.353	0.309	0.309
Mean					0.747	39.341	0.422	0.328	0.327

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Training AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Landcover	150km	1000bg	LQH_4.5	AICc	0.724	36.622	0.422	0.34	0.34
Landcover	150km	1000bg	H_5.5	AICc	0.721	36.534	0.418	0.348	0.348
Landcover	150km	1000bg	LQH_3	AICc	0.787	31.576	0.315	0.305	0.305
Landcover	150km	1000bg	LQH_5	seq	0.726	36.225	0.417	0.345	0.344
Landcover	150km	1000bg	LQHP_5	seq	0.723	45.165	0.487	0.351	0.352
Landcover	150km	1000bg	LQHP_1	seq	0.735	36.129	0.411	0.344	0.344
Mean					0.736	37.042	0.412	0.339	0.339
Landcover	150km	defaultbg	LQH_3	AICc	0.723	35.597	0.413	0.352	0.352
Landcover	150km	defaultbg	LQH_2	AICc	0.739	36.322	0.404	0.324	0.324
Landcover	150km	defaultbg	LQH_3.5	AICc	0.726	35.365	0.415	0.342	0.344
Landcover	150km	defaultbg	LQ_4.5	seq	0.717	37.273	0.418	0.348	0.348
Landcover	150km	defaultbg	LQ_5	seq	0.723	36.051	0.415	0.345	0.344
Landcover	150km	defaultbg	LQ_5.5	seq	0.714	35.647	0.415	0.352	0.352
Mean					0.724	36.043	0.413	0.344	0.344

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Training AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Combined	80km	500bg	L_6	AICc	0.769	35.944	0.438	0.295	0.294
Combined	80km	500bg	L_5.5	AICc	0.783	35.419	0.423	0.285	0.285
Combined	80km	500bg	L_4.5	AICc	0.779	34.409	0.41	0.293	0.294
Combined	80km	500bg	H_6	seq	0.771	40.85	0.465	0.302	0.303
Combined	80km	500bg	LQHP_5	seq	0.797	32.359	0.401	0.285	0.285
Combined	80km	500bg	LQHP_2.5	seq	0.813	32.437	0.388	0.272	0.271
Mean					0.785	35.237	0.421	0.289	0.289
Combined	80km	1000bg	H_5.5	AICc	0.779	39.513	0.444	0.299	0.299
Combined	80km	1000bg	H_6	AICc	0.772	40.914	0.466	0.301	0.301
Combined	80km	1000bg	H_4.5	AICc	0.776	38.164	0.436	0.299	0.299
Combined	80km	1000bg	H_0.5	seq	0.853	32.372	0.37	0.234	0.234
Combined	80km	1000bg	H_1	seq	0.825	32.622	0.392	0.265	0.266
Combined	80km	1000bg	H_2	seq	0.804	34.807	0.405	0.273	0.273
Mean					0.802	36.399	0.419	0.279	0.279

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Training AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Combined	80km	defaultbg	LQ_4	AICc	0.781	33.679	0.411	0.294	0.294
Combined	80km	defaultbg	LQH_4.5	AICc	0.773	33.976	0.423	0.296	0.296
Combined	80km	defaultbg	LQH_5	AICc	0.771	33.248	0.417	0.298	0.298
Combined	80km	defaultbg	LQHP_3.5	seq	0.805	32.214	0.39	0.275	0.275
Combined	80km	defaultbg	LQHP_2	seq	0.814	32.807	0.378	0.266	0.266
Combined	80km	defaultbg	LQHP_2.5	seq	0.809	30.837	0.364	0.271	0.271
Mean					0.792	32.793	0.397	0.283	0.283
Combined	150km	500bg	H_4	AICc	0.753	42.615	0.435	0.325	0.323
Combined	150km	500bg	H_5	AICc	0.742	46.007	0.474	0.329	0.331
Combined	150km	500bg	H_4.5	AICc	0.745	44.238	0.46	0.319	0.319
Combined	150km	500bg	H_4	seq	0.748	43.38	0.448	0.325	0.327
Combined	150km	500bg	LQH_2.5	seq	0.765	37.394	0.412	0.3	0.3
Combined	150km	500bg	LQHP_2.5	seq	0.799	33.856	0.373	0.276	0.276
Mean					0.759	41.248	0.434	0.312	0.313

Dataset type	Spatial resolution	BG points	Feature Class	Selection criteria	Training AUC	Equal TSS thresh	Equal TSS thresh2	Equal TSS area	Equal TSS omissio
Combined	150km	1000bg	L_6	AICc	0.725	37.092	0.432	0.342	0.342
Combined	150km	1000bg	L_5	AICc	0.745	37.392	0.421	0.327	0.327
Combined	150km	1000bg	L_5.5	AICc	0.725	38.233	0.443	0.339	0.339
Combined	150km	1000bg	LQHP_2	seq	0.806	33.661	0.362	0.268	0.269
Combined	150km	1000bg	LQHP_4.5	seq	0.793	34.513	0.379	0.277	0.276
Combined	150km	1000bg	LQHP_3	seq	0.794	31.958	0.354	0.284	0.284
Mean					0.765	35.475	0.398	0.306	0.306
Combined	150km	defaultbg	H_1.5	AICc	0.799	39.592	0.421	0.28	0.28
Combined	150km	defaultbg	LQ_3.5	AICc	0.749	35.453	0.412	0.322	0.323
Combined	150km	defaultbg	LQH_5	AICc	0.744	36.623	0.428	0.329	0.331
Combined	150km	defaultbg	LQH_0.5	seq	0.898	37.14	0.383	0.187	0.187
Combined	150km	defaultbg	H_6	seq	0.741	48.657	0.479	0.323	0.323
Combined	150km	defaultbg	H_5.5	seq	0.74	46.525	0.476	0.323	0.323
Mean					0.778	40.665	0.433	0.294	0.294

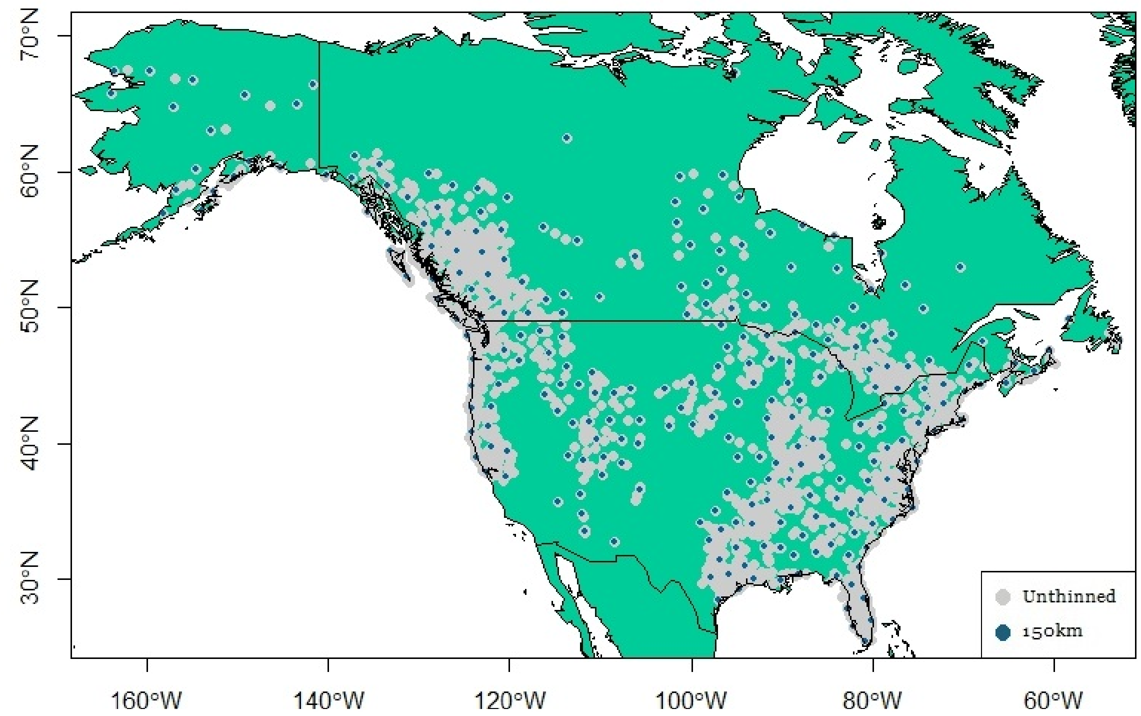

Figure 2.8.1. Occurrences of *Lontra canadensis*. Grey points are the cleaned unthinned points obtained from BISON, GBIF, and various Fish & Wildlife agencies. Blue points are those obtained from an 150km spatial thinning and used in the 150km resolution model

Chapter 3: Patterns of morphological variation in a broadly distributed semiaquatic carnivore, the North America river otter (*Lontra canadensis*)

3.1 Introduction

Variation is the fundamental driver of evolution, and variation both within and between populations is necessary for long-term species persistence. Higher standing variation *within* populations is associated with greater resilience and adaptability in response to change, decreased susceptibility to disease and parasites, higher population viability (reduced or no effects of inbreeding depression), and an increased ability to take advantage of variable or changing resources (Lacy 1997, Hedrick & Kalinowsky 2000, Sommer 2005, O'Grady et al 2006). Higher variation *between* populations is associated with increased species-level fitness, a higher likelihood that some populations will carry adaptive variants to changing environmental conditions, ability to colonize and adapt to multiple environments, and a higher potential for speciation (Guerra et al 1997, Webster et al 2018, Siegismund & Svejgaard Jensen 2001, Turingan et al 1995). As many species face habitat destruction and alteration across broad proportions of their distributions (Pimm et al 2014), as well as ongoing and accelerating impacts from climate change (Blois & Hadly 2009, Urban et al. 2015, Barnosky et al 2017, Dirzo et al 2014), understanding variation, how it is patterned within and between populations, and what its significance is within a species is critical to making informed conservation decisions (Richardson et al 2014, Chown 2012, Stockwell et al 2003, Messer & Petrov 2013, Des Roches et al 2018).

While the source of heritable variation is genetic, variation must be expressed phenotypically to be acted upon by natural selection. Phenotype can be expressed in a variety of ways, but one of the most direct means by which genetic variation interfaces with the environment is through an organism's morphology (Chown 2012, Rockman 2008). Morphology structures many aspects of an organism's existence – it dictates how an organism moves, obtains sustenance, what kinds of sustenance it is capable of obtaining and utilizing, how it reproduces, what predates on it, and how it interacts with its environment and conspecifics (Rockman 2008). Thus, understanding the relationship between variation in a species' morphology and its environment – such as the scale on which variation occurs, what variation is normal, or beneficial, or dependent on resource use – is necessary to understanding the ecological and evolutionary pressures that the species faces (Des Roches et al 2018, Richardson et al 2014). Morphological variation can be due to natural selection, mutation, phenotypic plasticity, historical factors, or genetic drift (Rockman 2008, Mitchell-Olds et al 2007). We cannot attribute morphological variation with complete certainty to any of these drivers based on morphology alone, but exploring how variation is distributed and how it interacts with environment and geography is a necessary first step in understanding a species ecology and adaptive capacity.

Potentially significant morphological variation may occur between populations, within populations or both, and may be maintained for a variety of reasons. Species with broad geographic ranges are expected to have more interpopulation variation than species with smaller ranges for several reasons. First, if populations are far apart from each other they are more likely to be genetically isolated from each other (isolation by distance, IBD). Reduced gene flow creates differentiation between populations regardless of other factors at play due to drift and similar mechanisms (Garant et al 2007). Second, species with broad geographic ranges generally experience greater environmental variation across their range, and are more likely to differentiate in response to this environmental variation (isolation by ecology, IBE) (Shafer et al 2013, Wang et al 2014). These different factors should act to increase morphological variation and population differentiation. Yet, broadly distributed species are often quite generalized ecologically and occur in a variety of habitats, which may act to maintain gene flow between populations and reduce population differentiation. Thus, range size or environmental variation across a range cannot necessarily be used as surrogates for morphological differentiation in broad ranging species. Instead, detailed investigations are needed to determine how variation is structured across the range in broadly distributed species.

North American river otters (*Lontra canadensis*) have a broad distribution: geographically, climatically, and ecologically (Lariviere & Walton 1998). Their range spans most of the North American continent, and connectivity between populations is highly variable (Lariviere & Walton 1998, Van Zyll de Jong 1972, Latch et al 2008). Given these attributes, a great deal of morphological variation is likely to exist across populations, particularly between those that are geographically isolated or experience different ecological conditions. Some evidence suggests this to be true: there are seven recognized subspecies across North America occupying different geographic ranges (Hall 1981, Lariviere & Walton 1998, Van Zyll de Jong 1987). Otters inhabiting different ecosystems tend to have different diets and hunting strategies that may call for different adaptations (Blundell et al 2004, Crowley et al 2013, Roberts et al 2008, Skyer 2006). Other evidence, however, calls this assumption into question: the subspecies categories have not been validated with modern techniques, and behavioral studies suggest that otters are quite flexible behaviorally, indicating behavioral modifications across environments might be a preferred adaptive strategy to morphological differentiation (Mowry et al 2015, Crowley et al 2013, Skyer 2006). Further, extensive reintroductions of river otters occurred in the 1980's and 1990's after local extirpations in parts of North America (Raesly 2001, Mowry et al 2015), in which members of one population of otters were relocated to a location with an entirely different ecology and climate. Eighteen of the 19 reintroduction locations maintained stable populations, regardless of difference between the original and novel habitats (Raesly 2001), suggesting very little local adaptation to specific environments. Here, we examine whether there is consistent morphological variation and structure in *Lontra canadensis* across subspecies or geographically-based groups, and, if there is, whether it is explained best by isolation by distance or isolation by ecology, in order to shed light on the role of morphology in otter relationships to their environments. We chose to measure crania because cranial shape has implications for locomotion and feeding strategies (Timm-Davis et al 2015, Jones et al 2015) and because this is the primary skeletal element museums retain.

3.2 Methods

3.2.1 Specimen Selection & Acquisition

In order to adequately capture variation across the species range, we aimed to sample at least 100 intact river otter crania (Hernandez-Romero et al 2015, Bolnick et al 2011). The goal was to provide the broadest spatial sample possible while also capturing potential variation within a region. We attempted to implement a systematic approach to spatial selection of specimens (such as a grid), but found that specimens were too unevenly distributed in space and time to apply such a selection process. Thus, we aimed for higher sampling density in a handful of locations (Pacific Northwest, Michigan, Florida) where there was an abundance of specimens, and a scattershot approach aimed at capturing specimens across as much geographic and environmental space as possible where there were few. We also aimed to sample at least five individuals from each of the subspecies identified by Hall (1981, also Lariviere & Walton 1998) (Hernandez-Romero et al 2015, Noback et al 2011). Our focus on the different subspecies is partially important to ensure sampling of previously identified variants, and to assess variation across these subspecies using modern methods. Parts of the historical range (such as Arizona and New Mexico) currently have very few populations of otters, but represent unusual habitats otters have historically occupied compared to currently occupied locations. Thus, we sampled specimens originally collected from any time over the last 100 years, in order to determine whether individuals in these extirpated populations had unique morphological characteristics. We also avoided sampling reintroduced animals, as we are interested in the evolutionary and relational significance of cranial shape and variation. Reintroduced animals did not arrive in their present locations naturally and they may not have occupied their present locations long enough to have experienced significant adaptation. This requirement eliminated animals from the last 40 years in many areas.

3.2.2 Data Collection & Processing

We surface scanned crania using a Geomagic Capture Mini 3-D scanner and turntable. We assembled and cleaned the 3-D images of the crania using Geomagic Wrap (3D Systems), then exported them as .ply files for use in IDAV Landmark Editor. We landmarked the crania using the Landmark application. We landmarked 48 points, 14 curves, and 2 patches, and exported the coordinates as .dta files for analysis in R.

We used the geomorph R package (version 3.3.1, Adams et al 2020) to perform a general Procrustes analysis (GPA) to align all specimens to the same orientation and centroid size. All subsequent analyses were performed on Procrustes-aligned data. We then performed several analyses to explore strength and direction of shape variation.

3.2.3 Aim 1 Analyses

We explored patterns in cranial morphology to examine how morphology varied across specimens (e.g., by sex) and whether it was consistent with previously identified subspecies. This was done using a variety of methods. We first performed a linear discriminant analysis (LDA) with leave-one-out cross-validation to examine group membership among crania by subspecies (as assigned by the museum or when the individual was catalogued), subspecies ((as assigned by location – see Fig. 1) Hall 1981, sex (to account for sex variation in other analyses), and centroid size (to determine whether individual size had an effect on overall shape). We also performed a Procrustes ANOVA to explore how cranial shape varies across specimens. While LDAs and ANOVAs are both methods that attempt to find linear combinations of measurements that summarize how one variable (e.g., shape) relates to subspecies or sex, LDA does this by utilizing categorical dependent variables and continuous independent variables. ANOVA does this by using categorical independent variables and a continuous dependent variable. In effect LDA tests the hypothesis that discrete categories, such as subspecies, are real and attempts to assign specimens to the appropriate category. ANOVA explores the variation present and examines statistically partitioned data, and how well the partitions correspond to the independent variables. Conducting multiple tests to explore how variation relates to discrete variables may be beneficial, particularly given the uncertainty of the subspecies categorizations. We also conducted a principal components analysis (PCA) of GPA. This analysis used the transformed shape coordinates to explore shape variation without assigning it a cause.

3.2.4 Aim 2 Analyses

We explored whether variation in cranial morphology was better explained by geographic distance or by environmental distance between specimens. We created distance matrices for geographic distance, climatic distance, and environmental distance. For geographic distances we extracted coordinate values at specimen locations and calculated straight line distances in meters. For climatic distances we downloaded the WorldClim 2 (http://www.worldclim.org/, Fick, S.E. and R.J. Hijmans, 2017) bioclimatic dataset in 2.5 arcminute grid cells in 2018. The WorldClim 2 dataset is a standard set of 19 historical climatic variables that capture different aspects of the climate system (Table 2). We extracted values for each of the 19 bioclimatic variables at specimen locations and calculated Euclidean distances between climatic values across specimen localities. For ecological distances, we used the MODIS Land Cover Dynamics Product MCD12Q1.006 from Land Process Distributed Active Archive Center (LPDAAC) in 2018. This assigns fine-scale grid cells to one of 17 different land cover type variables (Table 3). We decreased the resolution of the landcover layers from 500m to 2.5 arcminutes to match the resolution of the climate layer by aggregating each land cover variable to a percent cover of the variable across the new grid cell. We thus converted the single landcover product into 17 different variables (one raster for each of the land cover types), representing percent cover of each variable. We then extracted values for each of the 17 different landcover types at specimen locations and calculated Euclidean distances between ecological values across specimen localities. For morphological distance, we calculated Euclidean distances between GPA-aligned coordinate values of each specimen.

We initially tested a hypothesis of isolation by distance conducting Mantels test between morphological distance and geographic, climate, and ecological distances separately using the R package ecodist (version 2.0.7). We then conducted a Multiple Matrix Regression with Randomization (MMRR, Wang 2013) between distance matrices of morphological, geographic, climate, and ecological distances using code from (cite author/paper). This analysis allows us to test the relative influence of isolation by distance vs isolation by ecology on morphological differentiation.

3.3 Results

We 3D scanned 100 specimens from across the distribution of *Lontra canadensis,* though sampled specimens were very unevenly distributed across space and time due to the sampling restrictions and challenges outlined above. Sampling 5+ individuals per subspecies was not possible due to the sampling constraints: several specimens we received were ultimately unscannable due to damage, which meant that we were unable to acquire 5 scannable specimens for *L. canadensis kodiakensis, L. canadensis periclyzoma,* and *L. canadensis sonora* (Table 4).

3.3.1 Aim 1 Results

Accuracy for LDA-predicted group memberships is low, and lower with cross-validation. With cross-validation, LDA accuracy of group membership was 7.45% for museum-assigned subspecies, meaning that for location-assigned subspecies, only 7.5% of specimens were correctly assigned to their original location-based group. Accuracy was slightly better for other attributes: 15.15% for location-predicted subspecies, 29.487% for sex, and 14.49% for centroid size. These results indicate that LDA does not accurately predict group membership by museum-assigned subspecies, location-assigned subspecies, or centroid size. Specimen sex seems to be a slightly better indicator of shape, but still only 30% of individuals can be correctly classified by sex using this metric.

We conducted Procrustes ANOVAs on assigned subspecies, location-based subspecies, and sex (Table 5). All variables are significant at a 95% CI, however the percentage of shape variation explained by the different independent variables (R^2) is low. Sex explains 4% of shape variation, location-based subspecies explains 11.12% of shape variation, and museum-assigned subspecies explains 21.76% of shape variation. Procrustes ANOVAs are sensitive to the number of independent variables utilized, and since sex has two, location-based subspecies has seven, and museum-based subspecies has 17 groups, it is unsurprising that museum-based subspecies appears to explain a greater proportion of shape variation.

We also conducted a principal components analysis (PCA) of cranial shape on the GPA-aligned coordinates. PC1 explains 11% of the shape variation. There is clearly shape variation across specimens (Fig 2), and while certain location-assigned subspecies occupy slightly different shape spaces than others, there is too much overlap between members of subspecies, and too much distance between individual members of a subspecies, to identify a specimen to subspecies.

3.3.2 Aim 2 Results

There is an overall weak negative correlation between geographic distance and morphological distance (Mantel test: r = 0.1586, p = 0.001). However, there seems to be slightly different relationship between geographic and morphological distance across geographic distance classes (Fig 3): there is a weak but significant positive correlation (r= 0.11, p=0.001) at distances of less than 500 km, non-significant and neutral relationships for most distance classes, and then a significant but weak negative correlation (r= -0.05, p=0.001) at large distances, around 6,000km. There is no significant relationship between either climate or ecological distances and morphological distance.

MMRR results indicate a similar pattern to the Mantel test results, with weakly significant relationships between morphological distance and geographic distance. Overall, only 3% of morphological variation is explained by the combination of geographic distance, climate distance, and ecological distance (R^2 = 0.0309). Only geographic distance is significantly associated with morphological distance (p=0.001); ecological distance and climate distance are not significant. Plots of morphological distance vs geographic, ecological, and climate distance indicate relatively even distributions of morphological differentiation across each of these spaces (Fig 4).

3.4 Discussion

Environments are variable across space, and increasingly variable through time; characterizing morphological variation across a species range is important for understanding the functional ecological and evolutionary consequences of this environmental variation. The river otter’s broad geographic and environmental range and variable habitat connectivity across the range led us to hypothesize that there would be high inter-regional variation in morphology in this species. The categorization of seven distinct subspecies (Hall 1981, Koepfli & Wayne 1998) and variation in diet and hunting strategies across the range seemed to support this hypothesis, though successful introductions of otters into ecosystems outside their subspecies range (Raesly 2001, Mowry et al 2015), and recorded variation in diet and hunting strategies within populations called this hypothesis into question (Blundell et al 2004, Crowley et al 2013, Roberts et al 2008, Skyer 2006). Generally the relationships of otters to their habitats and the functional value of variation in this species are still not well understood. Thus, the aim of this study was to examine whether there is consistent morphological variation and structure in river otter crania, and if so whether it is best explained by isolation by distance or isolation by ecology.

Overall, our analyses show that there is a significant amount of morphological variation among members of *Lontra canadensis*. However, there is very little definable structure to that variation: a small amount of this variation may be explained by sex, but the sexes are not morphologically distinct categories (LDA, PCA, and ANOVA). Museum-assigned subspecies and centroid size do not explain patterns of shape variation. Location-assigned subspecies does not significantly explain shape variation either, and no distinct groups or strong shape trends can be elucidated from the PCA plots. While subspecies are named based on a variety of factors, these results bolster the lack of support for the historically defined subspecies and suggest that examination of genetic variation and gene flow will be needed, as well as further examination of teeth and postcranial elements, to determine whether there are any definable subspecies within *Lontra canadensis* and if so, the appropriate subspecies boundaries. Genetic experiments from other species, and more localized experiments in *L. canadensis* (Mowry et al 2015, Latch et al 2008, Brandt et al 2013, Ben-David & Golden 2009, Trinca et al 2012, Vianna et al 2010, 2011) suggest that populations are likely more genetically distinct from each other than they appear morphologically, though there is a notable gap in work on river otter genetics.

Similarly, there is a weak but significant, and potentially complex, relationship between geographic distance and morphological variation. It appears that morphological distance between specimens is highest at short distances (around 500 km), and generally non-significant at greater geographic distances. This indicates the possibility that diversifying selection across smaller spatial scales is more important than differentiation across broader populations. Otters may be differentiating morphologically between local habitats. However, we found no relationship between morphology and either climate or landcover variables, which potentially indicates that our variables are not adequately capturing ecological or habitat variables that matter to otters, such as size or structure of waterways. Further, there is no relationship between morphology and either climate or landcover attributes. A similar pattern has been found in the tropical soft grass mouse (*Akodon mollis*) (Alvarado-Serrano et al 2013), with high morphological variance among individuals, and low variance across habitats. This species has a similarly broad geographic and environmental range, and is apparently morphologically adapted to persist across a variety of environments. Puzachenko et al (2017) identified a similar pattern of high cranial morphological variation with no discernible relationship to climate or environment in marbled polecats.

Overall, there are no strong discernable drivers influencing morphological variation across North American river otters. Generally, this aligns with previous conclusions (DeNeve Weeks 2020, Chapters 1 & 2) that river otters are highly robust to living in a variety of environments and climates without undergoing major adaptive changes. For example, fossil evidence indicates that river otters have changed very little in the last ~2 million years (Kurten & Anderson 1980, Lariviere and Walton 1998, Prassack 2016). It may be that the river otter body plan is relatively well adapted to persist across a variety of semi-aquatic environments and thus it remains morphologically relatively constant over broad time and space. Additional support for the generality of the species comes from reintroduction experiments, which indicate that it is possible for otters to persist when moved from one habitat to an extremely different habitat (Raesly 2001). An alternative explanation is that our ecological and climatic variables do not adequately capture variables that matter to otters (DeNeve Weeks 2020, Chapter 2). We used 16 variables that primarily capture terrestrial landcover categories. Focusing on the aquatic variables instead may improve characterization of ecological factors influencing otter morphology.

Morphological variation across small scales, however, may be important to maintaining diversity in how otters interact with their environments, for example through individual otters specialization at a microhabitat scale to aspects of local environments**.** Newsome et al (2009) found that sea otters (*Enhydra lutris)* are diet generalists as a species or population, and diet specialists as individuals, where an individual otter specializes in a much smaller subset of prey types than the population overall consumes. Bolnick et al (2003) suggest that resource specialization on small scales is not an uncommon phenomenon in taxa where there is a variety of less-abundant prey species that may all require optimization of slightly different hunting strategies to effectively utilize (see also: Ferry-Graham et al 2oo2, Svanback & Bolnick 2005). It is possible that something similar is happening in river otters. It is known that in some habitats different otters use different hunting strategies than others (Blundell et al 2004, Crowley et al 2013, Roberts et al 2008, Skyer 2006), so river otters may maintain high levels of variation at local scales to take advantage of different microhabitats or prey types.

We generally expect that species with a broad geographic range which includes a variety of environment types will demonstrate adaptively-significant morphological variation across that range (Feijo et al 2020, Puzachenko et al 2017). However, while we found morphological variation across otters, we did not find much population structure in that variation, indicating that there is no discernable adaptive significance for morphological variation. It is possible that species that have good dispersal abilities and habitat connectivity (such as medium- or large-bodied carnivores) may generally demonstrate less differentiation in morphological variation across their ranges than expected. This is potentially good news from a conservation/reintroduction standpoint, as it may mean that genetically discrete populations are less functionally different than they appear to be. While preservation of genetic diversity is critical to a species continuing to survive across change, if populations of medium-large carnivores experience less functional variation across their ranges than expected, habitat mismatch or inbreeding depression in reintroductions and conservation activities may be less of a concern than originally thought in these populations.

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3.6 Tables

Table 3.1: Specimens sampled.

Museum ID*	Museum assigned subspecies	Location based subspecies	Latitude	Longitude	State	Sex	Collection Year
AMNH_184646	canadensis	canadensis	45.55	-78.6	Ontario	female	1960
AMNH_243525	pacifica	pacifica	43.65889	-123.3175		male	1970
AMNH_243650	pacifica	pacifica	44.75361	-123.4128	Oregon	female	1971
AMNH_243839	pacifica	pacifica	42.3996	121.8777	Oregon	male	1970
AMNH_255659		lataxina	26.945	-81.2838	Florida	female	1981
AMNH_275986		lataxina	41.88694	-75.41278	Pennsylvania	male	1968
CRCM_49_2		pacifica	46.5584	-122.275	Washington	female	1948
CRCM_49_398		pacifica	46.6195	-122.456	Washington	female	1949
CRCM_49_400		pacifica	46.5181	-123.827	Washington	male	1949
CRCM_49_4433		pacifica	47.3225	-122.311	Washington	female	1992
CRCM_49_75		pacifica	47.979	-122.202	Washington	female	1949
CRCM_49_77		pacifica	47.979	-122.202	Washington	male	1949
CRCM_52_49		pacifica	46.90549	-124.124	Washington	male	1952
CUMV_8356	degenera	canadensis	49.2828	-56.8245	Newfoundland		1953
CUMV_9942	canadensis	lataxina	44.43867	-74.2527	New York	female	1958
CUMV_9947	canadensis	lataxina	43.92972	-75.5894	New York	male	1958
FMNH_129320	canadensis	canadensis	44.49861	-88.18278	Wisconsin	female	982

Museum ID*	Museum assigned subspecies	Location based subspecies	Latitude	Longitude	State	Sex	Collection Year
FMNH_167045	lataxina	lataxina	40.359167	-91.434444	Illinois	female	1993
FMNH_167198	canadensis	canadensis	44.1917	-89.2506	Wisconsin	female	1994
FMNH_20168	periclyzoma	pericly	53	-132	British Columbia		1903
HSU_2941		pacifica	40.80194	-124.10333	California		2006
HSU_2989		pacifica	40.86667	-124.08167			2007
ISM_64670	canadensis	lataxina	41.86721	-90.1011	Illinois		1972
ISM_683788	canadensis	canadensis	47.8525	-92.6894	Minnesota		1955
ISM_684079	canadensis	canadensis	48.61667	-93.4167	Ontario		1959
ISM_686661	texensis	lataxina	29.7975	-93.325	Louisiana		1977
KU_2075	lataxina	lataxina	38.96	-95.21	Kansas	female	1882
MCZ_14395	pacifica	mira	49.66667	-125.833	British Columbia		1911
MCZ_34164	preblei	pacifica	60.989	138.1707	Yukon Territory	female	1936
MCZ_3537	lataxina	lataxina	35.83688	-78.5314	North Carolina	male	1894
MCZ_3538	lataxina	lataxina	35.83688	-78.5314	North Carolina	female	1894

Museum ID*	**Museum assigned subspecies**	**Location based subspecies**	**Latitude**	**Longitude**	**State**	**Sex**	**Collection Year**
MCZ_5638	canadensis	canadensis	44.73333	-65.51667		male	1896
MCZ_61896	canadensis	canadensis	42.69028	-70.6417	Massachusetts	male	1995
MCZ_62291	canadensis	canadensis	44.42583	-69.0069	Maine	female	1988
MCZ_7405	canadensis	canadensis	57.43333	-62.4167	Newfoundland and Labrador		1899
MCZ_7434	evexa	pacifica	49.06667	122.1167	British Columbia	female	1896
MCZ_7589	chimo	canadensis	55.08333	-59.1833	Newfoundland and Labrador		1901
MCZ_7591	preblei	canadensis	56.33333	-108.333	Newfoundland and Labrador		1901
MCZ_8848	preblei	canadensis	51.5258	56.8336	Newfoundland and Labrador	male	1899
MSB_138109	extera	pacifica	56.84417	-158.774	Alaska	female	1971
MSB_141050	optiva	pacifica	60.16667	-150.25	Alaska		2005
MSB_141051	optiva	pacifica	60.16667	-150.25	Alaska		2005
MSB_142906	nexa	pacifica	44.66361	-114.031	Idaho	male	2003

Museum ID*	Museum assigned subspecies	Location based subspecies	Latitude	Longitude	State	Sex	Collection Year
MSB_142921		pacifica	42.12944	-113.342	Idaho	male	2002
MSB_145873		pacifica	44.55278	-116.69222	Idaho	female	2002
MSB_228335		mira	42.88056	-115.79639	Idaho	female	2006
MSB_231452	pacifica	pacifica	65.2333333	-127.033333	Northwest Territories	male	2003
MSB_247546	periclyzoma	mira	55.63194	-132.9075	Alaska	male	
MSB_276133	periclyzoma	mira	56.95139	-134.948	Alaska	male	1998
MSB_276178	sonora	sonora	56.95139	-134.948	Alaska	female	1998
MSB_40072	sonora	sonora	35.07842	-106.663	New Mexico	female	1979
MSB_61218	nexa	pacifica	32.17528	-104.443	New Mexico	female	1981
MSB_92668	lataxina	lataxina	29.7000587	-93.9382757	Texas	male	1800
MVZ_19087	periclyzoma	pericly	53.1597751	-132.126688	British Columbia	female	1999
MVZ_19088	periclyzoma	pericly	53.1597751	-132.126688	British Columbia	male	1999
MVZ_25554	preblei	lataxina	45.0327	-110.7052		female	1917

Museum ID*	Museum assigned subspecies	Location based subspecies	Latitude	Longitude	State	Sex	Collection Year
MVZ_53503	evexa	pacifica	52.4667	-125.3167	British Columbia		1999
MVZ_61451	sonora	sonora	34.965427	-114.635501	California	male	1999
OMNH_53981	interiorensis	lataxina	36.16472	-89.4903	Tennessee	female	1982
OMNH_53988	interiorensis	lataxina	35.30046	-90.0898	Tennessee	male	1977
PSM_1084		pacifica	47.6564	-124.17	Washington		1940
PSM_12982		pacifica	46.635	-119.451	Washington	male	1968
PSM_24153		pacifica	47.35528	-123.1	Washington		1976
PSM_26372		pacifica	47.08885	-123.146	Washington	male	1977
PSM_2669		pacifica	47.95778	-124.365	Washington	female	1950
PSM_2670		pacifica	47.95778	-124.365	Washington	male	1950
PSM_2671		pacifica	47.95778	-124.365	Washington		1950
PSM_28459		pacifica	47.27917	-122.25083	Washington	male	1987
PSM_5167		pacifica	43.984	-122.164	Oregon	male	1914
ROM_19449		canadensis	47.45	-83.266667	Ontario	female	1948
ROM_20102		canadensis	48.48272	-84.95438	Ontario		1949
ROM_85351		canadensis	45.35	-79.28333	Ontario	male	1980

Museum ID*	Museum assigned subspecies	Location based subspecies	Latitude	Longitude	State	Sex	Collection Year
ROM_R9126		canadensis	47.833333	-83.4	ontario	female	1964
UAM_101245		mira	55.6288889	-131.971111	Alaska	female	2002
UAM_115822	kodiak	pacifica	57.089	-153.871	Alaska	male	2012
UAM_117483	extera	kodiacensis	57.7913	-155.329	Alaska	female	2013
UAM_24789	yukon	pacifica	64.73333	-156.933	Alaska	male	1993
UAM_32654		kodiacensis	59.45	-151.4	Alaska	female	2000
UAM_37067		pacifica	64.5	-165.416667	Alaska	female	2000
UAM_44547		pacifica	62.9666667	-144.883333	Alaska		2000
UAM_47108		pacifica	65.4666667	-165.783333	Alaska	female	2000
UAM_52538		pacifica	63.883	-152.233	Alaska	female	1985
UAM_92622		mira	55.5294444	-131.953333	Alaska	male	2002
UARK_87_138_146		lataxina	33.1285	-93.178	Arkansas	male	1984
UARK_87_138_67		lataxina	35.1012	-92.7677	Arkansas	female	1983
UF_20770	lataxina	lataxina	32.85528	-82.95	Georgia		1978
UF_32855		lataxina	27.8764	82.7779	Florida	male	1980
UF_32871		lataxina	30.441935	-82.94667	Florida	female	1981
UMMZ_173554		canadensis	44.5463	-84.5396	Michigan	male	1997

Museum ID*	Museum assigned subspecies	Location based subspecies	Latitude	Longitude	State	Sex	Collection Year
UMMZ_173600		canadensis	45.6002	-87.4795	Michigan	female	1996
UMMZ_173606		canadensis	46.0783	-88.6246	Michigan	female	1997
UMMZ_173637		canadensis	45.3514	-83.48889	Michigan	female	1997
UMMZ_173645		canadensis	46.3096	-84.7913	Michigan	male	1997
UMMZ_173656		canadensis	46.3983	-88.148	Michigan	male	1997
UMMZ_174215		canadensis	43.6203	-85.22639	Michigan	male	1994
UWBM_39350		pacifica	48.5994	-123.145	Washington	female	1976
UWBM_39358		pacifica	47.5934	-122.841	Washington	female	1990
UWBM_39359		pacifica	47.9172	-122.704	Washington	male	1992
UWBM_76894		pacifica	48.1181	-123.4307	Washington	male	1993

* AMNH = American Museum of Natural History; CRCM = Charles R. Conner Museum; CUMV = Cornell University Museum of Vertebrates; FMNH = Field Museum of Natural History; HSU = Humboldt State University Museum; ISM = Illinois State Museum; KU = University of Kansas Natural History Museum; MCZ = Harvard Museum of Comparative Zoology; MSB = University of New Mexico Museum of Southwest Biology; MVZ = Berkeley Museum of Vertebrate Zoology; OMNH = Sam Noble Museum at University of Oklahoma; PSM = Slater Museum of Natural History; ROM = Royal Ontario Museum; UAM = University of Alaska Museum of the North; UARK = University of Arkansas Museum; UF = Florida Museum of Natural History; UMMZ = University of Michigan Museum of Zoology; UWBM = University of Washington Burke Museum.

Table 3.2: WorldClim variables (www.worldclim.org) used to calculate climatic distance.

WorldClim Variable	Bioclimatic Variable
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Temperature Range
BIO3	Isothermality
BIO4	Temperature Seasonality
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Table 3.3: Landcover variables used to calculate environmental distance, from the Land Process Distributed Active Archive Center (lpdaac.usgs.gov)

Layer name	Landcover variable
evergreen_needleleaf	Evergreen needleleaf forest
evergreen_broadleaf	Evergreen broadleaf forest
deciduous_needleleaf	Deciduous needleleaf forest
deciduous_broadleaf	Deciduous broadleaf forest
mixed_forest	Mixed forest
closed_shrubland	Closed shrubland
open_shrubland	Open shrubland
woody_savanna	Woody savanna
savanna	Savanna
grassland	Grassland
permanent_wetland	Permanent wetland
cropland	Cropland
urban_built	Urban built
crop_natural_mosaic	Crop & natural mosaic
snow_ice	Snow & ice
barren	Barren
water	Water
NPP_mean	Net primary productivity

Table 3.4: Number of individuals scanned per location-based subspecies

Subspecies	Number of specimens
L. canadensis canadensis	24
L. canadensis kodiakensis	2
L. canadensis lataxina	19
L. canadensis mira	6
L. canadensis pacifica	42
L. canadensis periclyzoma	3
L. canadensis sonora	3

Table 3.5: Procrustes ANOVA results of museum-assigned subspecies, location-based subspecies, and sex. The asterisks after the p-value indicate the level of significance: *p ≤ 0.05 and **p ≤ 0.001.

	DF	**SS**	**MS**	**Rsq**	**F**	**Z**	**p**
Assigned subspecies	16	0.0442	0.0028	0.2176	1.4254	4.4251	0.001**
Location subspecies	6	0.0227	0.0038	0.1116	1.9257	6.0386	0.001**
Sex	3	0.008	0.0027	0.0395	1.3004	1.8511	0.038*

3.7 Figures

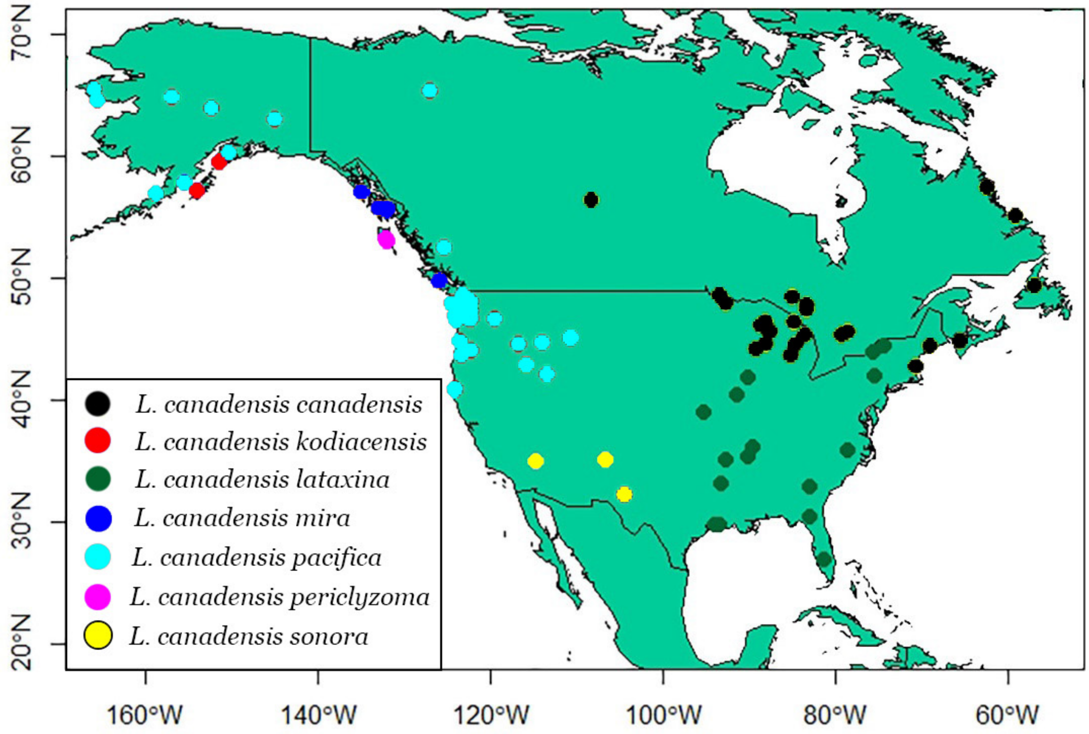

Figure 3.1: Specimens sampled by location-based subspecies.

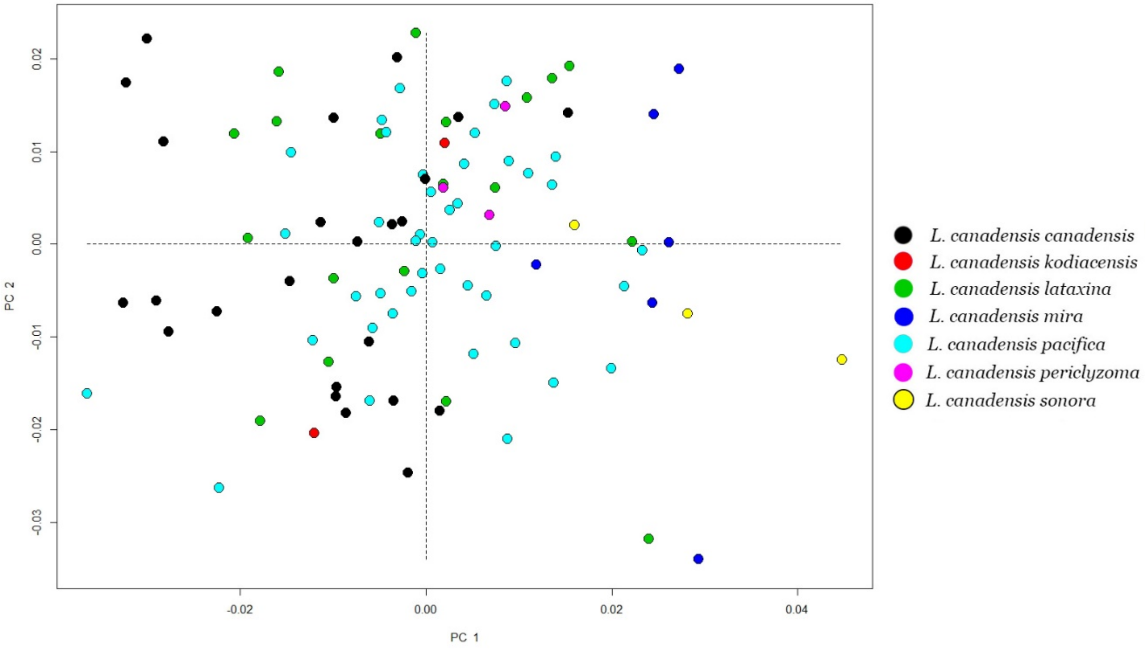

Figure 3.2: PCA plot of GPA-aligned coordinates of location-based subspecies

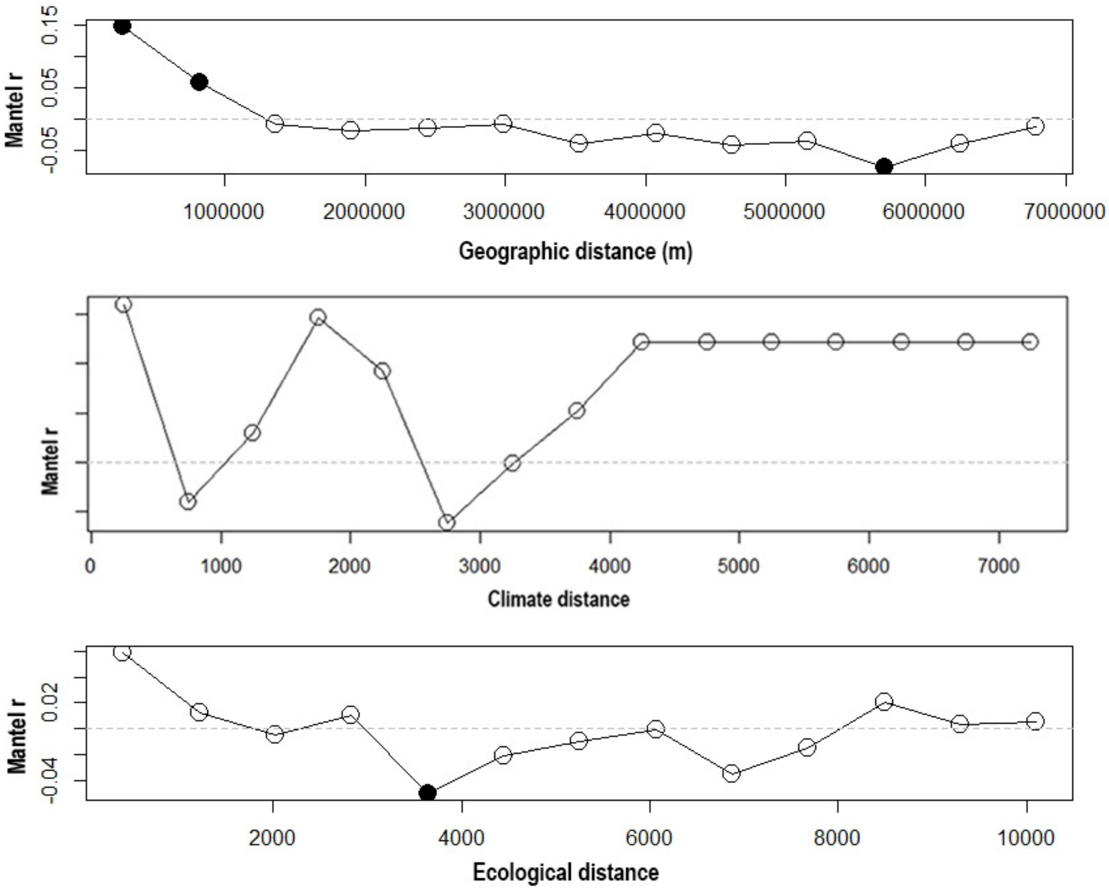

Figure 3.3: Mantel test of geographic distance, climate distance, and ecological distance vs morphological distance. Independent variable distance is on the X axis. The Y axis is the Mantel r value. Shaded circles indicate a significant relationship between geographic and morphological distance. Positive values indicate positive associations, and negative values indicate negative associations.

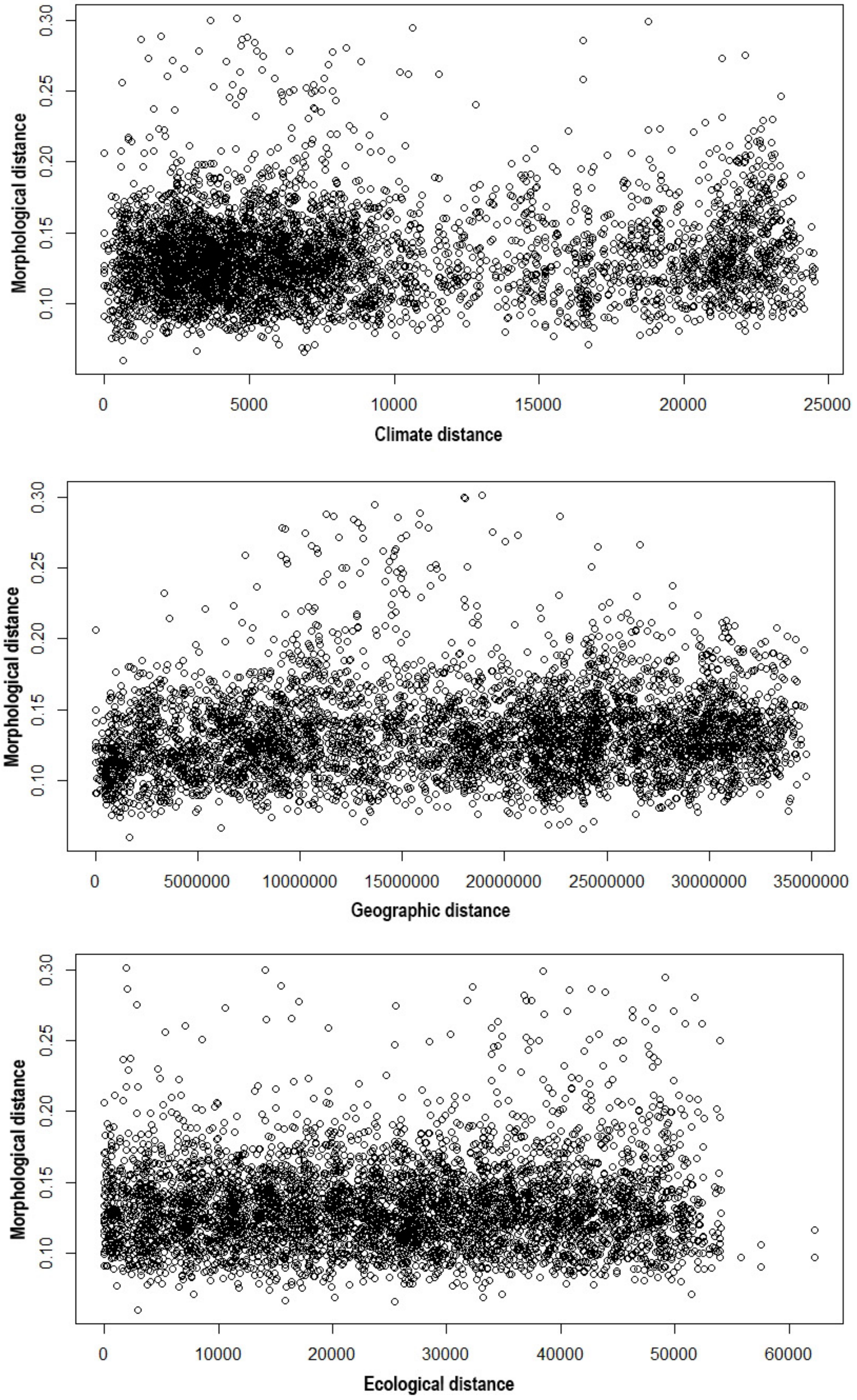

Figure 3.4: Multiple Matrix Randomized Regression geographic, climatic, and ecological distances vs morphological distance.