Contemporary and historic causes of biogeographic gradients in genetic diversity

by

Chloé Schmidt

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

Department of Biological Sciences

University of Manitoba

Winnipeg

Copyright © 2021 by Chloé Schmidt

Abstract

Genetic diversity is the most fundamental level of biodiversity, yet little is known about its broad spatial structure across taxa. Spatial variation in species richness, on the other hand, is the most notable and well-described biogeographic pattern in nature, but our understanding of its causes remains underdeveloped. Linking pattern to process requires shifting focus from the species level to population genetic diversity—the level at which evolution acts. Until recently, it was not possible to incorporate estimates of genome-wide diversity indicative of population processes into analyses of species richness patterns due to a lack of comparable multi-species, population-level data. To address this gap, I compiled publicly archived, raw, neutral, nuclear molecular genetic data to build an aggregated database of metrics of genetic composition in North American terrestrial vertebrates comprised of 99 species (44 mammal, 25 bird, 19 amphibian, and 11 reptile), totaling 58,946 individual genotypes from 1,682 sample sites across the United States and Canada. I hypothesized that these genetic and species levels of biodiversity are closely connected by demographic processes related to environmental carrying capacity determined by environmental energy availability, niche heterogeneity, and habitat loss and fragmentation due to human causes. I used statistical approaches to detect patterns of spatial structure and structural equation modelling to simultaneously analyze environmental effects on genetic and species levels. In general, genetic diversity and population connectivity were consistently negatively affected by habitat degradation both across urban-rural gradients and within urban areas. Accordant with known patterns, species diversity increased with energy and niche availability. Populations tended to have lower genetic diversity and were more genetically differentiated in species richness hotspots. Overall, these results suggest that genetic diversity and species richness are jointly affected by environmental carrying capacity related to historic and contemporary factors, but these relationships do not always hold across taxonomic groups. Understanding the relationships between genetic diversity, species richness, and environments is important because they contribute to ecosystem resilience in changing environments. The wealth of raw genetic data now available is exciting because of the new opportunities for exploring previously hidden levels of biodiversity it brings, and its value as a conservation tool.

Acknowledgements

First, I owe many thanks to my supervisor Colin Garroway for being unrelentingly positive these 4 years, and making research way more fun than I imagined it'd be. I'm grateful to my committee members Jay Treberg, Kevin Fraser, and Aleeza Gerstein for their input and guidance along the way. I also thank the past and present members of the PEEG crew, especially Riikka Kinnunen (and the 'squirrel girls', and the 'picnic-ers'), for many wacky days of squirrel-catching, of which unfortunately none made it into this thesis. It's like the old saying goes, you never know where your dissertation's gonna take you.

The importance of data sharing can't be understated for this project, and I am endlessly thankful to the numerous authors whose archived data contributed to the work presented herein (see Appendix), and to Mitchell Green who helped me build this dataset.

I of course want to thank my parents, my brother, and the rest of my family and friends for their support, asking lots of questions, and listening to lots of practice presentations. Last and certainly not least, a huge thanks goes to my creative writing professor Paul Blaney, for knowing I'd end up doing a PhD even when I didn't think so. Looks like the 16th time's the charm!

This work was funded by a Natural Sciences and Engineering Research Council of Canada Discovery Grant and a U. Manitoba Graduate Enhancement of Tri-council funding grant to Colin Garroway. I was additionally supported by a U. Manitoba Graduate Fellowship.

Table of contents

List of tables

List of figures

Contributions of authors

Chapter 2: C.S., R.P.K., J.B. and C.J.G. conceived of the study. C.S. and C.J.G. designed the study and wrote the first draft of the manuscript. C.S. built the dataset and conducted the statistical analysis with input from C.J.G. M.D. harvested raw data from repositories and built tools for data manipulation. All authors contributed to data interpretation and editing of subsequent manuscript drafts.

Chapter 3: C.S. and C.J.G. conceived of the study. C.S. collected and processed genetic data. C.S. performed the analysis with input from C.J.G. C.S. wrote the first draft of the manuscript, and C.J.G. contributed to editing subsequent drafts.

Chapter 4: C.S. and C.J.G. conceived of and designed the study. C.S. performed the analyses with input from C.J.G. and wrote the first draft of the manuscript. C.S. and C.J.G edited subsequent manuscript drafts.

Chapter 5: C.J.G. and C.S. conceptualized the study. C.S., S.D. and C.J.G. designed the study and C.S. conducted the statistical analysis with input from S.D. and C.J.G. All authors contributed to data interpretation. C.S. wrote the first draft of the manuscript and all authors participated in editing subsequent manuscript drafts.

Chapter 6: C.J.G., and C.S. conceived of the study. C.S., J.M.S, and C.J.G. designed the study and C.S. conducted the analyses with input from C.J.G. All authors contributed to data interpretation. C.S. wrote the first draft of the manuscript and all authors participated in editing subsequent manuscript drafts.

Chapter 7: C.S. and C.J.G. wrote the manuscript.

Chapter 1: Introduction

Effective biodiversity conservation requires a multilevel approach with targeted action directed at ecosystem, species, and genetic levels (Convention on Biological Diversity 2016; Pollock et al. 2017). The sustainability and resilience of ecosystems depend jointly on species and genetic diversity in ecological communities (Oliver et al. 2015). Although there are several facets of biodiversity, including phylogenetic, genetic, and trait or functional diversity (Pollock et al 2020), much attention has been devoted to the conservation of species biodiversity, which is more visible and more easily actionable, for instance through endangered species listing. Genetic diversity informs conservation practice; however, at present genetic monitoring tends to be limited to agriculturally important or charismatic species (Hoban et al. 2020). Population genetic diversity is the most fundamental level of biodiversity because it contributes to a population's capacity to adapt to environmental change. Compared to species richness, less is known about the spatial distribution of genetic diversity across species. Deeper knowledge about the broad-scale biogeography of genetic diversity is needed for conservation practitioners to take it into account more widely, for example in regional management and the designation of protected areas. Unprecedented human-caused global change makes it imperative to take multifaceted conservation approaches to preserve species and ensure they have the adaptive potential to withstand ongoing change (Pollock et al. 2020).

Macrogenetics approaches

Questions regarding the genetic variation of natural populations across species have always been central to population and conservation genetics. Molecular markers, which first came into common use in population genetics in the 1960s – 70s, led to a proliferation of tools, theory, and studies quantifying genetic variation in natural populations across diverse taxa (Charlesworth and Charlesworth 2017). The steady accumulation of molecular genetic data since then has opened the door to data synthesis approaches. In 1998, John Gillespie wrote that "sequencing is so easy that data are accumulating more rapidly than they can be interpreted" (Gillespie 1998). As early as 1976, conservation geneticists took

advantage of this rapid data accumulation to test the predictions of theoretical population genetics in real populations and to understand how species' life history, ecological traits, and environments shape genetic variation (Soulé 1976; Nevo 1978; Hamrick et al. 1979; Frankham 1995). Advances in sequencing and open data storage have renewed interest in repurposing publicly archived genetic data to address new questions, and this approach has now been formalized as a subdiscipline called *macrogenetics* (Leigh et al. *in review*; Blanchet et al. 2017; Schmidt and Garroway 2021). The central importance of spatiotemporal patterns of genetic diversity to evolutionary biology has been stressed from the beginning (Nevo 1978); however, the form of and statistical approaches used in macrogenetics has matured. Recent macrogenetics studies build on a rich tradition of mobilizing open genetic data and existing knowledge of evolutionary theory, population biology, and community ecology to understand how theory translates into real world population variation.

Biodiversity gradients

Spatial variation in species richness is the most notable and well-described biogeographic pattern in nature. The latitudinal species richness gradient—the pattern of increasing species richness towards the equator—has been known to naturalists for at least 3 centuries (Willig et al. 2003). Nearly 30 hypotheses have been proposed to explain the processes underlying the gradient, yet our understanding of its causes remains underdeveloped (Pontarp et al. 2019). Linking pattern to process requires shifting focus from the species level to population genetic diversity—the level at which evolution acts. Hypotheses for the species richness gradient generally fall into three broad categories: evolutionary speed, evolutionary time, and ecological limits. Evolutionary speed hypotheses suggest that rates of speciation and extinction vary across latitudes to create species richness clines. In high energy equatorial regions, shorter generation times and higher mutation rates may lead to faster speciation, but relative environmental stability ensures lower rates of extinction (Mittelbach et al. 2007). Evolutionary time hypotheses posit that populations in tropical regions, which were minimally affected by periods of glaciation, have had more time to evolve and speciate than those at higher latitudes

(Pontarp et al. 2019). Finally, ecological limits hypotheses are couched in demographic variation across latitudes, where high energy, resource rich tropical habitats are capable of supporting larger populations and communities (Currie et al. 2004; Storch et al. 2018). Larger populations have greater chances of persisting, and larger communities tend to have higher species diversity (Hubbell 2001).

Biodiversity gradients have been considered at other levels, including phylogenetic (Smith et al. 2005; Alexander Pyron and Wiens 2013; Igea and Tanentzap 2020) and functional diversity (Stevens et al. 2003; Devictor et al. 2007), but genetic diversity is the least explored. However, some hypotheses proposed to underlie the species richness gradient are applicable at a genetic level as well, particularly energy-richness hypotheses because they are directly related to carrying capacity and population size. Indeed, community ecology and population genetics are linked by demographic processes (Lowe et al. 2017), and these fields have borrowed hypotheses in the past. Ecological limits hypotheses are supported by the neutral theory of biodiversity (communities with more individuals tend to be more diverse; Hubbell 2001) which was inspired by the neutral theory of molecular evolution (Kimura 1983) in population genetics (larger populations have higher genetic diversity). Under ecological limits hypotheses, we could therefore expect a positive correlation between genetic diversity and species richness. Ecological limits are generally related to temperature and productivity gradients that set population-level carrying capacities, however, environmental heterogeneity and niche availability also limit the number of species able to coexist in an area (Allouche et al. 2012; Stein et al. 2014). Thus, the interplay between niche availability and energy availability likely regulates species diversity and genetic diversity simultaneously via population size. In this case, a positive correlation is not the default expectation, because more heterogeneous environments may support more specialized species at smaller population sizes.

Evolutionary time, evolutionary speed, and ecological limits hypotheses rarely consider contemporary environmental factors that may affect biodiversity. Yet, rapid environmental change is already affecting population genetic diversity—particularly that associated with human land use (Palumbi 2001; Johnson and Munshi-South 2017; Otto 2018; Schmidt et al. 2020). Predicting how human factors such as urbanization and climate change will affect

the distribution and adaptability of wildlife populations requires a cohesive understanding of the relationships between environments, species richness, and genetic diversity, and how these relationships might shift on short timescales. The clear conceptual links between genetic diversity and species richness suggest that it may be feasible to take conservation and management approaches targeting both of these levels at once. Although correlations between species and genetic diversity have been tested in species-specific contexts (Vellend 2005; Evanno et al. 2009; Watanabe and Monaghan 2017), such studies tend to focus on adaptive genetic variation in species that directly interact, such as plants and pollinators. It remains unclear how environments might shape genetic and species diversity through neutral processes affecting population size and gene flow (but see Evanno et al. 2009; Laroche et al. 2015), because these links have yet to be empirically tested across several species simultaneously.

Objectives

Until recently, it was not possible to incorporate estimates of genome-wide diversity indicative of population processes into analyses of species richness patterns due to a lack of comparable multi-species, population-level data. To address this gap, I took a macrogenetics approach and built a georeferenced database of genetic metrics by harvesting raw, archived molecular genetic data for 99 species (44 mammals, 25 birds, 19 amphibians, and 11 reptiles), totaling 58,946 individual genotypes from 1,682 sample sites in North America. Microsatellites are widely used in ecology and evolution studies and are among the most common nuclear marker available in public repositories (Schell 2018; Miles et al. 2019). The typical number of loci used in wildlife studies (~10 loci) provides a good estimate of neutral genome-wide variation with little information to gain from more dense sampling e.g. with more loci or single nucleotide polymorphism data (Mittell et al. 2015). For each site in this aggregated dataset, I estimated four metrics of genetic composition: gene diversity, allelic richness, effective population size (an indicator of the strength of drift), and population-specific F_{ST} (an index of population differentiation). I used this database to explore the effects of historical environments and contemporary threats to biodiversity in three major objectives. I first used the strong theoretical basis for

predicted evolutionary change in cities (Johnson and Munshi-South 2017) to understand whether urbanization, relative to more natural environments, consistently reduces genetic diversity in mammals and birds (Chapter 2), and amphibians (Chapter 3). Next, I tested whether variation in habitat quality and availability within cities consistently affects genetic diversity across terrestrial vertebrates (Chapter 4). Third, I identified continent-scale patterns in genetic diversity and the common causes of patterns of genetic diversity and species richness in mammals (Chapter 5) and amphibians (Chapter 6) considering them representative of endo- and ectotherm patterns. Finally, I explored the conservation utility of macrogenetics approaches with different genetic marker types (Chapter 7).

A note on methods

Much of this thesis relies on two concepts: the relationship between genetic diversity and population size, and quantifying spatial variation in genetic diversity. I'll briefly discuss these ideas here to provide some context, but they will be expanded upon in the following chapters.

The neutral theory of molecular evolution suggests that most mutations that persist in populations are selectively neutral or nearly so (Kimura 1983). Not being directly subject to selective processes, the frequencies of neutral genetic variants in a population are most strongly affected by the random sampling of gametes over time. This random fluctuation of allele frequencies in a population is *genetic drift*. The strength of genetic drift is inversely related to population size: it is strong in small populations where random sampling can have a greater effect on allele frequencies. It is weaker in large populations where the loss of alleles due to sampling will not have much impact on allele frequencies. Genetic drift can eventually cause neutral alleles to become randomly fixed or lost from a population, over time eroding genetic diversity. This is why, in general, large populations have more genetic diversity than small ones. But the relationship between the strength of genetic drift and population size only holds if we assume an ideal population with constant size, randomly mating individuals, and non-overlapping generations. Because this is almost never true in real populations, we use the *effective population size* in place of the census population size

(Charlesworth and Charlesworth 2010; Ellegren and Galtier 2016). The effective population size is the size of an ideal population which experiences the same amount of genetic drift as the measured population. We can think of it as the number of individuals contributing to the next generation. In this way, the effective population size is a measure of the strength of drift. Natural selection is more efficient when genetic drift is weak (Ellegren and Galtier 2016), thus populations with larger effective population sizes are in general better able to adaptively respond to new selection pressures.

Understanding the ways environments shape population size, structure, and genetic diversity requires identifying spatial variation in genetic composition. However, other factors than the ones we're interested in (e.g., urbanization) can affect patterns of genetic diversity. Some sources of variation in genetic diversity depend on species traits which affect the effective population size, such as body size or fecundity (Romiguier et al. 2014). Other sources of variation are spatial. For example, isolation-by-distance will generate a spatial signal in data where distant populations are more genetically distinct than nearby populations due to reduced gene flow. Or, populations in locations which were historically glacial refugia may still retain high levels of genetic diversity (Hewitt 2000). To isolate an effect of the factor of interest, we must first account for existing spatial patterns of genetic diversity. We can accomplish this using distance-based Moran's eigenvector maps (MEMs) (Borcard and Legendre 2002; Borcard et al. 2004; Dray et al. 2006; Legendre and Legendre 2012).

Distance-based MEMs are a type of eigenvector-based analysis used to model spatial structure in ecological data (Legendre and Legendre 2012). The eigenvectors identified with MEMs are conceptually similar to principal components generated in principal components analysis. MEMs capture spatial variation in the data and, like principal components, are orthogonal and can be used as covariates in regression models. MEMs were developed in community ecology but are used in landscape genetics to account for effects of unmeasured environmental variables (Manel et al. 2010; Garroway et al. 2013; Driscoe et al. 2019; Schmidt et al. 2020; Coscia et al. 2020). With MEMs, we can parse the amount of variation in genetic diversity attributable to spatial and non-spatial processes. MEMs are especially useful because they capture spatial variation at all spatial scales

detectable within the data. Distance-based MEM analysis produces n – 1 MEMs (n being the number of sample sites), but only the subset of eigenvectors corresponding to positive spatial autocorrelation are used. They are ordered according to spatial scale explained, with the first eigenvector explaining the broadest autocorrelation pattern. The eigenvalues associated with each MEM are proportional to Moran's I index of spatial autocorrelation (Dray et al. 2006). Here, I used MEMs to control for spatial autocorrelation and existing spatial patterns in genetic diversity to measure the effect of urbanization in Chapters 2 and 3. Then I got curious about what was causing all this spatial structure in the data, which led to Chapters 5 and 6.

References

Alexander Pyron, R., and Wiens, J.J. 2013. Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. Proc. R. Soc. B Biol. Sci. **280**(1770). doi:10.1098/rspb.2013.1622.

Allouche, O., Kalyuzhny, M., Moreno-Rueda, G., Pizarro, M., and Kadmon, R. 2012. Area-heterogeneity tradeoff and the diversity of ecological communities. Proc. Natl. Acad. Sci. U. S. A. **109**(43): 17495–17500. doi:10.1073/pnas.1208652109.

Blanchet, S., Prunier, J.G., and De Kort, H. 2017. Time to Go Bigger: Emerging Patterns in Macrogenetics. Trends Genet. **33**(9): 579–580. doi:10.1016/j.tig.2017.06.007.

Borcard, D., and Legendre, P. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecol. Modell. **153**(1–2): 51–68. doi:10.1016/S0304-3800(01)00501-4.

Borcard, D., Legendre, P., Avois-Jacquet, C., and Tuomisto, H. 2004. Dissecting the spatial structure of ecological data at multiple scales. Ecology **85**(7): 1826–1832.

Charlesworth, B., and Charlesworth, D. 2010. Elements of evolutionary genetics. Roberts & Company Publishers, Greenwood Village, Colorado, USA.

Charlesworth, B., and Charlesworth, D. 2017. Population genetics from 1966 to 2016.

Heredity **118**(1): 2–9. doi:10.1038/hdy.2016.55.

Convention on Biological Diversity. 2016. Strategic Plan for Biodiversity 2011–2020. Available from https://www.cbd.int/convention/articles/?a=cbd-02.

Coscia, I., Wilmes, S.B., Ironside, J.E., Goward-Brown, A., O'Dea, E., Malham, S.K., McDevitt, A.D., and Robins, P.E. 2020. Fine-scale seascape genomics of an exploited marine species, the common cockle *Cerastoderma edule*, using a multimodelling approach. Evol. Appl. **13**(8): 1854–1867. doi:10.1111/eva.12932.

Currie, D.J., Mittelbach, G.G., Cornell, H. V., Field, R., Guégan, J.F., Hawkins, B.A., Kaufman, D.M., Kerr, J.T., Oberdorff, T., O'Brien, E., and Turner, J.R.G. 2004. Predictions and tests of climate-based hypotheses of broad-scale variation in taxonomic richness. Ecol. Lett. **7**(12): 1121–1134. doi:10.1111/j.1461-0248.2004.00671.x.

Devictor, V., Julliard, R., Couvet, D., Lee, A., and Jiguet, F. 2007. Functional homogenization effect of urbanization on bird communities. Conserv. Biol. **21**(3): 741–751. doi:10.1111/j.1523-1739.2007.00671.x.

Dray, S., Legendre, P., and Peres-Neto, P.R. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol. Modell. **196**(3–4): 483–493. doi:10.1016/j.ecolmodel.2006.02.015.

Driscoe, A.L., Nice, C.C., Busbee, R.W., Hood, G.R., Egan, S.P., and Ott, J.R. 2019. Host plant associations and geography interact to shape diversification in a specialist insect herbivore. Mol. Ecol. **28**(18): 4197–4211. doi:10.1111/mec.15220.

Ellegren, H., and Galtier, N. 2016. Determinants of genetic diversity. Nat. Rev. Genet. **17**(7): 422–433. doi:10.1038/nrg.2016.58.

Evanno, G., Castella, E., Antoine, C., Paillat, G., and Goudet, J. 2009. Parallel changes in genetic diversity and species diversity following a natural disturbance. Mol. Ecol. **18**(6): 1137–1144. doi:10.1111/j.1365-294X.2009.04102.x.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: A review. Genet. Res. (Camb). **66**: 95–107. doi:10.1017/S0016672308009695.

Garroway, C.J., Radersma, R., Sepil, I., Santure, A.W., De Cauwer, I., Slate, J., and Sheldon, B.C. 2013. Fine-scale genetic structure in a wild bird population: The role of limited dispersal and environmentally based selection as causal factors. Evolution **67**(12): 3488–3500. doi:10.1111/evo.12121.

Gillespie, J. 1998. Population Genetics: A Concise Guide. Johns Hopkins University Press, Baltimore and London. doi:10.2307/2533705.

Hamrick, J.L., Linhart, Y.B., and Mitton, J.B. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Annu. Rev. Ecol. Syst. **10**: 173–200.

Hewitt, G.M. 2000. The genetic legacy of the Quaternary ice ages. Nature **405**(6789): 907–913.

Hoban, S., Bruford, M., D'Urban Jackson, J., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P.A., Paz-Vinas, I., Sjögren-Gulve, P., Segelbacher, G., Vernesi, C., Aitken, S., Bertola, L.D., Bloomer, P., Breed, M., Rodríguez-Correa, H., Funk, W.C., Grueber, C.E., Hunter, M.E., Jaffe, R., Liggins, L., Mergeay, J., Moharrek, F., O'Brien, D., Ogden, R., Palma-Silva, C., Pierson, J., Ramakrishnan, U., Simo-Droissart, M., Tani, N., Waits, L., and Laikre, L. 2020. Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. Biol. Conserv. **248**: 108654. doi:10.1016/j.biocon.2020.108654.

Hubbell, S.P. 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton NJ.

Igea, J., and Tanentzap, A.J. 2020. Angiosperm speciation cools down in the tropics. Ecol. Lett.: ele.13476. doi:10.1111/ele.13476.

Johnson, M.T.J., and Munshi-South, J. 2017. Evolution of life in urban environments. Science **358**(6363): eaam8327. doi:10.1126/science.aam8327.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge.

Laroche, F., Jarne, P., Lamy, T., David, P., and Massol, F. 2015. A neutral theory for interpreting correlations between species and genetic diversity in communities. Am. Nat. **185**(1): 59–69. doi:10.1086/678990.

Legendre, P., and Legendre, L. 2012. Numerical Ecology. *In* 3rd edition. Elsevier.

Leigh, D.M., van Rees, C.B., Millette, K.L., Breed, M.F., Schmidt, C., Bertola, L.D., Hand, B.K., Hunter, M.E., Jensen, E.L., Kershaw, F., Liggins, L., Luikart, G., Manel, S., Mergeay, J., Miller, J.M., Segelbacher, G., Hoban, S., and Paz-Vinas, I. (*In review*). Opportunities and challenges of macrogenetic studies. Nat. Rev. Genet.

Lowe, W.H., Kovach, R.P., and Allendorf, F.W. 2017. Population genetics and demography unite ecology and evolution. Trends Ecol. Evol. **32**(2): 141–152. doi:10.1016/j.tree.2016.12.002.

Manel, S., Poncet, B.N., Legendre, P., Gugerli, F., and Holderegger, R. 2010. Common factors drive adaptive genetic variation at different spatial scales in *Arabis alpina*. Mol. Ecol. **19**(17): 3824–3835. doi:10.1111/j.1365-294X.2010.04716.x.

Miles, L.S., Rivkin, L.R., Johnson, M.T.J., Munshi-South, J., and Verrelli, B.C. 2019. Gene flow and genetic drift in urban environments. Mol. Ecol. **28**(18): 4138–4151. doi:10.1111/mec.15221.

Mittelbach, G.G., Schemske, D.W., Cornell, H. V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., McCain, C.M., McCune, A.R., McDade, L.A., McPeek, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M., and Turelli, M. 2007. Evolution and the latitudinal diversity gradient: Speciation, extinction and biogeography. Ecol. Lett. **10**(4): 315–331. doi:10.1111/j.1461-0248.2007.01020.x.

Mittell, E.A., Nakagawa, S., and Hadfield, J.D. 2015. Are molecular markers useful predictors of adaptive potential? Ecol. Lett. **18**(8): 772–778. doi:10.1111/ele.12454.

Nevo, E. 1978. Genetic variation in natural populations: Patterns and theory. Theor. Popul. Biol. **13**(1): 121–177. doi:10.1016/0040-5809(78)90039-4.

Oliver, T.H., Heard, M.S., Isaac, N.J.B., Roy, D.B., Procter, D., Eigenbrod, F., Freckleton, R., Hector, A., Orme, C.D.L., Petchey, O.L., Proença, V., Raffaelli, D., Suttle, K.B., Mace, G.M., Martín-López, B., Woodcock, B.A., and Bullock, J.M. 2015. Biodiversity and resilience of ecosystem functions. Trends Ecol. Evol. **30**(11): 673–684. doi:10.1016/j.tree.2015.08.009.

Otto, S.P. 2018. Adaptation, speciation and extinction in the Anthropocene. Proc. R. Soc. B **285**(1891): 20182047. doi:10.1098/RSPB.2018.2047.

Palumbi, S.R. 2001. Humans as the world's greatest evolutionary force. Science **293**(5536): 1786–1790. doi:10.1017/CBO9781107415324.004.

Pollock, L.J., O'Connor, L.M.J., Mokany, K., Rosauer, D.F., Talluto, M. V., and Thuiller, W. 2020. Protecting Biodiversity (in All Its Complexity): New Models and Methods. Trends Ecol. Evol. **35**(12): 1119–1128. doi:10.1016/j.tree.2020.08.015.

Pollock, L.J., Thuiller, W., and Jetz, W. 2017. Large conservation gains possible for global biodiversity facets. Nature **546**(7656): 141–144. doi:10.1038/nature22368.

Pollock, L.J., O'Connor, L.M.J., Mokany, K., Rosauer, D.F., Talluto, M. V., and Thuiller, W. 2020. Protecting Biodiversity (in All Its Complexity): New Models and Methods. Trends Ecol. Evol. **35**(12): 1119–1128. doi:10.1016/j.tree.2020.08.015.

Pontarp, M., Bunnefeld, L., Cabral, J.S., Etienne, R.S., Fritz, S.A., Gillespie, R., Graham, C.H., Hagen, O., Hartig, F., Huang, S., Jansson, R., Maliet, O., Münkemüller, T., Pellissier, L., Rangel, T.F., Storch, D., Wiegand, T., and Hurlbert, A.H. 2019. The latitudinal diversity gradient: novel understanding through mechanistic eco-evolutionary models. Trends Ecol. Evol. **34**(3): 211–223. doi:10.1016/j.tree.2018.11.009.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., Chiari, Y., Dernat, R., Duret, L., Faivre, N., Loire, E., Lourenco, J.M., Nabholz, B., Roux, C., Tsagkogeorga, G., Weber, A.A.T., Weinert, L.A., Belkhir, K., Bierne, N., Glémin, S., and Galtier, N. 2014. Comparative population genomics in animals uncovers the determinants of genetic diversity. Nature **515**(7526): 261–263. doi:10.1038/nature13685.

Schell, C.J. 2018. Urban evolutionary ecology and the potential benefits of implementing genomics. J. Hered. **109**(2): 138–151. doi:10.1093/jhered/esy001/4807252.

Schmidt, C., Domaratzki, M., Kinnunen, R.P., Bowman, J., and Garroway, C.J. 2020. Continent-wide effects of urbanization on bird and mammal genetic diversity. Proc. R. Soc. B Biol. Sci. **287**(1920): 20192497. doi:10.1098/rspb.2019.2497.

Schmidt, C., and Garroway, C.J. 2021. The conservation utility of mitochondrial genetic diversity in macrogenetic research. Conserv. Genet.

Smith, S.A., Stephens, P.R., and Wiens, J.J. 2005. Replicate patterns of species richness, historical biogeography, and phylogeny in holarctic treefrogs. Evolution **59**(11): 2433–2450. doi:10.1111/j.0014-3820.2005.tb00953.x.

Soulé, M. 1976. Allozyme variation: its determinants in space in time. *In* Molecular Evolution. Sinauer Associates, Sunderland, MA. pp. 60–77.

Stein, A., Gerstner, K., and Kreft, H. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. Ecol. Lett. **17**(7): 866–880. doi:10.1111/ele.12277.

Stevens, R.D., Cox, S.B., Strauss, R.E., and Willig, M.R. 2003. Patterns of functional diversity across an extensive environmental gradient: Vertebrate consumers, hidden treatments and latitudinal trends. Ecol. Lett. **6**(12): 1099–1108. doi:10.1046/j.1461-0248.2003.00541.x.

Storch, D., Bohdalková, E., and Okie, J. 2018. The more-individuals hypothesis revisited: the role of community abundance in species richness regulation and the productivity–diversity relationship. Ecol. Lett. **21**(6): 920–937. doi:10.1111/ele.12941.

Vellend, M. 2005. Species diversity and genetic diversity: parallel processes and correlated patterns. Am. Nat. **166**(2): 199–215. doi:10.1086/431318.

Watanabe, K., and Monaghan, M.T. 2017. Comparative tests of the species-genetic diversity correlation at neutral and nonneutral loci in four species of stream insect. Evolution **71**(7): 1755–1764. doi:10.1111/evo.13261.

Willig, M.R., Kaufman, D.M., and Stevens, R.D. 2003. Latitudinal Gradients of Biodiversity: Pattern, Process, Scale, and Synthesis. Annu. Rev. Ecol. Evol. Syst. **34**(1): 273–309. doi:10.1146/annurev.ecolsys.34.012103.144032.

Chapter 2: Continent-wide effects of urbanization on bird and mammal genetic diversity

C. Schmidt[1], M. Domaratzki[2], R.P. Kinnunen[1], J. Bowman[3,4], C.J. Garroway[1]

[1]Department Biological Sciences, University of Manitoba, Winnipeg, MB, Canada R3T 2N2
[2]Department of Computer Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2
[3]Environmental and Life Sciences Graduate Program, Trent University, Peterborough, ON, Canada K9L 0G2
[4]Wildlife Research and Monitoring Section, Ontario Ministry of Natural Resources and Forestry, Peterborough, ON, Canada K9J 8M5

Status: published in Proceedings of the Royal Society B *(DOI: 10.1098/rspb.2019.2497*)

Abstract: Urbanization and associated environmental changes are causing global declines in vertebrate population sizes. In general, population declines of the magnitudes now detected should lead to reduced effective population sizes for animals living in proximity to humans and disturbed lands. This is cause for concern because effective population sizes set the rate of genetic diversity loss due to genetic drift, the rate of increase in inbreeding, and the efficiency with which selection can act on beneficial alleles. We predicted that the effects of urbanization should decrease effective population size and genetic diversity, and increase population-level genetic differentiation. To test for such patterns, we repurposed and reanalyzed publicly archived genetic data sets for North American birds and mammals. After filtering, we had usable raw genotype data from 85 studies and 41,023 individuals, sampled from 1,008 locations spanning 41 mammal and 25 bird species. We used census-based urban-rural designations, human population density, and the Human Footprint Index as measures of urbanization and habitat disturbance. As predicted, mammals sampled in more disturbed environments had lower effective population sizes and genetic diversity, and were more genetically differentiated from those in more natural environments. There were no consistent relationships detectable for birds. This suggests that, in general, mammal populations living in proximity to humans can be expected to have less capacity to respond adaptively to further environmental changes, and be more likely to suffer from effects of inbreeding.

Keywords: urbanization, genetic diversity, evolution, mammals

Background

Human activities are among the most prominent and efficient drivers of contemporary evolution (Palumbi 2001). In some cases, human-caused evolution in wild populations is well understood and predictable. For instance, we have a well-founded expectation that populations of pests and disease agents will respond adaptively to our attempts at controlling them (Palumbi 2001). It is also clear that humans inadvertently alter evolutionary change in wild populations through land use and habitat degradation (Johnson and Munshi-South 2017; Otto 2018). Whether the indirect effects of human activities on evolutionary change can cause predictable evolutionary outcomes is less well understood. We hypothesized that human land use, by limiting population size and fragmenting habitat, reduces effective population size and genetic diversity in wild populations leading to increased genetic differentiation. To investigate this prediction, we repurposed and reanalyzed publicly archived raw nuclear genetic data sets for North American birds and mammals to test for general relationships between urbanization and the genetic diversity of populations.

Urbanization is one of the most pervasive causes of habitat fragmentation and general landscape change. In addition to the ~700,000 km^2 occupied by cities (Liu et al. 2018), nearly 75% of the Earth's land surface has been modified by humans, primarily in support of city dwellers (Barnosky et al. 2012). This human-caused degradation of the planet's land surface has consistently reduced its capacity to support wildlife (WWF 2018). As a result, vertebrate populations have on average declined in size by ~60% between 1970 and 2014 (WWF 2018). Reductions in population size at this level should decrease genetic diversity by increasing the strength of genetic drift—allele frequency variation due to the random sampling of gametes from one generation to the next. Indeed, there have been general declines in the genetic diversity of populations since the industrial revolution (Leigh et al. 2019). While genetic drift is a neutral evolutionary process that operates independently of the selective value of alleles, it reduces the efficiency of deterministic evolutionary processes like selection by causing allele frequencies to randomly deviate from expected values. When drift is strong relative to selection, random gamete sampling becomes the predominant cause of allele frequency change. In addition, increased drift and inbreeding

can eventually lead to reduced mean fitness in small populations. If wildlife populations living in proximity to humans generally experience reductions in population size and connectivity, and thus increased drift, then they may systematically become less genetically diverse than those living in less disturbed environments. By altering a population's genetic composition in this way, human-caused environmental change could make evolutionary responses to such change less efficient.

The fragmented nature of cities leads to specific expectations about how evolutionary processes will be altered within them based on basic population genetic theory (Johnson and Munshi-South 2017) (Fig. 2.1). Assuming a finite population of constant size with individuals that randomly mate, die out, and are completely replaced by their offspring each generation, populations will lose genetic diversity at a rate inversely proportional to population size. In reality, natural populations always deviate from these assumptions. Fortunately, we can substitute the concept of effective population size for census population size and the predictive utility of the theory holds. The effective population size is the size of an idealized population which conforms to the preceding assumptions and produces the same rate of drift as observed in the measured population. We can think of effective population size as a measure of the rate at which genetic drift causes a population to lose genetic diversity. Nearly all violations of these assumptions cause the effective population size to be much lower than the census population size, underscoring that drift plays a more important role in determining genetic diversity and the efficiency of selection than what might be expected from census population size alone. Specifically, we predicted that reduced census population size and gene flow in cities would lead to smaller effective population sizes, decreased genetic diversity, and increased genetic differentiation in urban populations.

Collecting and processing raw publicly archived genetic data

We tested for general relationships between the human modification of terrestrial habitats and the genetic composition of North American mammals and birds using archived microsatellite data from 85 studies, including 41,023 individuals sampled at 1,008 georeferenced sample sites, spanning 66 species (Table S2.1, Table S2.2). In particular, we

studied the effects of urbanization and the human footprint (Sanderson et al. 2002). We conducted a systematic search of online repositories for all available bird and mammal microsatellite data available for North America and applied a series of filtering steps (see SI Methods) to build a database of georeferenced neutral genetic diversity in wild populations. Our approach was made possible due to the accumulation of data in public data archives, and a still-changing culture of open data in ecological and evolutionary research. Access to raw data originally generated for unrelated purposes allowed for a particularly powerful synthetic analysis. This was because we could consistently calculate population genetic parameters of interest for our question, whether or not they were presented in the original publications. In addition, the fact that these data were collected to address different questions reduces the likelihood that study system selection—perhaps a tendency to explore evolutionary responses to humans in systems where such responses are expected—biased our findings.

We chose to analyze data sets that used neutral microsatellite markers because microsatellites were the most common molecular marker type available in data repositories (Miles et al. 2019), and because the evolutionary processes that we are interested in are best measured with neutral markers. Although the number of loci surveyed in microsatellite studies is often small relative to surveys of genome-wide markers, the typical number of microsatellites used (~10 loci) in fact estimates genome-wide diversity well with little gain in accuracy with additional genotyping (Mittell et al. 2015). Variation in microsatellite loci will likely capture recent, fine-scale changes in population structure due to their high mutation rates and variability. While questions about adaptive genetic variation are also interesting, adaptive diversity is currently more difficult to generally define and interpret than neutral genetic diversity, and there are still relatively few data sets suitable for this type of multi-population and multi-species analysis.

We tested for effects of urbanization and the human footprint on estimates of four population genetic parameters calculated for each site: effective population size, gene diversity, allelic richness, and the fixation index F_{ST} (196 bird sites, of which 129 sampled non-migratory species and were reanalyzed separately, and 812 mammal sites, Fig. 2.2;

Table S2.1). We estimated contemporary effective population size of the parental generation using a single sample linkage disequilibrium method to quantify genetic drift (Hill 1981; Waples 2006; Do et al. 2014). Of available methods, this approach is one of the more accurate and it is relatively robust to departures from underlying assumptions about population structure (Gilbert and Whitlock 2015). Estimators of effective population size perform poorly when sampling error swamps signals of genetic drift, and this meant that effective population size was not estimable at some sites, which we excluded from analysis (see SI Methods for details). Gene diversity (Nei 1973) is a measure of genetic diversity that accounts for the evenness and abundance of alleles, and it is not significantly affected by sample size or rare alleles (Charlesworth and Charlesworth 2010). We calculated rarefied allelic richness, the number of alleles per locus corrected for sample size, as a second measure of genetic diversity (Leberg 2002). To quantify genetic differentiation among sites, we estimated site-specific F_{ST} (Weir and Goudet 2017).

Modelling strategy

We focused our analyses on the continental United States and Canada due to the historical and demographic similarities of cities and land-usage in this region (La Sorte et al. 2007), and to ensure that species have had broadly similar exposures to past climate variation (Hewitt 2000). We chose three indices of urbanization and human presence. First, we classified a sample as coming from an urban or rural site based on United States Census Bureau (U.S. Census Bureau 2016) and Statistics Canada (Statistics Canada 2016) classifications of urban areas and population centers which are designated based on population density (Appendix S2). Second, we measured human population density at each site, which may capture aspects of the continuous nature of the effects of human presence that would not be apparent in the binary urban-rural classification. Lastly, we used the Human Footprint Index (Sanderson et al. 2002) as a measure of human presence because it incorporates data from multiple land use types including human population density, built-up areas, nighttime lights, land cover, and human access to coastlines, roads, railways, and navigable rivers.

Current levels of genetic diversity will reflect many past processes in addition to urbanization and human-caused environmental degradation more generally. Such processes include exposure to Pleistocene glaciations as well as species-specific life history traits, such as body mass and longevity, each of which could shape effective population size and thus genetic diversity. Because exposure to past environments (Hewitt 2000; Shafer et al. 2010) and life history trait variation (Stearns 1992) vary spatially, we expect the effects of such processes to create spatial variation in genetic diversity. We can account for such spatial patterns by including variables describing spatial patterns in genetic diversity directly in our models, even when the variables themselves are unmeasured (Manel et al. 2010). This can be accomplished with distance-based Moran's Eigenvector Maps, or dbMEMs (Borcard et al. 2004; Dray et al. 2006; Manel et al. 2010). Briefly, dbMEMs are orthogonal spatially explicit eigenvectors that summarize spatial autocorrelation (Moran's I) patterns in data across all scales. We used dbMEMs that described spatial variation in our measures of the genetic composition of sample sites in our regression models to explicitly account for processes causing spatial patterns in the data (Dray et al. 2006, 2012). Neutral genetic diversity also varies with species life history traits which may lack spatial structure (Romiguier et al. 2014). We therefore included species as a random effect, allowing both slopes and intercepts to vary, in a generalized mixed modeling framework to capture variation in genetic diversity not already accounted for by dbMEMs (see SI for detailed methods).

We used Bayesian generalized linear mixed models to test for relationships between genetic diversity and urbanization (Bürkner 2017, 2019). We treated each of our four population genetic parameters (effective population size, allelic richness, gene diversity, and site-specific F_{ST}) as dependent variables in a series of regression models. Each genetic parameter was fit to each urbanization variable (urban-rural, human population density, and Human Footprint) in separate models that also contained terms for species as a random effect, and spatial variables (dbMEMs) when they were important descriptors of spatial patterns in genetic data. Finally, we fit a null model to each population genetic parameter that contained the random effect for species and spatial variables only. The defining feature of such hierarchical models is that they are models of models – parameter

estimates and intercepts were estimated for each species and the distribution of these species-specific estimates allows us to generalize effects of urbanization across species. We fit these models for bird and mammal data independently. Migratory behavior in birds may affect spatial patterns in genetic diversity depending on where samples were taken, and whether they were sampled during the breeding season. Therefore, we also ran these models separately for non-migratory birds only (7 species, 129 sites; Table S2.1).

Results and Discussion

Relationships between all measures of urbanization and the genetic composition of mammal populations were consistently in the predicted directions (see the position of parameter estimates and the breadth of 95% credible intervals in Fig 3a). Effective population size, allelic richness, and gene diversity tended to be negatively related to the measures of urbanization, and sites sampled in areas with greater human presence tended to be the most genetically differentiated (Fig. 2.3a; Table 2.1). Contrasting these trends, we found no clear evidence for consistent effects of urbanization and the human footprint on the genetic composition of non-migratory bird samples when analyzed alone (Fig. 2.3b; Table 2.1), or when migratory and non-migratory species were combined for analyses (Fig. S2.1).

To assess model fits we estimated marginal R^2 (R^2_m), the variance explained by the fixed effects, and conditional R^2 (R^2_c), the variance explained by both fixed and random effects (Nakagawa and Schielzeth 2013). For mammals, all models containing indices of human disturbance explained more variation in the genetic composition of populations than null models (Table 2.1). Human population density explained the most variation in each measure of the genetic composition of mammal sample sites (effective population size: R^2_m 0.28; R^2_c 0.25; gene diversity R^2_m 0.12; R^2_c 0.70; allelic richness R^2_m 0.07; R^2_c 0.70; F_{ST} R^2_m 0.22; R^2_c 0.35) except allelic richness, where explained variance was similar among all urban predictors (Table 2.1).

The lack of consistent evidence for genetic effects of urbanization on birds may in part be due to the limited number of data sets available compared to data availability for mammals

and features of those species. Data from seven species remained after excluding migratory species: the California scrub jay (*Aphelocoma californica)*, black-capped chickadee (*Poecile atricapillus)*, boreal chickadee (*Poecile hudsonicus)*, barn owl (*Tyto alba)*, cactus wren (*Campylorhynchus brunneicapillus)*, spotted owl (*Strix occidentalis)*, and Ridgway's rail (*Rallus obsoletus).* These species have distinctive ecological and life history traits which vary such that we might not expect to find consistent effects of human presence for these data (Fig. S2.3). For example, the first four species are human commensals whose population sizes may be expected to increase in proximity to humans. Indeed, exploration of the species-specific effects underlying our mixed model suggests that genetic diversity increases with urbanization for each of these species except barn owls. In contrast, Ridgway's rail and spotted owl are specialized to California salt marshes and old growth forests, respectively, and thus may respond negatively to human presence. However, our results could also be attributable to birds' motility. Cities and their surrounding areas are characterized by disjoint patches of habitat interspersed among paved surfaces, buildings, and grassy or agricultural areas (Marzluff and Ewing 2001). Birds' ability to fly may buffer against the effects of habitat fragmentation and allow for gene flow from undisturbed populations (Buchmann et al. 2013) in situations where mammal movements would be more restricted. Indeed, a global analysis of 57 mammal species found that the movements of individuals living in areas with a high Human Footprint Index were considerably reduced relative to those in less disturbed areas (Tucker et al. 2018), which suggests that fragmentation could underlie the patterns we detect in mammals.

In addition to an overall pattern in mammals of reduced gene flow and stronger drift, we note that individual species varied in the strength of their responses to urbanization in our analyses (Fig. S2.2). This variation could be due to differences in ecological or life history traits which render species amenable to or susceptible to urbanization. Surprisingly, we found even synanthropic mammals (e.g., red foxes *Vulpes vulpes*, and mule deer *Odocoileus hemionus*) were negatively affected by urbanization at a genetic level (Fig. S2.2).

Few other studies have synthetically reanalyzed raw molecular genetic data from online repositories to test for effects of human land-use on genetic diversity. Miraldo et al.

(Miraldo et al. 2016) and Millette et al. (Millette et al. 2020) reanalyzed raw mtDNA sequence data at a global scale across multiple taxa to assess spatial patterns of variation in sequence diversity, and to explore their relationships with measures of human disturbance. These studies arrived at somewhat contradictory results. Miraldo et al. (Miraldo et al. 2016) looked at sequence variation in two mitochondrial genes in mammals, *cytochrome b* and *cytochrome oxidase subunit I*, and found that while genetic variation in *cytochrome oxidase subunit I* increased in less disturbed environments, *cytochrome b* variation was not obviously related to human disturbance. Millette et al.'s more recent work (Millette et al. 2020) spanned more taxa, including both birds and mammals, and examined variation across spatial scales as well as temporal variation in genetic diversity as measured at *cytochrome oxidase subunit I*. Interestingly, they detected no overarching trend for a loss of genetic diversity associated with proximity to humans (measured by human population density), and no systematic decline in diversity through time. They found considerable spatial, temporal, and taxonomic variation in diversity trends.

How can we explain our results in light of this previous work? Our first thought is that differences could be due to the general lack of relationship between mtDNA diversity and population size (Bazin et al. 2006). Habitat fragmentation and reduced population sizes are hypothesized to be the leading mechanisms causing reduced genetic diversity – if these processes are not captured well by mtDNA markers, trends may be difficult to detect. Additionally, the studies by Miraldo et al. (Miraldo et al. 2016) and Millette et al. (Millette et al. 2020) were global in scope. This is certainly a strength of their work, but underlying spatial differences may be harder to detect and control for at this scale. By focusing on North America, we attempted to control for variation in the timing and nature of disturbances that would otherwise be difficult at a global scale. Taken together, it is clear that there are interesting spatial trends in genetic variation, and the exploration of their underlying causes warrants further study.

While there has not to our knowledge been other synthetic analyses of raw nuclear genetic data similar to ours, there have been recent syntheses based on published measures of genetic variation (DiBattista 2008; Miles et al. 2019). Bautista (2008) found that allelic

richness and heterozygosity (an amalgam of expected and observed) tended to be lower for mammals described as coming from disturbed populations relative to those from undisturbed populations and, similar to us, found no differences for bird species. Miles et al. (2019) too found that allelic richness tended to be reduced in urban populations across a diverse array of taxa. Expected heterozygosity and F_{ST} tended to be lower in urban areas, but this trend was not universal and not significant. They also noted clear instances where urbanization appeared to facilitate gene flow and diversity. We too find some positive effects of urbanization (Fig S2) but our results more strongly suggest a general negative and less idiosyncratic effect of urbanization on the genetic composition of mammal populations. We suspect that differences in effect sizes and consistency between our work and these previous studies are in part due to our having access to raw genetic data sets. This meant we could calculate our genetic and disturbance measures consistently across our entire data set. For example, we could avoid procedures that might add noise to relationships such as combining two measures of heterozygosity and, by calculating a site specific F_{ST} metric, not have to take means of pairwise measures. Because our populations were georeferenced we were able to take a spatially explicit population-level approach that compared genetic composition to the same measures of disturbance across space. An advantage of using literature-based data is that the sample size in terms of species numbers was higher than ours for each of these literature-based syntheses. Regardless, these syntheses and ours paint a consistent picture of the effects of urbanization on genetic diversity.

Urbanization and the broader human footprint are leading causes of the current high rates of species and population-level biodiversity losses (McKinney 2006; Aronson et al. 2014). The population genetic patterns we detected reflect patterns in genome-wide nuclear genetic diversity that are ultimately the result of disturbances related to human presence at the ecosystem, community, and population levels. The consistent effects for mammals across our three measures of human disturbance suggest that this pattern is not confined to just urban spaces – human land use is an issue for the genetic diversity of species in general. This has considerable importance for understanding the current nature and future consequences of biodiversity loss. While monitoring individuals within populations is the

best tool for detecting population trends and assessing risk, such direct monitoring of many species is not logistically possible. Our results suggest that calls for intraspecific genetic monitoring programs are warranted and feasible (Mimura et al. 2017). A relatively small number of genetic markers reflects genome-wide diversity well (Mittell et al. 2015) and is capable of detecting the effects of human presence. In fact, publicly archiving data with publications, originally intended to ensure data posterity, safe-keeping, and research reproducibility, could be better utilized for this task. Access to raw molecular data sets will continue to increase for the foreseeable future, and can be used for monitoring regardless of the original purpose. However, for this to be a useful component of genetic monitoring, more researchers will need to adhere to the standards and best practices for data sharing to maximize reusability (Whitlock 2011; Michener 2015). This includes using standardized file and metadata formats that are clearly communicated in data package metadata, and including all relevant methodological information. In the data searches presented here, a majority (192/313 = 61%) of datasets were excluded because they did not meet our study criteria (Data S1). However, an additional 36 datasets were excluded for reasons associated with difficulty accessing or interpreting data (Data S1): for example, not being able to download files (i.e., only metadata was available, or only select datasets were deposited), or unclear methodological detail (i.e., no species designations, delineation between study groups was unclear, or lack of spatial reference). We were able to resolve such issues in many cases by contacting the authors, however this might not always be practical for larger studies and limits the ability to automate the data collection process.

Relative to populations in more natural environments, mammal populations in proximity to humans have a reduced capacity to spread beneficial alleles in response to selection pressures, have reduced genetic diversity which can reduce mean population fitness (Reed and Frankham 2003; Szulkin et al. 2010), and are more genetically isolated from natural populations. We are extensively and irreversibly creating environmental change while simultaneously reducing the capacity of some populations to evolve in response. Reducing fragmentation and facilitating population connectivity are therefore key to preserving genetic diversity in mammals. Current estimates suggest that by 2050, just 10% of the planet's surface will be unaltered by humans (WWF 2018). Land transformation processes

are eroding genetic diversity in mammals, compounding direct effects of habitat loss in a way that threatens the long-term existence of populations that persist.

Acknowledgements

CS, RPK, and CJG were supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to CJG. CS and RPK were additionally supported by a U. Manitoba Graduate Enhancement of Tri-council funding grant to CJG. We would like to thank Grace Ji for creating the program we used to format raw data. We also thank Kyle Lefort, Paul O'Brien, the students of the Autumn 2018 section of Evolutionary Biology (BIOL3300) at U. Manitoba, and two anonymous reviewers for their comments on earlier drafts of the manuscript.

References

Aronson, M.F.J., La Sorte, F.A., Nilon, C.H., Katti, M., Goddard, M.A., Lepczyk, C.A., Warren, P.S., Williams, N.S.G., Cilliers, S., Clarkson, B., Dobbs, C., Dolan, R., Hedblom, M., Klotz, S., Kooijmans, J.L., Kühn, I., MacGregor-Fors, I., McDonnell, M., Mörtberg, U., Pyšek, P., Siebert, S., Sushinsky, J., Werner, P., and Winter, M. 2014. A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. Proc. R. Soc. B Biol. Sci. **281**(1780): 20133330. doi:10.1098/rspb.2013.3330.

Barnosky, A.D., Hadly, E.A., Bascompte, J., Berlow, E.L., Brown, J.H., Fortelius, M., Getz, W.M., Harte, J., Hastings, A., Marquet, P.A., Martinez, N.D., Mooers, A., Roopnarine, P., Vermeij, G., Williams, J.W., Gillespie, R., Kitzes, J., Marshall, C., Matzke, N., Mindell, D.P., Revilla, E., and Smith, A.B. 2012. Approaching a state shift in Earth's biosphere. Nature **486**(7401): 52–58. Nature Publishing Group. doi:10.1038/nature11018.

Bazin, E., Glémin, S., and Galtier, N. 2006. Population size does not influence mitochondrial

genetic diversity in animals. Science. **312**(5773): 570–572. doi:10.1126/science.1122033.

Borcard, D., Legendre, P., Avois-Jacquet, C., and Tuomisto, H. 2004. Dissecting the spatial structure of ecological data at multiple scales. Ecology **85**(7): 1826–1832.

Buchmann, C.M., Schurr, F.M., Nathan, R., and Jeltsch, F. 2013. Habitat loss and fragmentation affecting mammal and bird communities: The role of interspecific competition and individual space use. Ecol. Inform. **14**: 90–98. Elsevier B.V. doi:10.1016/j.ecoinf.2012.11.015.

Bürkner, P.-C. 2019. brms: Bayesian Regression Models using "Stan." Available from https://cran.r-project.org/package=brms.

Bürkner, P.C. 2017. brms: An R Package for Bayesian Multilevel Models Using Stan. J. Stat. Softw. **80**(1): 1–28. doi:10.18637/jss.v080.i01.

Charlesworth, B., and Charlesworth, D. 2010. Elements of evolutionary genetics. Roberts & Company Publishers, Greenwood Village, Colorado, USA.

DiBattista, J.D. 2008. Patterns of genetic variation in anthropogenically impacted populations. Conserv. Genet. **9**(1): 141–156. doi:10.1007/s10592-007-9317-z.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., and Ovenden, J.R. 2014. NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol. Ecol. Resour. **14**(1): 209–214. doi:10.1111/1755-0998.12157.

Dray, S., Legendre, P., and Peres-Neto, P.R. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol. Modell. **196**(3–4): 483–493. doi:10.1016/j.ecolmodel.2006.02.015.

Dray, S., Pélissier, R., Couteron, P., Fortin, M.-J., Legendre, P., Peres-Neto, P.R., Bellier, E., Bivand, R., Blanchet, F.G., De Cáceres, M., Dufour, A.-B., Heegaard, E., Jombart, T., Munoz, F., Oksanen, J., Thioulouse, J., and Wagner, H.H. 2012. Community ecology in the age of multivariate multiscale spatial analysis. Ecol. Monogr. **82**(3): 257–275. doi:10.1890/11-1183.1.

Gilbert, K.J., and Whitlock, M.C. 2015. Evaluating methods for estimating local effective population size with and without migration. Evolution. **69**(8): 2154–2166. doi:10.1111/evo.12713.

Hewitt, G.M. 2000. The genetic legacy of the Quaternary ice ages. Nature **405**(6789): 907–913.

Hill, W.G. 1981. Estimation of effective population size from data on linkage disequilibrium. Genet. Res. (Camb). **38**: 209–216. doi:10.1017/S0016672300020553.

Johnson, M.T.J., and Munshi-South, J. 2017. Evolution of life in urban environments. Science. **358**(6363): eaam8327. doi:10.1126/science.aam8327.

Leberg, P.L. 2002. Estimating allelic richness: Effects of sample size and bottlenecks. Mol. Ecol. **11**: 2445–2449. doi:10.1046/j.1365-294X.2002.01612.x.

Liu, X., Hu, G., Chen, Y., Li, X., Xu, X., Li, S., Pei, F., and Wang, S. 2018. High-resolution multi-temporal mapping of global urban land using Landsat images based on the Google Earth Engine Platform. Remote Sens. Environ. **209**: 227–239. Elsevier. doi:10.1016/j.rse.2018.02.055.

Manel, S., Poncet, B.N., Legendre, P., Gugerli, F., and Holderegger, R. 2010. Common factors drive adaptive genetic variation at different spatial scales in Arabis alpina. Mol. Ecol. **19**(17): 3824–3835. doi:10.1111/j.1365-294X.2010.04716.x.

Marzluff, J.M., and Ewing, K. 2001. Restoration of fragmented landscapes for the conservation of birds: A general framework and specific recommendations for urbanizing landscapes. Restor. Ecol. **9**(3): 280–292. doi:10.1007/978-0-387-73412-5_48.

McKinney, M.L. 2006. Urbanization as a major cause of biotic homogenization. Biol. Conserv. **127**(3): 247–260. doi:10.1016/j.biocon.2005.09.005.

Michener, W.K. 2015. Ecological data sharing. Ecol. Inform. **29**(P1): 33–44. doi:10.1016/j.ecoinf.2015.06.010.

Miles, L.S., Rivkin, L.R., Johnson, M.T.J., Munshi-South, J., and Verrelli, B.C. 2019. Gene flow and genetic drift in urban environments. Mol. Ecol. **28**(18): 4138–4151. doi:10.1111/mec.15221.

Millette, K.L., Fugère, V., Debyser, C., Greiner, A., Chain, F.J.J., and Gonzalez, A. 2020. No consistent effects of humans on animal genetic diversity worldwide. Ecol. Lett. **23**(1): 55–67. doi:10.1111/ele.13394.

Mimura, M., Yahara, T., Faith, D.P., Vázquez-Domínguez, E., Colautti, R.I., Araki, H., Javadi, F., Núñez-Farfán, J., Mori, A.S., Zhou, S., Hollingsworth, P.M., Neaves, L.E., Fukano, Y., Smith, G.F., Sato, Y.-I., Tachida, H., and Hendry, A.P. 2017. Understanding and monitoring the consequences of human impacts on intraspecific variation. Evol. Appl. **10**(2): 121–139. doi:10.1111/eva.12436.

Miraldo, A., Li, S., Borregaard, M.K., Florez-Rodriguez, A., Gopalakrishnan, S., Rizvanovic, M., Wang, Z., Rahbek, C., Marske, K.A., and Nogues-Bravo, D. 2016. An Anthropocene map of genetic diversity. Science. **353**(6307): 1532–1535. doi:10.1126/science.aaf4381.

Mittell, E.A., Nakagawa, S., and Hadfield, J.D. 2015. Are molecular markers useful predictors

of adaptive potential? Ecol. Lett. **18**(8): 772–778. doi:10.1111/ele.12454.

Nakagawa, S., and Schielzeth, H. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. **4**(2): 133–142. doi:10.1111/j.2041-210x.2012.00261.x.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. **70**(12): 3321–3323. doi:10.1073/pnas.70.12.3321.

Otto, S.P. 2018. Adaptation, speciation and extinction in the Anthropocene. Proc. R. Soc. B **285**(1891): 20182047. doi:10.1098/RSPB.2018.2047.

Palumbi, S.R. 2001. Humans as the world's greatest evolutionary force. Science. **293**(5536): 1786–1790. doi:10.1017/CBO9781107415324.004.

Reed, D.H., and Frankham, R. 2003. Correlation between Fitness and Genetic Diversity. Conserv. Biol. **17**(1): 230–237. doi:10.1046/j.1523-1739.2003.01236.x.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., Chiari, Y., Dernat, R., Duret, L., Faivre, N., Loire, E., Lourenco, J.M., Nabholz, B., Roux, C., Tsagkogeorga, G., Weber, A.A.T., Weinert, L.A., Belkhir, K., Bierne, N., Glémin, S., and Galtier, N. 2014. Comparative population genomics in animals uncovers the determinants of genetic diversity. Nature **515**(7526): 261–263. doi:10.1038/nature13685.

Sanderson, E.W., Jaiteh, M., Levy, M.A., Redford, K.H., Wannebo, A. V., and Woolmer, G. 2002. The Human Footprint and the Last of the Wild. Bioscience **52**(10): 891. doi:10.1641/0006-3568(2002)052[0891:THFATL]2.0.CO;2.

Shafer, A.B.A., Cullingham, C.I., Côté, S.D., and Coltman, D.W. 2010. Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North

America. Mol. Ecol. **19**(21): 4589–4621. doi:10.1111/j.1365-294X.2010.04828.x.

La Sorte, F.A., McKinney, M.L., and Pyšek, P. 2007. Compositional similarity among urban floras within and across continents: Biogeographical consequences of human-mediated biotic interchange. Glob. Chang. Biol. **13**(4): 913–921. doi:10.1111/j.1365-2486.2007.01329.x.

Statistics Canada. 2016. Population Centres Census Division Cartographic Boundary File. 2016 Census. Statistics Canada Catalogue no. 92-160-X. Available from https://www12.statcan.gc.ca/census-recensement/2011/geo/bound-limit/bound-limit-2016-eng.cfm.

Stearns, S.C. 1992. The Evolution of Life Histories. Oxford University Press, New York.

Szulkin, M., Bierne, N., and David, P. 2010. Heterozygosity-fitness correlations: A time for reappraisal. Evolution. **64**(5): 1202–1217. doi:10.1111/j.1558-5646.2010.00966.x.

Tucker, M.A., Böhning-Gaese, K., Fagan, W.F., Fryxell, J.M., Moorter, B. Van, Alberts, S.C., Ali, A.H., Allen, A.M., Attias, N., Avgar, T., Bartlam-Brooks, H., Bayarbaatar, B., Belant, J.L., Bertassomi, A., Beyer, D., Bidner, L., Beest, F.M. Van, Blake, S., Blaum, N., Bracis, C., Brown, D., Bruyn, P.J.N. De, Cagnacci, F., Calabrese, J.M., Camilo-Alves, C., Chamaillé-Jammes, S., Chiaradia, A., Davidson, S.C., Dennis, T., Destefano, S., Diefenbach, D., Douglas-Hamilton, I., Fennessy, J., Fichtel, C., Fiedler, W., Fischer, C., Fischhoff, I., Fleming, C.H., Ford, A.T., Fritz, S.A., Gehr, B., Goheen, J.R., Gurarie, E., Hebblewhite, M., Heurich, M., Hewison, A.J.M., Hof, C., Hurme, E., Isbell, L.A., Janssen, R., Jeltsch, F., Kaczensky, P., Kane, A., Kappeler, P.M., Kauffman, M., Kays, R., Kimuyu, D., Koch, F., Kranstauber, B., Lapoint, S., Leimgruber, P., Linnell, J.D.C., López-López, P., Markham, A.C., Mattisson, J., Medici, E.P., Mellone, U., Merrill, E., Mourão, G.D.M., Morato, R.G.,

Morellet, N., Morrison, T.A., Díaz-Muñoz, S.L., Mysterud, A., Nandintsetseg, D., Nathan, R., Niamir, A., Odden, J., O'hara, R.B., Oliveira-Santos, L.G.R., Olson, K.A., Patterson, B.D., Paula, R.C. De, Pedrotti, L., Reineking, B., Rimmler, M., Rogers, T.L., Rolandsen, C.M., Rosenberry, C.S., Rubenstein, D.I., Safi, K., Saïd, S., Sapir, N., Sawyer, H., Schmidt, N.M., Selva, N., Sergiel, A., Shiilegdamba, E., Silva, J.P., Singh, N., Solberg, E.J., Spiegel, O., Strand, O., Sundaresan, S., Ullmann, W., Voigt, U., Wall, J., Wattles, D., Wikelski, M., Wilmers, C.C., Wilson, J.W., Wittemyer, G., Zięba, F., Zwijacz-Kozica, T., and Mueller, T. 2018. Moving in the Anthropocene: Global reductions in terrestrial mammalian movements. Science. **359**(6374): 466–469. doi:10.1126/science.aam9712.

U.S. Census Bureau. 2016. TIGER/Line Shapefiles: Urban Areas (machinereadable data files). Available from https://www.census.gov/geo/maps-data/data/tiger-line.html.

Waples, R.S. 2006. A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv. Genet. **7**(2): 167–184. doi:10.1007/s10592-005-9100-y.

Weir, B.S., and Goudet, J. 2017. A unified characterization of population structure. Genetics **206**: 2085–2103.

Whitlock, M.C. 2011. Data archiving in ecology and evolution: Best practices. Trends Ecol. Evol. **26**(2): 61–65. doi:10.1016/j.tree.2010.11.006.

WWF. 2018. Living Planet Report - 2018: Aiming higher. *Edited By*M. Grooten and R.E.A. Almond. WWF, Gland, Switzerland. Available from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:LIVING+PLANET+REPORT+2004#0.

Table 2.1. Model summaries for mammals, non-migratory birds, and all birds. Four models were constructed per response variable, each including one of three proxies of urbanization: urban-rural category, human population density (popden), and Human Footprint Index (HFI). The fourth model did not include any measure of urbanization and had only dbMEMs as fixed effects (spatial model), or, where no dbMEMs were selected, a null model. Coefficient of variation, R^2, values are an indicator of model fit; marginal R^2 describes the proportion of variation explained by fixed effects, while conditional R^2 is the variation explained by both fixed and random effects.

Class	variable	sites	Fixed effects		coefficient	95% CI		Marginal R^2	Conditional R^2
			dbMEMS	covariate		lower	upper		
Mammals	effective population size	639	5						
				urban-rural	-0.51	-0.87	-0.16	0.04	0.24
				popden	-1.10	-2.44	-0.28	0.28	0.25
				HFI	-0.27	-0.47	-0.05	0.05	0.25
				none	--	--	--	0.01	0.22
	gene diversity	812	13						
				urban-rural	-0.18	-0.33	-0.04	0.04	0.69
				popden	-0.35	-0.68	-0.12	0.12	0.70
				HFI	-0.12	-0.21	-0.02	0.04	0.70
				none	--	--	--	0.03	0.69
	allelic richness	812	21						
				urban-rural	-0.11	-0.29	0.05	0.07	0.69
				popden	-0.15	-0.33	0.00	0.07	0.70
				HFI	-0.11	-0.18	-0.03	0.07	0.70
				none	--	--	--	0.06	0.69
	F_{ST}	795	10						
				urban-rural	0.25	0.02	0.50	0.10	0.33
				popden	0.47	0.11	0.96	0.22	0.35
				HFI	0.15	0.04	0.28	0.11	0.34

				none	--	--	--	0.09	0.32
Birds (non-migratory)	effective population size	87	0						
				urban-rural	-0.20	-1.27	0.80	0.01	0.20
				popden	-0.21	-1.05	0.49	0.03	0.20
				HFI	-0.37	-0.87	-0.15	0.07	0.22
				none	--	--	--	0.00	0.19
	gene diversity	129	3						
				urban-rural	0.01	-0.38	0.41	0.03	0.80
				popden	0.13	-0.09	0.42	0.03	0.80
				HFI	0.04	-0.18	0.25	0.03	0.80
				none	--	--	--	0.02	0.80
	allelic richness	129	0						
				urban-rural	0.10	-0.77	0.82	0.02	0.24
				popden	-0.09	-0.42	0.23	0.01	0.17
				HFI	0.05	-0.37	0.41	0.02	0.22
				none	--	--	--	0.00	0.16
	F_{ST}	128	2						
				urban-rural	-0.13	-0.70	0.41	0.06	0.13
				popden	-0.05	-0.45	0.31	0.06	0.13
				HFI	-0.06	-0.38	0.24	0.06	0.13
				none	--	--	--	0.04	0.10
Birds (all)	effective population size	125	1						
				urban-rural	-0.18	-0.85	0.50	0.08	0.17
				popden	-0.20	-0.74	0.31	0.10	0.17
				HFI	-0.12	-0.48	0.30	0.08	0.18
				none	--	--	--	0.06	0.15
	gene diversity	196	2						

			urban-rural	-0.03	-0.20	0.15	0.00	0.90
			popden	0.04	-0.07	0.15	0.00	0.90
			HFI	-0.03	-0.05	0.07	0.00	0.90
			none	--	--	--	0.00	0.90
allelic richness	196	1						
			urban-rural	0.20	-0.27	0.62	0.03	0.35
			popden	-0.03	-0.24	0.20	0.02	0.29
			HFI	0.11	-0.12	0.32	0.03	0.33
			none	--	--	--	0.01	0.30
F_{ST}	190	1						
			urban-rural	0.00	-0.35	0.34	0.02	0.05
			popden	0.04	-0.22	0.34	0.02	0.05
			HFI	0.08	-0.12	0.31	0.03	0.07
			none	--	--	--	0.01	0.04

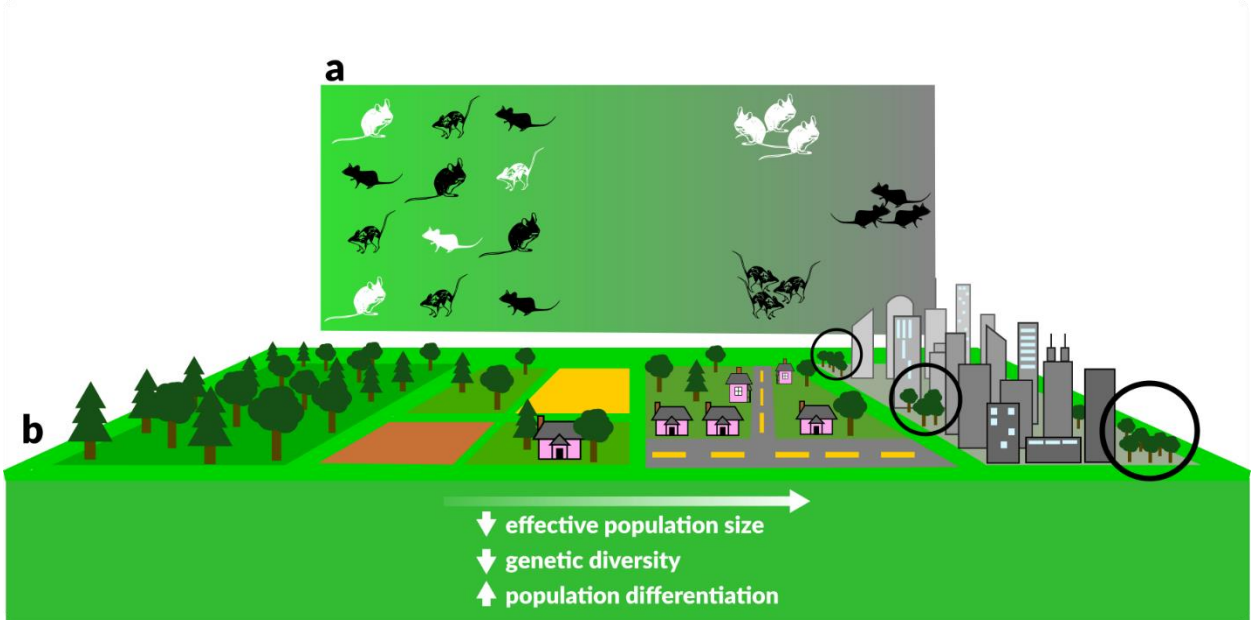


Figure 2.1. Urbanization is expected to cause smaller effective population sizes, lower genetic diversity, and increased population differentiation in comparison to natural habitats (a). As habitats become increasingly urbanized, they experience greater fragmentation (b), resulting in smaller patch sizes with lower connectivity. Smaller patches limit supportable population sizes wherein genetic drift becomes the predominant evolutionary force and movement between patches in urbanized areas (black circles) becomes difficult, reducing gene flow.

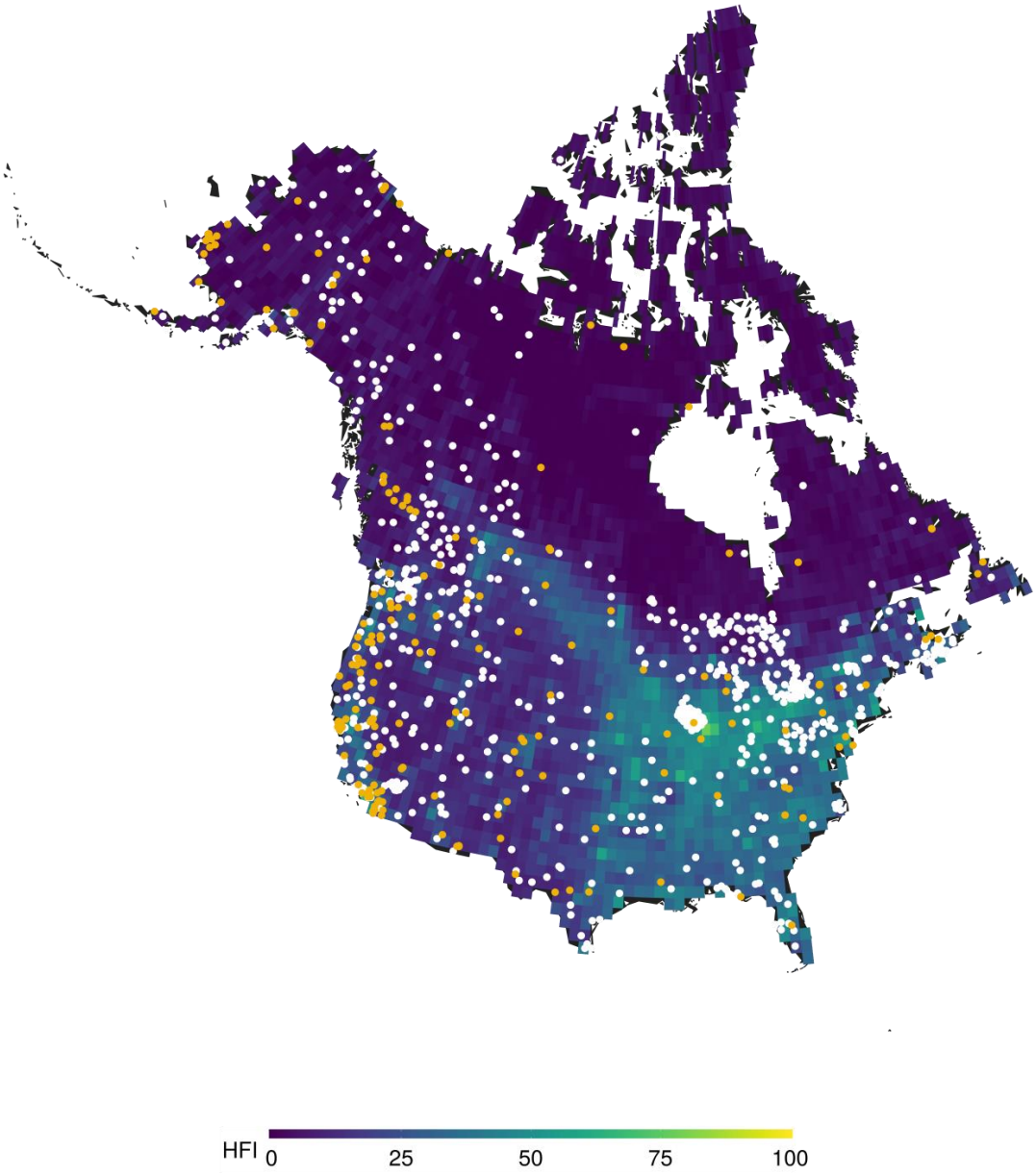


Figure 2.2. Map of 1,008 sample sites for the 66 mammal and bird species native to North America examined in this study. 812 sites were mammals (white points) and 129 birds (orange points). Using microsatellite markers, we calculated effective population size, gene diversity, allelic richness, and population-specific F_{ST} for each site. Sites are overlaid on a map of the Human Footprint Index (HFI) where values range from 0 (wild habitat) to 100 (disturbed habitat). Note HFI resolution was reduced for the purposes of visualization.

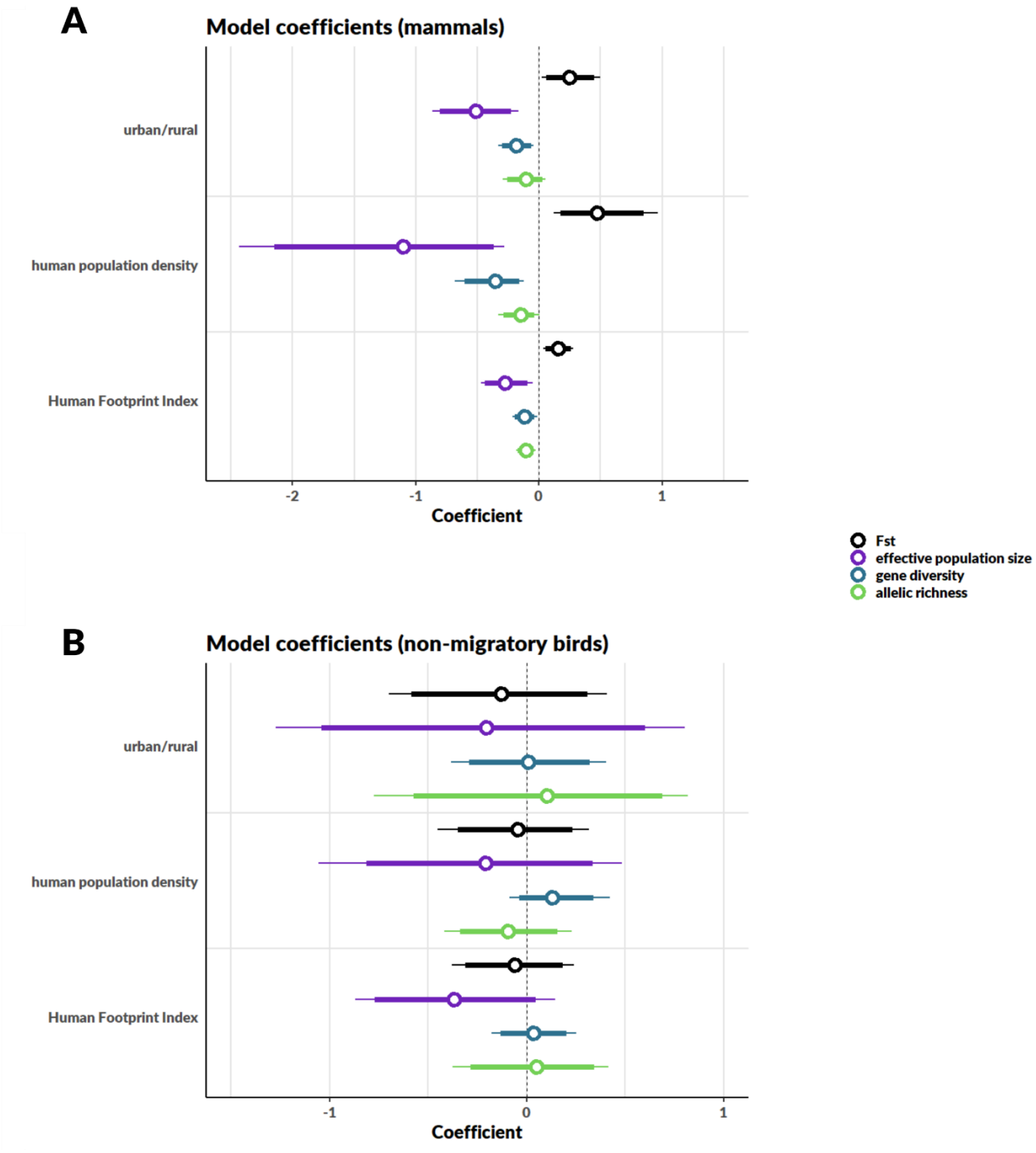


Figure 2.3. GLMM coefficients for fixed urban and human disturbance effects in mammals (top), and non-migratory birds (bottom; for bird results including migratory species, refer

to Fig. S2.1 and SI Table S2.2). Open circles represent coefficient estimates, bold lines are 90% credible intervals, and narrow lines are 95% credible intervals. Sample size differed between variables, i.e. sites where effective population size was not calculable were excluded, and calculation of population-specific FST for all sites within a study required at least two sample sites. Mammals: effective population size n = 639; gene diversity and allelic richness n = 812; FST n = 795. Birds (non-migratory): effective population size n = 87; gene diversity and allelic richness n = 129; F_{ST} n = 128.

Chapter 3: Inconsistent effects of urbanization on amphibian genetic diversity

Chloé Schmidt, Colin J Garroway

Department of Biological Sciences, 50 Sifton Rd, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada

Status: revision requested from Molecular Ecology *(preprint DOI: 10.1101/2020.08.16.253104)*

Abstract: Habitat loss and fragmentation are leading causes of vertebrate population declines. These declines are thought to be partly due to decreased connectivity and habitat loss reducing animal population sizes in human transformed habitats. With time this can lead to reduced effective population size and genetic diversity which restricts the ability of wildlife to cope with environmental change through genetic adaptation. However, it is not well understood whether these effects are generally applicable across taxa. Here, we repurposed and synthesized raw microsatellite data from online repositories for 19 amphibian species sampled at 554 georeferenced sites in North America. For each site, we estimated gene diversity, allelic richness, effective population size, and population differentiation. Using binary urban-rural census designations, and continuous measures of human population density, the Human Footprint Index, and percent impervious surface cover, we tested for generalizable effects of human land use on amphibian genetic diversity. We found no consistent relationships between urbanization and any of our genetic metrics. These results contrast with consistent negative effects of urbanization in mammals and species-specific positive and negative effects in birds. In the context of widespread amphibian declines, our results suggest that habitat loss and local extinction of populations in human transformed habitats is a more immediate concern than declining genetic diversity in populations that persist.

Keywords: heterozygosity, habitat fragmentation, anthropogenic disturbance, ectotherm, frogs, salamanders

Introduction

Populations of terrestrial vertebrates are experiencing declines globally due to habitat loss and the conversion of natural land for human purposes (WWF 2018). Proportionately more amphibians (41%) are threatened with extinction than are mammals (25%), reptiles (22%), or birds (13%) (Hoffmann et al. 2010). Due to data deficiencies for many amphibians this risk is likely underestimated (Stuart et al. 2004). Habitat transformation by humans is among the foremost threats to vertebrate biodiversity, in terms of both species losses and declining population sizes (Hamer and McDonnell 2008). Amphibians are clearly sensitive to habitat degradation (Hamer and McDonnell 2008; Collins et al. 2009), but we do not yet know whether amphibians exhibit generalizable population genetic responses to urbanization and other human land uses.

Genetic diversity is the most fundamental level of biodiversity. It is important for conservation because it reflects a population's ability to adaptively respond to environmental change (Pereira et al. 2013). Small populations typically have lower genetic diversity, higher rates of inbreeding depression, experience stronger effects of genetic drift, and have a reduced capacity to respond to environments adaptively and purge deleterious alleles via selection— this reduces long-term population viability (Frankham 1995). These genetic effects are a consequence of low effective population size (Falconer and Mackay 1996). The effective population size is the size of an ideal population with constant size, randomly mating individuals, and non-overlapping generations that produces the same rate of genetic drift as the measured population. Over time, the random loss or fixation of alleles caused by drift becomes the dominant force driving evolutionary change in populations with small effective sizes, regardless of census population size.

Habitat fragmentation and loss due to urbanization is a major cause of population decline (WWF 2018). This is because it divides populations and impedes dispersal between them, leading to greater genetic differentiation and smaller effective population sizes, which in turn reduces genetic diversity and strengthens genetic drift (Johnson and Munshi-South 2017; Miles et al. 2019; Schmidt et al. 2020). Mammals are the most well-studied taxa in urban evolutionary ecology (Schell 2018; Miles et al. 2019) and they tend to adhere to the

expectation of increased drift and genetic differentiation in disturbed environments (DiBattista 2008; Schmidt et al. 2020). Whether these effects are generalizable across other groups is unclear (Miles et al. 2019). For example, birds' flight ability may buffer them against the effects of habitat fragmentation, and species-specific effects of urbanization on genetic diversity in birds suggests that other factors associated with urban habitats primarily affect population dynamics (Schmidt et al. 2020).

Urbanization can have particularly negative consequences for amphibian populations due to their specialized habitat requirements. Declines in abundance are associated with the reduction and isolation of wetlands, wetland vegetation, and forest cover (Hamer and McDonnell 2008; Collins et al. 2009). Some species, especially those that rely on vernal pools, are unable to complete their life cycles in urban environments where aquatic and terrestrial habitats are separated by roads and other urban infrastructure (Hamer and McDonnell 2008). Locally, amphibians have patchy distributions that form metapopulation structures at regional scales. Limited population connectivity due to wetland destruction and fragmentation can sever connections within the metapopulation network, causing population isolation (Hamer and McDonnell 2008). The genetic diversity of amphibian populations could thus be especially vulnerable to continued land-use change. Genetic diversity and population connectivity were reduced in urban populations of some species (Arens et al. 2007; Noël et al. 2007; Munshi-South et al. 2013a), while other species appear to be more tolerant of urbanization or had higher genetic diversity in urban populations (Hamer and McDonnell 2008; Browne et al. 2009; Furman et al. 2016; Lourenço et al. 2017; Nowakowski et al. 2018; Fusco et al. 2020).

Here, we tested for general effects of urbanization on gene diversity, allelic richness, effective population size, and population-specific F_{ST} across a sample of North American amphibians by repurposing archived georeferenced microsatellite data. We harvested data amounting to 13,680 individual genotypes from 19 species sampled at 554 sites in Canada and the United States (Table S3.2). Synthesizing and repurposing data collected for different questions is powerful because it is unlikely that study system selection, for instance a focus on systems where strong effects are suspected, would bias our findings.

Additionally, this approach allowed us to consistently calculate both genetic and environmental variables that were not necessarily presented in the original papers. We focused on North America to control for effects of regional history on genetic diversity and population structure (Hewitt 2000; Schmidt et al. 2020). Additionally, the southeastern United States is an important hotspot for salamander species diversity. We focused on four measures of landscape change that encompass different aspects of urbanization and human presence. We considered census-based urban/rural designations as a broad scale categorical measure of urbanization. We used human population density measured per square kilometer as a finer scale continuous measure of human presence. The Human Footprint Index was a more comprehensive fine-scale measure of human presence and disturbance which incorporates population density, built-up areas, night-time lights, land cover, and human access to coastlines, roads, railways and navigable rivers (WCS and CIESIN 2005). Lastly, we measured the percent of impervious surface cover at each site which was available at a finer resolution (30 m).

Methods

Data assembly. We conducted a systematic search of online repositories for raw microsatellite data in February 2019 using the DataONE interface (Jones et al. 2017) through R (R Core Team 2019). We focused on microsatellites because they are widely used for genetic monitoring of wildlife populations, and raw data is commonly archived in online repositories (Miles et al. 2019). Neutral microsatellite markers provide good estimates of genome-wide diversity (Mittell et al. 2015) in addition to their capturing useful information about population demography. We searched for species names using a list of amphibian species native to North America from the IUCN Red List database (for example, "*Ambystoma maculatum*"), in addition to the terms: "microsat*", "short tandem*", or "single tandem*". We obtained 51 search results. Twenty-three results were duplicates (owing to different data versions archived in the Dryad repository) and were removed (see Data S1 for complete list of search results).

We screened the remaining data for suitability according to the following criteria: raw, neutral microsatellite data available (i.e., called allele sizes); species present in data were on search list; the location of data collection was North America; and the original study design would not have effects on genetic diversity, such as island populations, or populations having recently undergone a bottleneck for reasons unrelated to urbanization. In some cases, multiple datasets from one research group included the same species sampled at overlapping sites. In these cases we kept the dataset with a greater number of sample locations to avoid resampling the same populations. Ultimately 20 out of 28 unique datasets were retained for reanalysis (Table S3.2). If sample coordinates were unavailable, locations were georeferenced in ArcMap Desktop version 10.3.1 (ESRI, Redlands, CA) using sample site maps published in papers.

Next, we imported selected datasets into R using the adegenet package (Jombart et al. 2017). We calculated two measures of genetic diversity, gene diversity (Nei 1973) and allelic richness. We also calculated effective population size, and population-specific F_{ST} (Weir and Goudet 2017). Gene diversity is a measure of the spread and evenness of alleles in a population. It primarily takes into account allele frequencies and is therefore is only minimally affected by different sample sizes (Charlesworth and Charlesworth 2010). Allelic richness is a count of alleles in a population that is typically standardized to a minimum sample size by rarefaction (Leberg 2002). Here, we set a minimum sample size of 5 individuals. Gene diversity and allelic richness were calculated in adegenet (Jombart et al. 2017). We estimated the effective population size of the parental generation using the linkage disequilibrium method (Waples and Do 2010) in Neestimator v. 2 (Waples and Do 2008; Do et al. 2014). This method is among the more precise, especially for smaller effective population sizes (Waples and Do 2010). However, in large populations signals of drift are overwhelmed by sampling error which hinders estimation of effective population size. If too few individuals or loci were sampled to provide information on the strength of genetic drift, an estimate of infinity is returned. We excluded these estimates from our analyses (Fig. S3.1). Finally, population-specific F_{ST} is a measure of population differentiation which estimates how far populations in a sample have diverged from a

common ancestor (Weir and Goudet 2017). This method requires at least 2 populations per dataset, and we were unable to estimate F_{ST} when this requirement was not met. Population-specific F_{ST} was calculated with the 'betas' function in the hierfstat package (Goudet and Jombart 2015).

Human influence. We focused on four metrics which capture varying aspects of the effects of humans and urbanization. First, we assigned each site to urban or non-urban categories depending on whether they fell within census-designated urban areas and population centers in the United States and Canada (Statistics Canada 2016; U.S. Census Bureau 2016). Next, we measured the average human population density per km^2 and the Human Footprint Index (WCS and CIESIN 2005) at each site within a buffer zone. We used 1, 5, 10, and 15 km buffers around each site to test whether relationships between genetic metrics and human presence were scale-dependent. Finally, we measured percent impervious surface at each site with no buffer because data was available at a finer, 30 meter resolution (Brown de Colstoun et al. 2017). We also assessed the effects of roads at each site (University and Georgia 2013) independent from the Human Footprint Index, as this metric may have more proximate effects on amphibian population size and structure. However, total road length was strongly correlated with human population density and the Human Footprint Index, therefore we present these analyses in the SI. There was no evidence for scale dependence, and we report results from the 10 km buffer for human population density and the Human Footprint Index in the main text as this allowed for comparison to results for mammals and birds from previous work using this analytical approach (Schmidt et al. 2020). Full results for all scales are presented in SI Fig. S3.2.

Analysis. We examined the effects of human land transformation on genetic diversity (gene diversity and allelic richness), effective population size, and population differentiation (population-specific F_{ST}). However, there are likely spatial patterns in the distribution of genetic diversity present in the data dues to sample site arrangements (closer sites are more similar), local environment, population structure and demography, historical factors such as climate and glaciation cycles, or species life history (e.g. dispersal distance). Following Schmidt et al. (2020), we controlled for factors that could create spatial structure

in the data with distance-based Moran's eigenvector maps (MEMs). This method produces spatial eigenvectors with positive eigenvalues directly proportional to Moran's *I* index of spatial autocorrelation. MEMs capture spatial variation across all scales perceivable by the data (Borcard and Legendre 2002; Borcard et al. 2004; Dray et al. 2006). For example, in a multi-species context, broad scale spatial variation may be generated by climatic factors which can affect several species simultaneously, while patterns at finer scales could result from local environmental variation or species traits. MEMs are used in landscape genetics to control for spatial patterns caused by unknown factors, and as proxies for unmeasured environmental variables in regressions (Manel et al. 2010). We computed MEMs in the adespatial package (version 0.3.8) (Dray et al. 2017) and used a forward selection procedure to select MEMs that described important spatial variation in gene diversity, allelic richness, effective population size, and population-specific F_{ST} (Blanchet et al. 2008).

Next, we tested for effects of human presence on the population genetics of sample sites using hierarchical models in a Bayesian framework. For each genetic measure (gene diversity, allelic richness, effective population size, and population-specific F_{ST}) we fitted a series of five linear mixed models. Four models each included a measure of human presence (urban/rural classification, human population density, Human Footprint Index, and impervious surface cover) and selected MEMs, while the fifth model was a null model which included MEMs only or was intercept-only if no spatial autocorrelation was detected. We allowed relationships to vary for each species by including species as a random effect allowing slopes and intercepts to vary. Random slope and intercept models can be interpreted as first estimating the effects of human presence across populations within species, then generalizing coefficient estimates across species. Using this random effect structure also allows us to examine how species-specific effects contribute to the overall coefficient estimate, e.g., whether coefficients with credible intervals overlapping 0 are due to no effects across species, or a combination of species with positive and negative effects. Effective population size and human population density were log-transformed, and all variables were scaled and centered before analysis allowing us to compare effect sizes across models. Bayesian regression models were run using brms (Bürkner 2019) with 4

chains, 5000 iterations (1000 burn-in iterations), and default uniform priors. We calculated marginal (R^2_m) and conditional R^2 (R^2_c) values to determine the proportion of variation explained by fixed effects, and fixed and random effects together, respectively.

Finally, we also fit phylogenetically controlled models by fitting a phylogenetic covariance matrix generated with the 'taxize' and 'ape' packages in R (Chamberlain and Szocs 2013; Paradis and Schliep 2019). Phylogenetic controls are typically fit to account for the possibility that unreplicated events in a species group's evolutionary history drive patterns in data. Urbanization is a recent phenomenon, and so any relationships we find would indeed be replicated. We were primarily interested in whether species, regardless of their evolutionary relationships, tend to lose genetic diversity in response to urbanization. Nevertheless, phylogenetically controlled models could be interesting from other perspectives. Parameter estimates from phylogenetic regressions did not differ from models without phylogenetic control, and so we present these results in the SI (Fig. S3.3, Table S3.1).

Results

Data synthesis

From 20 previously published datasets, we obtained raw microsatellite genotypes from 13,680 individuals of 19 species sampled at 554 sites in Canada and the United States (Fig. 3.1; Table S3.2). The median number of individuals sampled per site was 21 (range: 5 – 299), and the mean number of sampled loci was 11.15 (range: 5 – 20). The average gene diversity across species was 0.68 ± 0.16 SD, and for allelic richness was 5.16 ± 2.11 SD. Species-specific summaries are presented in Table S3.2.

We were able to measure population-specific F_{ST} for all but 2 sample sites where only a single site was sampled. Mean F_{ST} was 0.11 ± 0.15 SD. Finally, we were only able to estimate effective population size for 387 sites. The median effective population size was 45.5 individuals, however, the range varied widely between sites (range: 1.3 – 7847.8).

Effects of urbanization

Human presence and urbanization did not tend to have strong effects on amphibian genetic diversity or population structure (Fig. 3.2, Table 3.1). Census-based urban/rural classification and percent impervious surface cover had no detectable effects on any genetic metric. There was a trend of higher genetic diversity and effective population sizes, and lower population differentiation at sites with higher Human Footprint Index and population density. However, the only clear effects were a negative effect of the Human Footprint Index on population-specific F_{ST} and a positive effect of human population density on effective population size (Fig. 3.2). Responses to population density varied consistently in strength and direction regardless of spatial scale, and effects of the Human Footprint Index began to be distinguishable with a 10 km buffer (Fig. S3.2). Urban predictors had particularly low explanatory power for effective population size and allelic richness relative to null models (Table 3.1). For gene diversity, models with population density or Human Footprint Index—continuous measures of urbanization—explained the largest amount of variation (19% and 17% respectively; Table 3.1). These relationships remained after accounting for phylogenetic relatedness (Fig. S3.3; Table S3.1). Effects of human presence on genetic diversity varied slightly between species (Fig. 3.3). Genetic diversity in Cope's giant salamander (*Dicamptodon copei*), the Rocky Mountain tailed frog (*Ascaphus montanus*), and the spring peeper (*Pseudacris crucifer*) tended to increase in more urban habitats whereas genetic diversity decreased with urbanization for northern dusky salamanders (*Desmognathus fuscus*) and California red-legged frogs (*Rana draytonii*) (Fig. 3.3). There was no clear direction of effect for the remaining 14 species.

Discussion

In general, genetic diversity and population structure in North American amphibians were not related to the measures of urbanization and human disturbance we tested. Responses to human presence measured by the Human Footprint Index and population density

trended in the same directions. In contrast to our expectations, amphibians showed a tendency towards lower levels of population differentiation, higher effective population sizes, and greater genetic diversity in transformed environments. Species-specific responses to urbanization (Fig. 3.3) suggest that pronounced positive effects in a few species drove this pattern: Cope's giant salamander, the Rocky Mountain tailed frog, and the spring peeper had higher genetic diversity in increasingly urban habitats. Cope's giant salamander and the Rocky Mountain tailed frog are both species with restricted ranges that were only sampled in low to moderately disturbed areas (Fig. 3.3), and are sensitive to anthropogenic disturbance (Spear and Storfer 2010; Trumbo et al. 2013; Metzger et al. 2015). It is unclear what may have caused the positive relationships with genetic diversity and population connectivity we find here. Species- or population-specific evolutionary history likely contributed to these results, however our analysis speaks to general interspecific patterns, and species-specific causes of variable responses to urbanization would require additional focal work.

Spring peepers on the other hand are an early successional species with a large range in eastern North America. In general, widely distributed generalist species, pond breeders, and species with aquatic development are more tolerant of habitat disturbance (Hamer and McDonnell 2008; Nowakowski et al. 2018). This may explain the positive trend we found in spring peepers as well as the absence of effects in other widely distributed species, including spotted salamanders (*Ambystoma maculatum*), streamside salamanders (*A. barbouri*), and wood frogs (*Lithobates sylvaticus*). Human-made ponds and still waters in moderately transformed areas (e.g., agricultural land, parks, golf courses) may provide important breeding habitats for some amphibian species (Babbitt and Tanner 2000; Dimauro and Hunter 2002; Barry et al. 2008; Brand and Snodgrass 2010; Saarikivi et al. 2013; Nowakowski et al. 2018). Indeed, abundance in a closely related species, the boreal chorus frog (*P. maculata*), was also increased in more urban environments, perhaps due to a preference for open habitats, or increased availability of potential breeding habitats near roads (Browne et al. 2009).

Genetic diversity and population connectivity were unaffected by urbanization in the remaining 16 species. However, these findings should not be taken as indicating that the loss of genetic diversity due to urbanization is not a concern for amphibians. Three non-mutually exclusive factors could have contributed to this result which we discuss in turn: sample locations, temporal population size fluctuations, and sensitivity to habitat loss. First, a majority of sample sites in our aggregated dataset were located in natural to moderately transformed landscapes (Human Footprint Index < 50; Fig. S3.4), and relatively few species were sampled in highly urbanized habitats. The only species with populations consistently sampled in highly urbanized sites was the northern dusky salamander, which showed a marked, albeit non-significant, decline in genetic diversity with increasing human disturbance (Fig. 3.3) (Munshi-South et al. 2013b). It is thus possible that our ability to detect effects on genetic diversity and population structure was limited because populations in densely developed urban habitats were unsampled, or perhaps unavailable for sampling due to extirpations and population declines.

Moreover, using macrogenetics approaches to test the effects of urbanization on amphibian genetic diversity may generally be difficult to detect due to unstable population sizes. Amphibian abundances at sites can vary across orders of magnitude from year to year (Collins et al. 2009). It may be that for many species, genetic signals of urbanization are swamped by population fluctuations associated with recurrent bottlenecks. Indeed, allelic richness—the number of alleles—is more sensitive to population bottlenecks than is gene diversity, which measures the evenness and spread of alleles (Corunet and Luikart 1996). Allelic richness data might thus be noisier than gene diversity for amphibians. Highly variable population sizes could have caused the discrepancy in model explanatory power we find between these two measures of genetic diversity: proximity to humans and spatial patterns explained substantially more of the variation in gene diversity (10-19%) than allelic richness (2-4%; Table 3.1).

Finally, amphibians' complex physiology, life history, and habitat requirements likely make them more sensitive than other vertebrates to human-caused habitat loss (Gibbs 1998), especially considering that increased human presence is associated with declines in

wetland area and connectivity (Gibbs 2000). Compared to birds and mammals, amphibian movements are naturally more constrained due to strict physiological and environmental requirements (e.g., proximity to aquatic and terrestrial environments, presence of ephemeral ponds, forest cover, and soil moisture), which also contributes to patchy population distributions (Collins et al. 2009). Thus in general, habitat fragmentation and loss related to human disturbance may limit dispersal and reduce the probability of recolonizing areas where species were previously extirpated (Guzy et al. 2012). At a 30 m resolution, most sites included in our analyses were located in areas with 0 % impervious surface cover, even if other metrics at broader scales indicated higher levels of urbanization. This suggests that urban amphibian populations can maintain levels of genetic diversity comparable to natural populations in small habitat patches in urban areas. Our results therefore suggest that with regards to urbanization, the outcomes of habitat loss and degradation—such as local extinction—are of a more immediate concern than gradual long-term declines owing to reduced genetic diversity in this taxon. This would explain losses at species and population levels without our detecting declines in genetic diversity. This is concerning because temporary wetlands which may benefit several species are typically not a priority for habitat conservation, and due to their small size, can dry out easily and are more vulnerable to pollution in human-dominated habitats (Hamer and McDonnell 2008).

As habitat specialists, amphibian populations in general appear to be more threatened by habitat loss in cities compared to other vertebrate taxa. Varied life histories and ecological needs across taxa play an integral role in how species cope with land-use change. While patterns of reduced genetic diversity and increased differentiation in mammals are quite consistent (DiBattista 2008; Schmidt et al. 2020), this pattern seems to not generally apply to all vertebrate classes. In addition to a lack of discernible general effects in amphibians, responses to urbanization in birds were equally likely to be negative or positive depending on species (Schmidt et al. 2020). This suggests that vagility is a major trait determining how different taxa responds to urbanization, and that perhaps variation in other traits has secondary role. In light of previous findings (DiBattista 2008; Miles et al. 2019; Schmidt et

al. 2020) and our results for amphibians, it appears that there is no one size fits all answer to questions about the effects of urbanization on genetic diversity (Miles et al. 2019). Nevertheless, although the direction of effect might vary, it is clear that urbanization alters the genetics of wildlife populations quite broadly.

Acknowledgements: We thank Mitchell Green for his assistance collecting and formatting raw microsatellite data from online repositories. We would also like to thank the authors of the original studies whose data contributed to this work. C.S. and C.J.G. were supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to C.J.G. C.S. was also supported by a U. Manitoba Graduate Fellowship, and a U. Manitoba Graduate Enhancement of Tri-council funding grant to C.J.G.

References

Arens, P., Van Der Sluis, T., Van't Westende, W.P.C., Vosman, B., Vos, C.C., and Smulders, M.J.M. 2007. Genetic population differentiation and connectivity among fragmented Moor frog (Rana arvalis) populations in the Netherlands. Landsc. Ecol. **22**(10): 1489–1500. doi:10.1007/s10980-007-9132-4.

Babbitt, K.J., and Tanner, G.W. 2000. Use of temporary wetlands by anurans in a hydrologically modified landscape. Wetlands **20**(2): 313–322. doi:10.1672/0277-5212(2000)020[0313:UOTWBA]2.0.CO;2.

Barry, D.S., Pauley, T.K., and Maerz, J.C. 2008. Amphibian use of man-made pools on clear-cuts in the Allegheny Mountains of West Virginia, USA. Appl. Herpetol. **5**(2): 121–128. doi:10.1163/157075408784648772.

Blanchet, G.F., Legendre, P., and Borcard. 2008. Forward selection of explanatory variables. Ecology **89**(9): 2623–2632.

Borcard, D., and Legendre, P. 2002. All-scale spatial analysis of ecological data by means of

principal coordinates of neighbour matrices. Ecol. Modell. **153**(1–2): 51–68. doi:10.1016/S0304-3800(01)00501-4.

Borcard, D., Legendre, P., Avois-Jacquet, C., and Tuomisto, H. 2004. Dissecting the spatial structure of ecological data at multiple scales. Ecology **85**(7): 1826–1832.

Brand, A.B., and Snodgrass, J.W. 2010. Value of artificial habitats for amphibian reproduction in altered landscapes: Contributed paper. Conserv. Biol. **24**(1): 295–301. doi:10.1111/j.1523-1739.2009.01301.x.

Brown de Colstoun, E.C., Huang, C., Wang, P., Tilton, J.C., Tan, B., Phillips, J., Niemczura, S., Ling, P.-Y., and Wolfe, R.E. 2017. Global Man-made Impervious Surface (GMIS) Dataset From Landsat. NASA Socioeconomic Data and Applications Center (SEDAC), Palisades, NY. Available from https://doi.org/10.7927/H4P55KKF.

Browne, C.L., Paszkowski, C.A., Foote, A.L., Moenting, A., and Boss, S.M. 2009. The relationship of amphibian abundance to habitat features across spatial scales in the Boreal Plains. Écoscience **16**(2): 209–223. doi:10.2980/16-2-3220.

Bürkner, P.-C. 2019. brms: Bayesian Regression Models using “Stan.” Available from https://cran.r-project.org/package=brms.

Chamberlain, S., and Szocs, E. 2013. taxize - taxonomic search and retrieval in R. F1000Research. Available from http://f1000research.com/articles/2-191/v2.

Charlesworth, B., and Charlesworth, D. 2010. Elements of evolutionary genetics. Roberts & Company Publishers, Greenwood Village, Colorado, USA.

Collins, J.P., Crump, M.L., and Lovejoy III, T.E. 2009. Extinction in Our Times: Global Amphibian Decline. Oxford University Press, Oxford.

Corunet, J.M., and Luikart, G. 1996. Power Analysis of Two Tests for Detecting Recent Population Bottlenecks From Allele Frequency Data. Genetics **144**: 2001–2014.

DiBattista, J.D. 2008. Patterns of genetic variation in anthropogenically impacted populations. Conserv. Genet. **9**(1): 141–156. doi:10.1007/s10592-007-9317-z.

Dimauro, D., and Hunter, M.L. 2002. Pools in Managed Forests. For. Sci. **48**(2502): 397–406.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., and Ovenden, J.R. 2014. NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol. Ecol. Resour. **14**(1): 209–214. doi:10.1111/1755-0998.12157.

Dray, S., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., and Wagner, H.H. 2017. adespatial: Multivariate Multiscale Spatial Analysis. Available from https://cran.r-project.org/package=adespatial.

Dray, S., Legendre, P., and Peres-Neto, P.R. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol. Modell. **196**(3–4): 483–493. doi:10.1016/j.ecolmodel.2006.02.015.

Falconer, D.S., and Mackay, T.F.C. 1996. Introduction to Quantitative Genetics. *In* 4th edition. Longman, Harlow, Essex, UK.

Frankham, R. 1995. Conservation Genetics. Annu. Rev. Genet. **29**: 305–332. Available from www.annualreviews.org.

Furman, B.L.S., Scheffers, B.R., Taylor, M., Davis, C., and Paszkowski, C.A. 2016. Limited genetic structure in a wood frog (*Lithobates sylvaticus*) population in an urban landscape inhabiting natural and constructed wetlands. Conserv. Genet. **17**(1): 19–30. Springer Netherlands. doi:10.1007/s10592-015-0757-6.

Fusco, N.A., Pehek, E., and Munshi-South, J. 2020. Urbanization reduces gene flow but not genetic diversity of stream salamander populations in the New York City metropolitan area. Evol. Appl. (May): 1–18. doi:10.1111/eva.13025.

Gibbs, J.P. 1998. Distribution of woodland amphibians along a forest fragmentation gradient. Landsc. Ecol. **13**(4): 263–268. doi:10.1023/A:1008056424692.

Gibbs, J.P. 2000. Wetland loss and biodiversity conservation. Conserv. Biol. **14**(1): 314–317. doi:10.1046/j.1523-1739.2000.98608.x.

Goudet, J., and Jombart, T. 2015. hierfstat: Estimation and Tests of Hierarchical F-Statistics. Available from https://cran.r-project.org/package=hierfstat.

Guzy, J.C., Mccoy, E.D., Deyle, A.C., Gonzalez, S.M., Halstead, N., and Mushinsky, H.R. 2012. Urbanization interferes with the use of amphibians as indicators of ecological integrity of wetlands. J. Appl. Ecol. **49**(4): 941–952. doi:10.1111/j.1365-2664.2012.02172.x.

Hamer, A.J., and McDonnell, M.J. 2008. Amphibian ecology and conservation in the urbanising world: A review. Biol. Conserv. **141**(10): 2432–2449. doi:10.1016/j.biocon.2008.07.020.

Hewitt, G.M. 2000. The genetic legacy of the Quaternary ice ages. Nature **405**(6789): 907–913.

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Bohm, M., Brooks, T.M., Butchart, S.H.M., Carpenter, K.E., Chanson, J., Collen, B., Cox, N.A., Darwall, W.R.T., Dulvy, N.K., Harrison, L.R., Katariya, V., Pollock, C.M., Quader, S., Richman, N.I., Rodrigues, A.S.L., Tognelli, M.F., Vie, J.-C., Aguiar, J.M., Allen, D.J., Allen, G.R., Amori, G., Ananjeva, N.B., Andreone, F., Andrew, P., Ortiz, A.L.A., Baillie, J.E.M., Baldi, R., Bell, B.D., Biju, S.D., Bird, J.P., Black-Decima, P., Blanc, J.J., Bolanos, F., Bolivar-G., W., Burfield, I.J., Burton, J.A., Capper, D.R., Castro, F., Catullo, G., Cavanagh, R.D., Channing, A., Chao, N.L., Chenery, A.M., Chiozza, F., Clausnitzer, V., Collar, N.J., Collett, L.C., Collette, B.B., Fernandez, C.F.C., Craig, M.T., Crosby, M.J., Cumberlidge, N., Cuttelod, A., Derocher, A.E., Diesmos, A.C., Donaldson, J.S., Duckworth, J.W., Dutson, G., Dutta, S.K., Emslie, R.H., Farjon, A., Fowler, S., Freyhof, J., Garshelis, D.L., Gerlach, J., Gower, D.J., Grant, T.D., Hammerson, G.A., Harris, R.B., Heaney, L.R., Hedges, S.B., Hero, J.-M., Hughes, B., Hussain, S.A., Icochea M., J., Inger, R.F., Ishii, N., Iskandar, D.T., Jenkins, R.K.B., Kaneko, Y., Kottelat, M., Kovacs, K.M., Kuzmin, S.L., La Marca, E., Lamoreux, J.F., Lau, M.W.N., Lavilla, E.O., Leus, K., Lewison, R.L., Lichtenstein, G., Livingstone, S.R., Lukoschek, V., Mallon, D.P., McGowan, P.J.K., McIvor, A., Moehlman, P.D., Molur, S., Alonso, A.M., Musick, J.A., Nowell, K., Nussbaum, R.A., Olech, W., Orlov, N.L., Papenfuss, T.J., Parra-Olea, G., Perrin, W.F., Polidoro, B.A., Pourkazemi, M., Racey, P.A., Ragle, J.S., Ram, M., Rathbun, G., Reynolds, R.P., Rhodin,

A.G.J., Richards, S.J., Rodriguez, L.O., Ron, S.R., Rondinini, C., Rylands, A.B., Sadovy de Mitcheson, Y., Sanciangco, J.C., Sanders, K.L., Santos-Barrera, G., Schipper, J., Self-Sullivan, C., Shi, Y., Shoemaker, A., Short, F.T., Sillero-Zubiri, C., Silvano, D.L., Smith, K.G., Smith, A.T., Snoeks, J., Stattersfield, A.J., Symes, A.J., Taber, A.B., Talukdar, B.K., Temple, H.J., Timmins, R., Tobias, J.A., Tsytsulina, K., Tweddle, D., Ubeda, C., Valenti, S. V., Paul van Dijk, P., Veiga, L.M., Veloso, A., Wege, D.C., Wilkinson, M., Williamson, E.A., Xie, F., Young, B.E., Akcakaya, H.R., Bennun, L., Blackburn, T.M., Boitani, L., Dublin, H.T., da Fonseca, G.A.B., Gascon, C., Lacher, T.E., Mace, G.M., Mainka, S.A., McNeely, J.A., Mittermeier, R.A., Reid, G.M., Rodriguez, J.P., Rosenberg, A.A., Samways, M.J., Smart, J., Stein, B.A., and Stuart, S.N. 2010. The Impact of Conservation on the Status of the World's Vertebrates. Science **330**(6010): 1503–1509. doi:10.1126/science.1194442.

Johnson, M.T.J., and Munshi-South, J. 2017. Evolution of life in urban environments. Science. **358**(6363): eaam8327. doi:10.1126/science.aam8327.

Jombart, T., Kamvar, Z.N., Collins, C., Lustrik, R., Beugin, M.-P., Knaus, B.J., Solymos, P., Mikryukov, V., Schliep, K., Maié, T., Morkovsky, L., Ahmed, I., Cori, A., Calboli, F., and Ewing, R.J. 2017. adegenet: Exploratory Analysis of Genetic and Genomic Data. Available from https://cran.r-project.org/package=adegenet.

Jones, M.B., Slaughter, P., Nahf, R., Boettiger, C., Jones, C., Read, J., Walker, L., Hart, E., and Chamberlain, S. 2017. dataone: R Interface to the DataONE REST API. Available from https://cran.r-project.org/package=dataone.

Leberg, P.L. 2002. Estimating allelic richness: Effects of sample size and bottlenecks. Mol. Ecol. **11**: 2445–2449. doi:10.1046/j.1365-294X.2002.01612.x.

Lourenço, A., Álvarez, D., Wang, I.J., and Velo-Antón, G. 2017. Trapped within the city: Integrating demography, time since isolation and population-specific traits to assess the genetic effects of urbanization. Mol. Ecol. **26**: 1498–1514. doi:10.1111/mec.14019.

Manel, S., Poncet, B.N., Legendre, P., Gugerli, F., and Holderegger, R. 2010. Common factors drive adaptive genetic variation at different spatial scales in Arabis alpina. Mol. Ecol. **19**(17): 3824–3835. doi:10.1111/j.1365-294X.2010.04716.x.

Metzger, G., Espindola, A., Waits, L.P., and Sullivan, J. 2015. Genetic Structure across Broad Spatial and Temporal Scales: Rocky Mountain Tailed Frogs (Ascaphus montanus; Anura: Ascaphidae) in the Inland Temperate Rainforest. J. Hered.: 700–710. doi:10.1093/jhered/esv061.

Miles, L.S., Rivkin, L.R., Johnson, M.T.J., Munshi-South, J., and Verrelli, B.C. 2019. Gene flow and genetic drift in urban environments. Mol. Ecol. **28**(18): 4138–4151. doi:10.1111/mec.15221.

Mittell, E.A., Nakagawa, S., and Hadfield, J.D. 2015. Are molecular markers useful predictors of adaptive potential? Ecol. Lett. **18**(8): 772–778. doi:10.1111/ele.12454.

Munshi-South, J., Zak, Y., and Pehek, E. 2013a. Data from: Conservation genetics of extremely isolated urban populations of the Northern Dusky Salamander (Desmognathus fuscus) in New York City. doi:10.5061/dryad.q1nc0.

Munshi-South, J., Zak, Y., and Pehek, E. 2013b. Conservation genetics of extremely isolated urban populations of the northern dusky salamander (Desmognathus fuscus) in New York City. PeerJ **1**: 1–16. doi:10.7717/peerj.64.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. **70**(12): 3321–3323. doi:10.1073/pnas.70.12.3321.

Nowakowski, A.J., Frishkoff, L.O., Thompson, M.E., Smith, T.M., and Todd, B.D. 2018. Phylogenetic homogenization of amphibian assemblages in human-altered habitats across the globe. Proc. Natl. Acad. Sci. U. S. A. **115**(15): E3454–E3462. doi:10.1073/pnas.1714891115.

Noël, S., Ouellet, M., Galois, P., and Lapointe, F.-J. 2007. Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander. Conserv. Genet. (8): 599–606. doi:10.1007/s10592-006-9202-1.

Paradis, E., and Schliep, K. 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in {R}. Bioinformatics **35**: 526–528.

Pereira, H.M., Ferrier, S., Walters, M., Geller, G.N., Jongman, R.H.G., Scholes, R.J., Bruford, M.W., Brummitt, N., Butchart, S.H.M., Cardoso, A.C., Coops, N.C., Dulloo, E., Faith, D.P., Freyhof, J., Gregory, R.D., Heip, C., Höft, R., Hurtt, G., Jetz, W., Karp, D.S., McGeoch, M.A., Obura, D., Onoda, Y., Pettorelli, N., Reyers, B., Sayre, R., Scharlemann, J.P.W., Stuart, S.N., Turak, E., Walpole, M., and Wegmann, M. 2013. Essential biodiversity variables. Science **339**(6117): 277–278. doi:10.1126/science.1229931.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. Vienna, Austria. Available from http://www.r-project.org/.

Saarikivi, J., Knopp, T., Granroth, A., and Merilä, J. 2013. The role of golf courses in maintaining genetic connectivity between common frog (Rana temporaria) populations in an urban setting. Conserv. Genet. **14**(5): 1057–1064. doi:10.1007/s10592-013-0495-6.

Schell, C.J. 2018. Urban evolutionary ecology and the potential benefits of implementing genomics. J. Hered. **109**(2): 138–151. doi:10.1093/jhered/esy001/4807252.

Schmidt, C., Domaratzki, M., Kinnunen, R.P., Bowman, J., and Garroway, C.J. 2020. Continent-wide effects of urbanization on bird and mammal genetic diversity. Proc. R. Soc. B Biol. Sci. **287**(1920): 20192497. doi:10.1098/rspb.2019.2497.

Spear, S.F., and Storfer, A. 2010. Anthropogenic and natural disturbance lead to differing patterns of gene flow in the Rocky Mountain tailed frog, *Ascaphus montanus*. Biol. Conserv. **143**(3): 778–786. Elsevier Ltd. doi:10.1016/j.biocon.2009.12.021.

Statistics Canada. 2016. Population Centres Census Division Cartographic Boundary File. 2016 Census. Statistics Canada Catalogue no. 92-160-X. Available from https://www12.statcan.gc.ca/census-recensement/2011/geo/bound-limit/bound-limit-2016-eng.cfm.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., and Waller, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. Science. **306**(5702): 1783–1786. doi:10.1126/science.1103538.

Trumbo, D.R., Spear, S.F., Baumsteiger, J., and Storfer, A. 2013. Rangewide landscape genetics of an endemic Pacific northwestern salamander. Mol. Ecol. **22**(5): 1250–1266. doi:10.1111/mec.12168.

U.S. Census Bureau. 2016. TIGER/Line Shapefiles: Urban Areas (machinereadable data files). Available from https://www.census.gov/geo/maps-data/data/tiger-line.html.

University, C. for I.E.S.I.N.-C.-C., and Georgia, I.T.O.S.-I.-U. of. 2013. Global Roads Open Access Data Set, Version 1 (gROADSv1). NASA Socioeconomic Data and Applications Center (SEDAC), Palisades, NY. Available from https://doi.org/10.7927/H4VD6WCT.

Waples, R.S., and Do, C. 2008. LDNE: A program for estimating effective population size from data on linkage disequilibrium. Mol. Ecol. Resour. **8**(4): 753–756. doi:10.1111/j.1755-0998.2007.02061.x.

Waples, R.S., and Do, C. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evol. Appl. **3**(3): 244–262. Wiley/Blackwell (10.1111). doi:10.1111/j.1752-4571.2009.00104.x.

WCS, and CIESIN. 2005. Last of the Wild Project, Version 2, 2005 (LWP-2): Global Human Footprint Dataset (Geographic). NASA Socioeconomic Data and Applications Center (SEDAC), Palisades, NY. doi:10.7927/H4M61H5F.

Weir, B.S., and Goudet, J. 2017. A unified characterization of population structure. Genetics **206**: 2085–2103.

WWF. 2018. Living Planet Report - 2018: Aiming higher. *Edited By*M. Grooten and R.E.A. Almond. WWF, Gland, Switzerland. Available from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:LIVING+PLANET+REPORT+2004#0.

Table 3.1. Model summaries for the effects of urbanization and human presence on population genetic composition. Five models were run for each response variable, four of which included a measure of human presence. The last was a null model including only spatial predictors as fixed effects if spatial autocorrelation was detected, otherwise was an intercept-only model. Marginal R^2 refers to the proportion of variation explained by fixed effects, and conditional R^2 is the variation explained by both fixed and random effects.

Variable	**Covariate**	**Coefficient (95% CI)**	**Marginal; Conditional R^2**
effective population size	urban/rural	0.35 (-0.13 – 0.79)	0.01; 0.32
n = 387	human population density	0.16 (0.03 – 0.31)	0.03; 0.32
	Human Footprint Index	0.15 (-0.01 – 0.34)	0.02; 0.32
	% impervious surface	0.05 (-0.04 – 0.13)	0.00; 0.31
	none	–	0.00; 0.31
gene diversity			
n = 554	urban/rural	-0.14 (-0.92 – 0.71)	0.10; 0.74
	human population density	0.21 (-0.07 – 0.52)	0.19; 0.76
	Human Footprint Index	0.28 (-0.04 – 0.59)	0.17; 0.77
	% impervious surface	-0.04 (-0.25 – 0.20)	0.02; 0.73
	none	–	0.11; 0.74
allelic richness			
n = 554	urban/rural	-0.16 (-0.50 – 0.21)	0.03; 0.59
	human population density	0.02 (-0.09 – 0.16)	0.03; 0.59
	Human Footprint Index	0.07 (-0.08 – 0.26)	0.04; 0.60
	% impervious surface	0.02 (-0.14 – 0.27)	0.01; 0.60
	none	–	0.02; 0.59
F_{ST}			
n = 552	urban/rural	0.04 (-1.25 – 1.28)	0.15; 0.58
	human population density	-0.27 (-0.65 – 0.07)	0.18; 0.59
	Human Footprint Index	-0.44 (-0.86 – -0.02)	0.19; 0.61
	% impervious surface	0.09 (-0.23 – 0.37)	0.05; 0.55
	none	–	0.18; 0.56

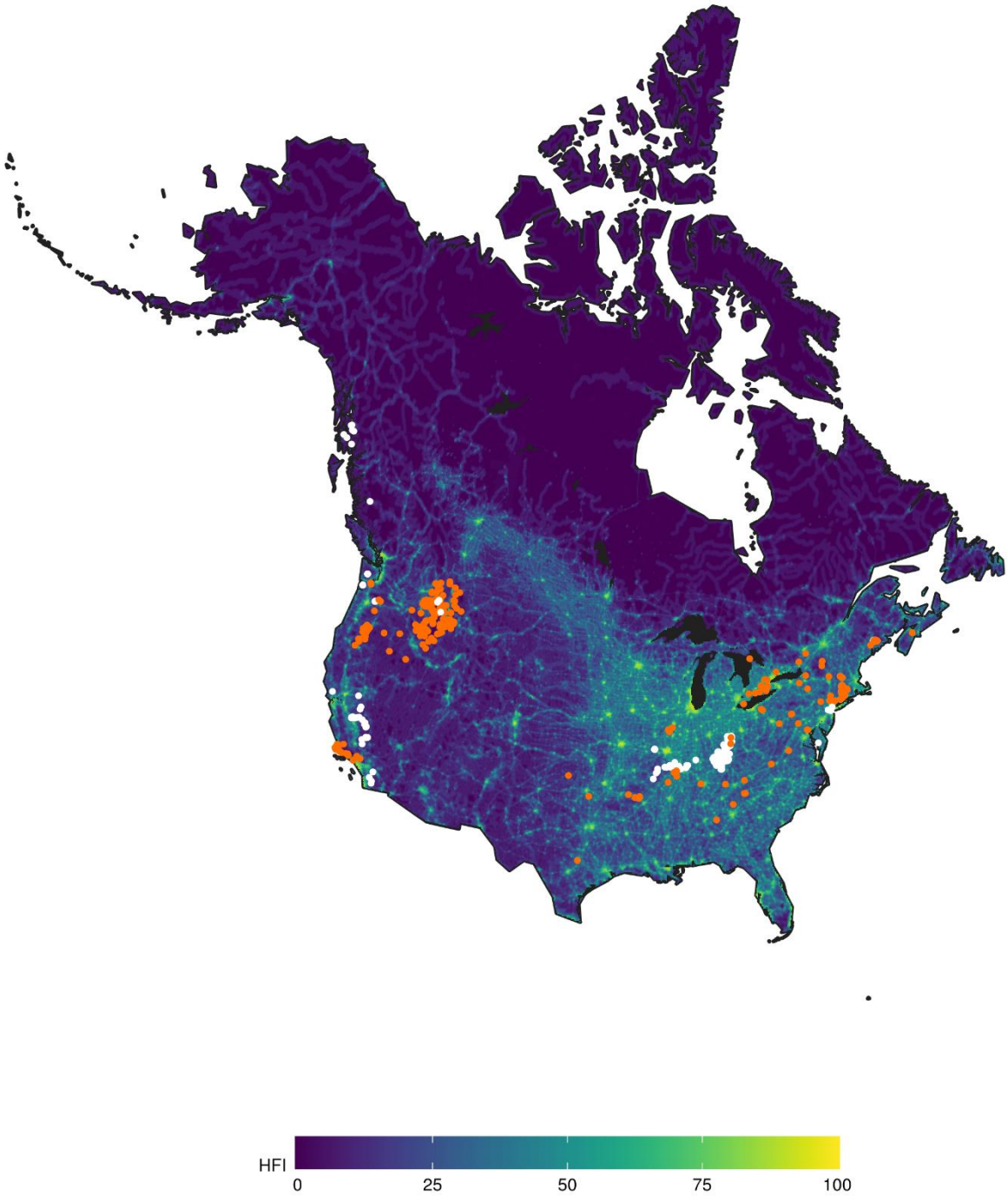


Figure 3.1. Map showing the locations of 554 sample sites from 19 species included in our analyses. White points are salamanders, and orange points are frogs. We harvested raw microsatellite data sampled from each site to estimate gene diversity, allelic richness, effective population size, and population-specific F_{ST}. Sites are overlaid on a map of the Human Footprint Index (HFI), which ranges from 0 (most wild) to 100 (most transformed).

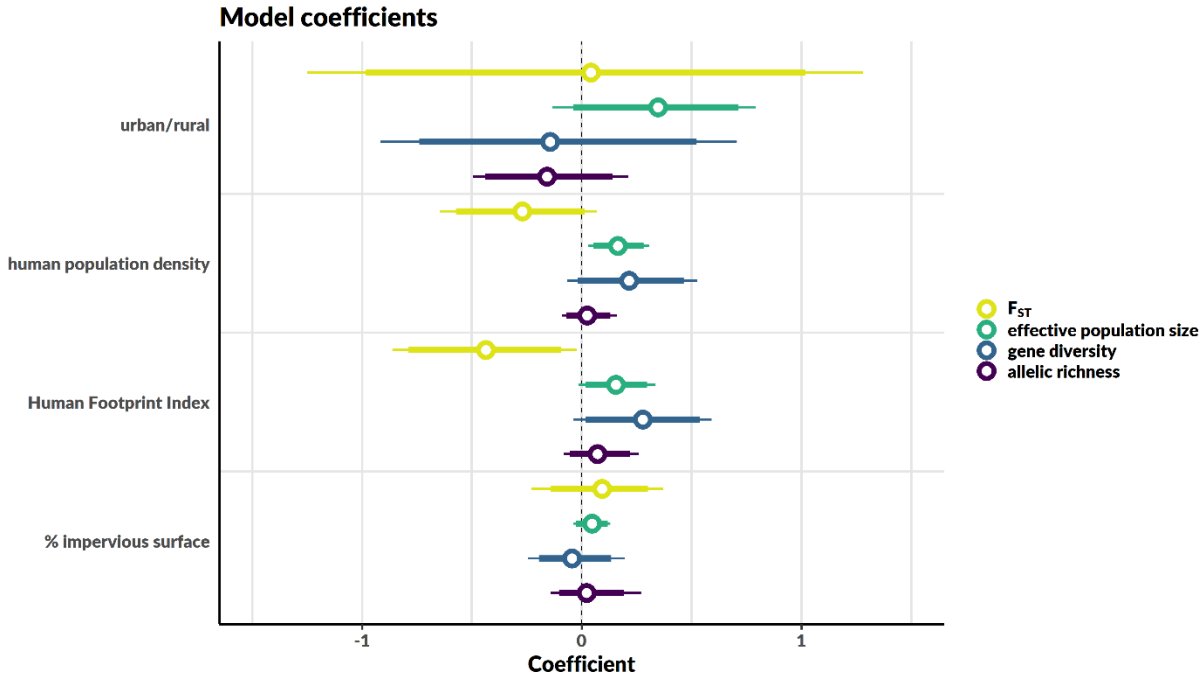


Figure 3.2. Coefficients from Bayesian linear mixed models. Effect sizes (open circles) are shown with 90% (bold lines) and 95% (thin lines) credible intervals. Note sample sizes differed between genetic measures (see Table 3.1). Effects appear generally small and inconsistent, however, there is a positive relationship between effective population size and human population density, and a negative relationship between population-specific F_{ST} and the Human Footprint Index.

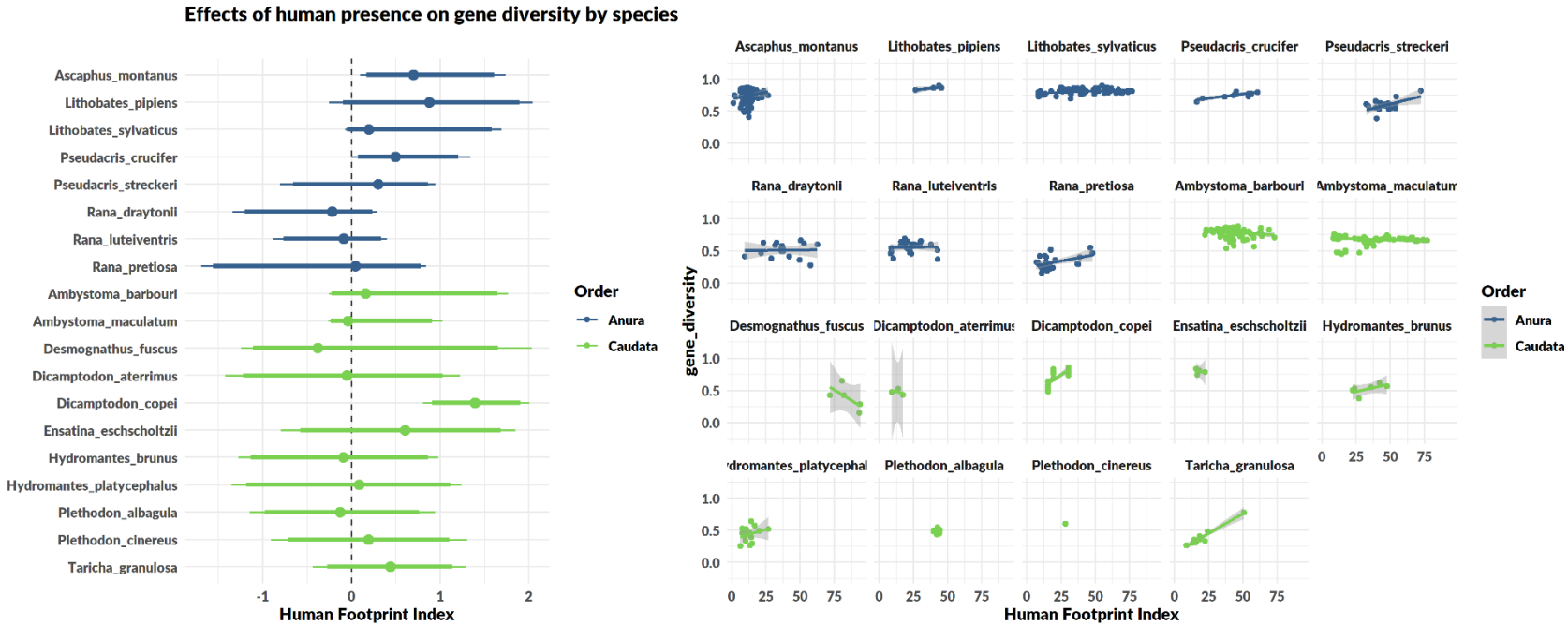


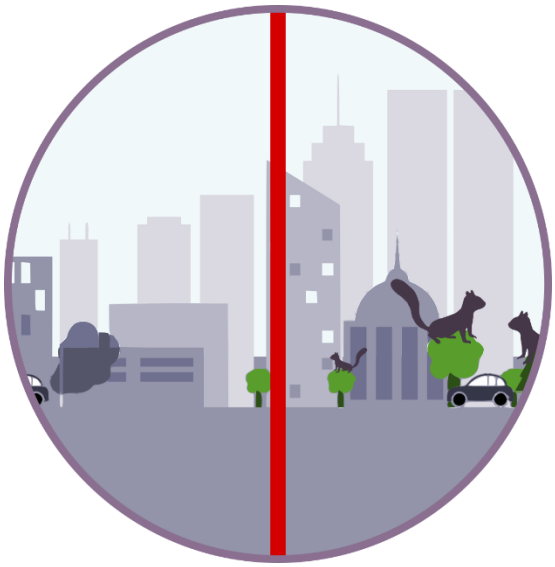
Figure 3.3. Species-specific effects of the Human Footprint Index on genetic diversity. The left graph shows species-specific coefficients for the effect of the Human Footprint Index on gene diversity; the right graphs are gene diversity plotted against the Human Footprint Index for each species. Grey shading around regression lines represent 95% confidence intervals. Few species respond positively to urbanization (Rocky mountain tailed frog, *Ascaphus montanus*; Cope's giant salamander, *Dicamptodon copei*; and spring peeper *Pseudacris crucifer*). In most species however, genetic diversity appears to have no relationship with measures of urbanization and human presence.

Chapter 4: Systemic racism alters wildlife genetic diversity

Chloé Schmidt, Colin J Garroway

Department of Biological Sciences, 50 Sifton Rd, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada

Status: under review in PNAS



Abstract: Humans are the defining feature of urban ecosystems, but the ways that our social decision-making affect evolution in urban wildlife populations are not well understood (Schell et al. 2020; Des Roches et al. 2021). In the United States, systemic racism—specifically government-mandated 'redlining' policies—have had lasting effects on the built structure of cities due to the racial segregation of neighborhoods (Schell et al. 2020). However, it is not known whether varying habitat structure and natural resource availability due to segregation affects evolution in urban wildlife. We repurposed public genetic data from 35 terrestrial vertebrate species sampled in 202 urban locations to show that systemic racism creates environments that reduce the efficiency of selection and neutral genetic diversity, and cause animal populations to become less well connected. The relationship between neighborhood racial composition and animal genetic diversity was mediated by habitat disturbance. This suggests that systemic racism alters evolutionary processes acting on urban wildlife populations in ways that negatively affect their chances of persistence. Limited capacity to support large, well-connected wildlife populations reduces access to nature and builds on existing environmental inequities shouldered by predominantly non-white neighborhoods.

Keywords: urban evolution, redlining, effective population size, population isolation, Human Footprint Index

Introduction

Historic and ongoing systemic racism and racial segregation have played a prominent role in the development and structure of cities in the United States (Rothstein 2017; Schell et al. 2020). One of the most direct causes of present-day racial segregation was the government-sponsored practice of *redlining*, which graded neighborhoods based on desirability and systematically excluded racial and ethnic minorities—namely Black Americans—from homeownership in better-ranked neighborhoods. During the suburb boom in the 1950s, discriminatory redlining policies and practices related to lending, insurance, zoning, and public housing collectively encouraged white Americans to move into new suburban communities and simultaneously pushed Black Americans and other racial and ethnic minorities to reside towards urban cores (Rothstein 2017). Lower-ranking redlined neighborhoods subsequently received less public investment and typically became densely populated, had more industrial infrastructure, and less green space (reviewed in Rothstein 2017; Schell et al. 2020). Racial segregation and spatial isolation were often reinforced by physical barriers such as highways, railroad tracks, and sometimes walls (Rothstein 2017). These practices, although outlawed in the Fair Housing Act of 1968, created a socially-structured geography associated with socioeconomic and environmental inequity that persists in American cities (Rothstein 2017; Watkins and Gerrish 2018).

Evolutionary effects of systemic racism

Accumulating knowledge of the effects of systemic racism on the structure of urban environments now allows us to turn an eye towards its effects on evolution in urban wildlife. In a comprehensive review, Schell et al. (Schell et al. 2020) show that socioeconomic decision-making and racial inequality have created environmental conditions that can alter the distribution and demography of wildlife in cities in ways that should cause evolutionary change (Schell et al. 2020; Des Roches et al. 2021). However, these ideas have received little empirical attention. Residential racial segregation creates disparities in natural resource availability, land use, pollution, and habitat connectivity,

such that neighborhoods that historically excluded minorities tend to be better wildlife habitat (Schell et al. 2020). This means that local environmental carrying capacities in cities are likely predicted by the racial makeup of neighborhoods. In general, larger, more connected populations have better chances of persisting in an area, are less strongly affected by genetic drift, and have higher genetic diversity which determines their capacity to respond to environmental change. Cities are now where people primarily interact with and benefit from nature (Fuller and Irvine 2013), and their design is becoming increasingly important for the conservation of native biodiversity (Aronson et al. 2014). Managing wildlife for conservation and human well-being requires a comprehensive understanding of eco-evolutionary processes in cities, and this extends to identifying the ways that human social patterns shape evolution in urban wildlife.

We test the hypothesis that systemic racism produces urban environments that alter population demography and thus evolutionary change in city-dwelling populations of amphibians, birds, mammals, and reptiles across the continental United States. It is now clear that urbanization and human land use in general affect the genetic composition of wildlife populations when compared to populations in more natural environments (Johnson and Munshi-South 2017; Leigh et al. 2019; Schmidt et al. 2020). How evolutionary processes shape genetic diversity within cities is less well understood. We predicted that levels of genetic diversity and connectivity among urban wildlife populations would vary with the racial composition of neighborhoods, increasing in predominantly white, less environmentally disturbed areas. The effect of systemic racism on ecological and evolutionary change in urban wildlife is likely mediated by differential resource distribution and habitat degradation (Schell et al. 2020). We explored this idea by testing the effects of the racial composition of neighborhoods on genetic diversity alone, and while statistically controlling for habitat degradation.

Residential segregation has contributed to the present marked wealth disparities across racial groups in the United States (Pager and Shepherd 2008; Reskin 2012). Although discriminatory housing policies are now illegal, Black Americans are still less likely to own homes, prospective homeowners are steered towards predominantly Black neighborhoods,

and homes they do own are less likely to appreciate (Reskin 2012), presenting a racialized barrier to wealth accumulation. The ecological effects of wealth on wildlife can be notable. Wealth, or "luxury effects", alter habitat quality, population dynamics, and the species composition of urban wildlife communities. However, the strength of luxury effects vary regionally and across taxa (Jenerette et al. 2011; Roman et al. 2018; Schell et al. 2020). Additionally, the greatest disparities in urban forest cover across the racial mosaic appear on public, rather than private land, reflecting the effect of biased municipal investment in communities rather than the effects of individual wealth (Watkins and Gerrish 2018). For these reasons the environmental effects of structural racism cannot be captured by neighborhood wealth alone, and here we focus on habitat disturbance more generally.

Quantifying genetic diversity in terrestrial vertebrates

We tested our hypothesis by building a database of georeferenced publicly archived, raw, neutral microsatellite data sets (Schmidt et al. 2020) (Fig. 4.1; Methods). We aggregated 6,284 individual genotypes from 7 amphibian, 11 bird, 14 mammal, and 3 reptile species native to North America. The typical numbers of microsatellite loci used in molecular ecological research estimate genome-wide diversity well (Mittell et al. 2015). We conducted a systematic search for microsatellite data in online data repositories in R (R Core Team 2019) using a list of species names for terrestrial vertebrates native to North America (Methods). By repurposing raw data, we were able to consistently calculate our chosen metrics of genetic composition and environmental variation across the entire aggregated dataset. For each sample site, we calculated the effective population size using a linkage disequilibrium method (Do et al. 2014), gene diversity (Nei 1973), standardized allelic richness, and measured genetic divergence using site-specific F_{ST} (Weir and Goudet 2017). The effective population size is an estimate of the strength of genetic drift, gene diversity and allelic richness are two measures of genetic diversity, and site-specific F_{ST} was our estimate of relative genetic differentiation among sites. We excluded sites not located within US Census-designated urban areas (U.S. Census Bureau 2016).

We calculated the percentage of residents identifying as white in census blocks located within 0.5, 1, and 5 km of each sample site in our dataset using demographic data from the 2010 US Census (Manson et al. 2019). We present results from 0.5 km buffers here, but note results were consistent across all scales (Fig. S4.1). We chose this metric of neighborhood racial segregation because white Americans are the most separated demographic. According to the 2010 Census, the average white American lives in a predominantly white neighborhood, while other racial groups typically live in more diverse neighborhoods (Logan and Stults 2011). Thus, the proportion of white residents in a neighborhood captures environmental heterogeneity caused by racial segregation well. We quantified disturbance at each site with the Human Footprint Index (WCS and CIESIN 2005). The Human Footprint Index measures human-caused habitat transformation from the most wild to the most disturbed. It provides a broad index of habitat degradation by incorporating human population density, roads, railways, access to navigable rivers, built-up areas, land cover, and nighttime lights. The percentage of white residents in a neighborhood was negatively correlated with the Human Footprint Index (Pearson's r = -0.52; 95% confidence interval: -0.60 – -0.43), demonstrating that in our dataset, predominately non-white neighborhoods were located in more disturbed environments.

We tested the effect of racial composition on the genetic composition of species at sample sites using Bayesian hierarchical models (Bürkner 2019). We controlled for variation across taxonomic class and species using a random effect structure with random intercepts for species nested in class, allowing slopes and intercepts to vary within species (Methods). Here, random slope and intercept models estimate the effect of racial composition on each species, and the distribution of species-specific parameter estimates shrink towards an overall mean— the effect size across all species. This is a feature of hierarchical models that is highlighted in a multi-species context. Shrinkage allows levels of a random effect to inform each other, yielding more robust estimates of effect size, at the same time detecting general effects across species that may be difficult to detect in single-species analyses (Harrison et al. 2018). Moran's I tests detected no residual spatial autocorrelation in the models.

Effects of racial segregation on genetic variation

We detected consistent relationships between genetic composition and the racial composition of neighborhoods (Fig. 4.2). Species tended to have larger effective population sizes, higher genetic diversity, and were less genetically differentiated in neighborhoods with higher proportions of white residents (Fig. 4.2; Table 4.1). This result suggests that demographic and evolutionary processes in urban wildlife vary within American cities in ways that make population persistence in minority neighborhoods more difficult.

We then fit separate models relating the Human Footprint Index alone, and both the Human Footprint Index and the racial composition of neighborhoods together, to our measures of genetic composition. This was to explore whether the effects of the neighborhood racial composition might be mediated by habitat degradation and reduced natural resource availability. Specifically, we used adjusted R^2 values to determine whether models including both racial composition and the Human Footprint Index explained more variation than either covariate alone. In each model the Human Footprint Index was negatively related to genetic diversity, effective population size, and connectivity (Table 4.1). The amount of variation explained by racial composition and human disturbance was low across all models (Table S4.1). For all genetic metrics, the proportion of variance explained by models including both racial composition and the Human Footprint Index was similar to the variation explained using only one of these covariates (Table 4.1). These results suggest that segregation drives the unequal distribution of resources and creates landscape heterogeneity that shapes demography and genetic diversity in urban wildlife.

Ecologically, our results suggest that majority non-white neighborhoods support smaller, more fragmented, less genetically diverse wildlife populations. Source-sink dynamics could potentially create this pattern, because wildlife from natural and less disturbed sites further from city centers have limited access to urban cores (Schell et al. 2020). Notably, the effects of racial composition and habitat degradation on genetic composition were consistent across taxa and cities. Previous work along urban-rural gradients suggests that mammal populations were generally negatively affected by increasing human disturbance,

but responses in birds were species-specific with both increases and decreases in diversity detected (Schmidt et al. 2020), and effects were not detectable in amphibians (Schmidt and Garroway 2020). When considering habitat variation within cities, it appears urban wildlife populations, regardless of taxa or location, tend to be larger and harbor higher genetic diversity in less disturbed habitat patches. This was true even for common urban species in our dataset, including coyotes and black-capped chickadees. These results are concerning because urban biodiversity is important for human mental and physical well-being (Russell et al. 2013), and disparities in access to nature build on existing health-related environmental disamenities in minority neighborhoods including greater exposure to air pollution and heat-island effects (Bailey et al. 2017; Schell et al. 2020).

Urban evolutionary ecology research is only beginning to more deeply explore the effects of spatial heterogeneity within cities generated by human social processes (Schell et al. 2020; Des Roches et al. 2021). This will require more informed, comprehensive sampling of urban habitats. In our dataset, 87% of sites were located in predominantly white neighborhoods. If we are to fully consider environmental heterogeneity within and across cities to understand the spectrum of ways humans affect their environments, better sampling by a more diverse research community is needed. However, racial diversity is particularly low in ecology and evolution (Graves 2019; O'Brien et al. 2020). Black students, for example, earned just 1% of ecology and evolutionary biology doctorates awarded in the United States in 2019 (National Science Foundation and National Center for Science and Engineering Statistics 2019). Research in urban evolutionary ecology will become an increasingly important resource for decision-makers and city planners to make cities sustainable habitats for wildlife while meeting human needs (Des Roches et al. 2021). It is clear that systemic racism is altering evolutionary processes acting on urban wildlife populations. To achieve environmental equity, and ultimately cities that support humans and wildlife alike, it is necessary to confront systemic biases and social processes in urban evolutionary ecology research.

Methods

Data compilation

To create the database of genetic metrics, we performed 3 systematic searches of online data repositories between 2018 and 2020 using the DataONE interface for R (Jones et al. 2017) with the keywords "str", "microsat*", single tandem*", "short tandem*", and species name (e.g. "*Alces alces*"). DataONE is a network of public data repositories, such as Dryad. We used existing datasets described in (Schmidt et al. 2020); (Schmidt and Garroway 2020) where detailed methods for dataset assembly can be found. We augmented this dataset in February 2019 with data from reptiles, and in November 2020 with additional mammal data using the same inclusion criteria. In brief we retained datasets with neutral microsatellite datasets sampled from native species located in North America where study design would not influence genetic diversity (e.g., island or managed populations). We retrieved 68 total search results for reptiles, 28 of which were duplicates. In total 11 datasets met our inclusion criteria. For additional mammal data we obtained 37 search results, of which 10 were duplicates and 8 were added to our database. We measured effective population sizes, allelic richness, gene diversity, and population-specific F_{ST} for each sample site from raw microsatellite datasets. We estimated effective population size of the parental generation using the linkage disequilibrium method in Neestimator (Do et al. 2014). We were unable to estimate effective population size when sampling error overwhelms signals of genetic drift, as is the case when too few individuals were sampled or populations are extremely large. We calculated allelic richness and gene diversity using the adegenet package in R (Jombart et al. 2017). Allelic richness is sensitive to the number of sampled individuals, thus we standardized this measure to the minimum sample size across the entire dataset (5 individuals; (Leberg 2002). Gene diversity (Nei 1973) is a heterozygosity metric that is minimally affected by sample size variation (Charlesworth and Charlesworth 2010). Finally, population-specific F_{ST} (Weir and Goudet 2017) is a relative measure of genetic differentiation that estimates how far populations have diverged from a common ancestor in a sample. We computed this metric with the hierfstat package (Goudet and Jombart 2015), and note that it can only be computed when at least 2 populations were sampled per dataset.

Because this study focuses on the effects of human demographics within cities, we excluded non-urban sites from this analysis based on whether they were located within the boundaries of census-designated urban areas (U.S. Census Bureau 2016). Data from 43 studies were ultimately included, and the final dataset consisted of 268 sites across all taxa (Tables 1, 2). Of these, we were able to estimate effective population size at 226 sites, thus we retained these sites for analysis for effective population size, gene diversity, and allelic richness. Site-specific F_{ST} was estimable at 222 sites. The datasets included a site-level measure of the Human Footprint Index (WCS and CIESIN 2005) from previous analyses (Schmidt and Garroway 2020; Schmidt et al. 2020). We then obtained demographic data from the United States Census Bureau through the IPUMS National Historical Geographic Information System (Manson et al. 2019). Demographic data is from census blocks, the smallest census geographic unit. For each site, we measured the percent of the population identifying as white within 3 buffer sizes: 0.5, 1, and 5 km. Note sample sizes differed across these scales when sites were not located near populated blocks within the designated buffer size ($n_{0.5km}$ = 202 sites; n_{1km} = 215 sites, n_{5km} = 226 sites).

Statistical analysis

All analyses were conducted in R version 4.0.1 (R Core Team 2020). To test for the effects of residential racial segregation (% white residents in neighborhood) on the genetic diversity of wild populations we used Bayesian linear mixed models implemented in the brms package (Bürkner 2017). We log-transformed effective population size, and scaled and centered all variables prior to analysis.

Our modelling strategy incorporated a random effect structure to account for variation across taxonomic class and species. We included random intercepts for species nested in class, allowing slopes to vary within species. Random slope models provide more conservative parameter estimates due to shrinkage, where the distribution of group-level effects are drawn towards the overall mean effect (Harrison et al. 2018). Shrinkage to the overall effect is strongest for groups with fewer observations, allowing them to borrow

strength from better sampled-groups. In this way, knowledge is shared across grouping levels of a random factor because we assume they are drawn from a common statistical population—whereas in fixed-effect only models, groups are assumed to be independent. The benefits of shrinkage in random slopes and intercept models are especially salient from a macrogenetics perspective. Species- or city-specific analyses often yield varying results (e.g., Miles et al. 2019), but when analyzing raw data aggregated across broader spatial or taxonomic contexts, random slope and intercept models can provide better estimates of general effects.

We treated previous results from a different dataset showing the effect of the Human Footprint Index on mammal gene diversity, allelic richness, effective population size, and population-specific F_{ST} (Schmidt et al. 2020) as suitable priors given the negative correlation between the percentage of white residents in a neighborhood and the Human Footprint Index. We assigned informative normally distributed priors (allelic richness: mean = 0.11± 0.04 SD; gene diversity: mean = 0.12 ± 0.05 SD; effective population size: mean = 0.27 ± 0.22 SD; F_{ST}: mean = -0.15 ± 0.06 SD) and ran all models with 4 chains and minimum 10000 iterations. We used Moran's *I* tests to test for spatial autocorrelation in model residuals. We used marginal and conditional R^2 to see the amount of variation explained by fixed, and fixed and random effects respectively (Table S4.1) (Nakagawa and Schielzeth 2013). Next, we used the same modeling approach to test for the effects of Human Footprint Index alone, and the joint effects of racial segregation and the Human Footprint Index on genetic composition in another series of models. Finally, to compare explanatory ability between univariate models and models including both racial composition and Human Footprint Index, we used adjusted R^2 values. Adjusted R^2 for Bayesian models calculates the amount of variation explained using leave-one-out cross validation taking into account model complexity. If models including both covariates explain more variation than models with either covariate, this suggests that the effect of neighborhood racial composition and environmental disturbance on genetic composition are to some extent independent. If the opposite is true, it is more likely that racial

composition affects genetic composition due to its correlation with environmental disturbance.

Acknowledgments: We are grateful to the authors of the original datasets for making their data public. We thank Mitchell Green for assisting with data collection. We would like to thank the Population Ecology and Evolutionary Genetics group at the University of Manitoba, Jeff Bowman, Ruth Rivkin, Alicia Korpach, Alice Schmidt for providing feedback on earlier drafts of the manuscript. C.S. and C.J.G. were supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to C.J.G. C.S. was also supported by a U. Manitoba Graduate Fellowship and a U. Manitoba Graduate Enhancement of Tri-council funding grant to C.J.G.

References

Aronson, M.F.J., La Sorte, F.A., Nilon, C.H., Katti, M., Goddard, M.A., Lepczyk, C.A., Warren, P.S., Williams, N.S.G., Cilliers, S., Clarkson, B., Dobbs, C., Dolan, R., Hedblom, M., Klotz, S., Kooijmans, J.L., Kühn, I., MacGregor-Fors, I., McDonnell, M., Mörtberg, U., Pyšek, P., Siebert, S., Sushinsky, J., Werner, P., and Winter, M. 2014. A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. Proc. R. Soc. B Biol. Sci. **281**(1780): 20133330. doi:10.1098/rspb.2013.3330.

Bailey, Z.D., Krieger, N., Agénor, M., Graves, J., Linos, N., and Bassett, M.T. 2017. Structural racism and health inequities in the USA: evidence and interventions. Lancet **389**(10077): 1453–1463. Elsevier Ltd. doi:10.1016/S0140-6736(17)30569-X.

Bürkner, P.-C. 2019. brms: Bayesian Regression Models using "Stan." Available from https://cran.r-project.org/package=brms.

Bürkner, P.C. 2017. brms: An R Package for Bayesian Multilevel Models Using Stan. J. Stat. Softw. **80**(1): 1–28. doi:10.18637/jss.v080.i01.

Charlesworth, B., and Charlesworth, D. 2010. Elements of evolutionary genetics. Roberts & Company Publishers, Greenwood Village, Colorado, USA.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., and Ovenden, J.R. 2014. NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol. Ecol. Resour. **14**(1): 209–214. doi:10.1111/1755-0998.12157.

Fuller, R.A., and Irvine, K.N. 2013. Interactions between people and nature in urban environments. *In* Urban Ecology. *Edited by* K.J. Gaston. Cambridge University Press, Cambridge. pp. 134–171. doi:10.1017/CBO9780511778483.008.

Goudet, J., and Jombart, T. 2015. hierfstat: Estimation and Tests of Hierarchical F-Statistics. Available from https://cran.r-project.org/package=hierfstat.

Graves, J.L. 2019. African Americans in evolutionary science: Where we have been, and what's next. Evol. Educ. Outreach **12**(1): 1–10. Springer US. doi:10.1186/s12052-019-0110-5.

Harrison, X.A., Donaldson, L., Correa-Cano, M.E., Evans, J., Fisher, D.N., Goodwin, C.E.D., Robinson, B.S., Hodgson, D.J., and Inger, R. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ **6**: e4794. doi:10.7717/peerj.4794.

Jenerette, G.D., Harlan, S.L., Stefanov, W.L., and Martin, C.A. 2011. Ecosystem services and urban heat riskscape moderation: Water, green spaces, and social inequality in Phoenix, USA. Ecol. Appl. **21**(7): 2637–2651. doi:10.1890/10-1493.1.

Johnson, M.T.J., and Munshi-South, J. 2017. Evolution of life in urban environments. Science. **358**(6363): eaam8327. doi:10.1126/science.aam8327.

Jombart, T., Kamvar, Z.N., Collins, C., Lustrik, R., Beugin, M.-P., Knaus, B.J., Solymos, P., Mikryukov, V., Schliep, K., Maié, T., Morkovsky, L., Ahmed, I., Cori, A., Calboli, F., and Ewing, R.J. 2017. adegenet: Exploratory Analysis of Genetic and Genomic Data. Available from https://cran.r-project.org/package=adegenet.

Jones, M.B., Slaughter, P., Nahf, R., Boettiger, C., Jones, C., Read, J., Walker, L., Hart, E., and Chamberlain, S. 2017. dataone: R Interface to the DataONE REST API. Available from https://cran.r-project.org/package=dataone.

Leberg, P.L. 2002. Estimating allelic richness: Effects of sample size and bottlenecks. Mol. Ecol. **11**: 2445–2449. doi:10.1046/j.1365-294X.2002.01612.x.

Leigh, D.M., Hendry, A.P., Vázquez-Domínguez, E., and Friesen, V.L. 2019. Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. Evol. Appl. **12**(8): 1505–1512. doi:10.1111/eva.12810.

Logan, J.R., and Stults, B.J. 2011. The Persistence of Segregation in the Metropolis: New Findings from the 2010 Census. *In* Census Brief prepared for Project US2010. Available from http://www.s4.brown.edu/us2010.

Manson, S., Schroeder, J., Van Riper, D., and Ruggles, S. 2019. IPUMS National Historical Geographic Information System: Version 14.0 [Database] Minneapolis, MN: IPUMS. doi:10.18128/D050.V14.0.

Miles, L.S., Rivkin, L.R., Johnson, M.T.J., Munshi-South, J., and Verrelli, B.C. 2019. Gene flow and genetic drift in urban environments. Mol. Ecol. **28**(18): 4138–4151. doi:10.1111/mec.15221.

Mittell, E.A., Nakagawa, S., and Hadfield, J.D. 2015. Are molecular markers useful predictors of adaptive potential? Ecol. Lett. **18**(8): 772–778. doi:10.1111/ele.12454.

Nakagawa, S., and Schielzeth, H. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. **4**(2): 133–142. doi:10.1111/j.2041-210x.2012.00261.x.

National Science Foundation, and National Center for Science and Engineering Statistics. 2019. Doctorate Recipients from U.S. Universities: 2019. Alexandria, VA. Available from https://www.nsf.gov/statistics/sed/2018/nsf18304/.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. **70**(12): 3321–3323. doi:10.1073/pnas.70.12.3321.

O'Brien, L.T., Bart, H.L., and Garcia, D.M. 2020. Why are there so few ethnic minorities in ecology and evolutionary biology? Challenges to inclusion and the role of sense of belonging. Soc. Psychol. Educ. **23**(2): 449–477. Springer Netherlands.

doi:10.1007/s11218-019-09538-x.

Pager, D., and Shepherd, H. 2008. The sociology of discrimination: Racial discrimination in employment, housing, credit, and consumer markets. Annu. Rev. Sociol. **34**: 181–209. doi:10.1146/annurev.soc.33.040406.131740.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. Vienna, Austria. Available from http://www.r-project.org/.

R Core Team. 2020. R: A Language and Environment for Statistical Computing. Vienna, Austria. Available from https://www.r-project.org/.

Reskin, B. 2012. The race discrimination system. Annu. Rev. Sociol. **38**: 17–35. doi:10.1146/annurev-soc-071811-145508.

Des Roches, S., Brans, K.I., Lambert, M.R., Rivkin, L.R., Savage, A.M., Schell, C.J., Correa, C., De Meester, L., Diamond, S.E., Grimm, N.B., Harris, N.C., Govaert, L., Hendry, A.P., Johnson, M.T.J., Munshi-South, J., Palkovacs, E.P., Szulkin, M., Urban, M.C., Verrelli, B.C., and Alberti, M. 2021. Socio-eco-evolutionary dynamics in cities. Evol. Appl. **14**(1): 248–267. doi:10.1111/eva.13065.

Roman, L.A., Pearsall, H., Eisenman, T.S., Conway, T.M., Fahey, R.T., Landry, S., Vogt, J., van Doorn, N.S., Grove, J.M., Locke, D.H., Bardekjian, A.C., Battles, J.J., Cadenasso, M.L., van den Bosch, C.C.K., Avolio, M., Berland, A., Jenerette, G.D., Mincey, S.K., Pataki, D.E., and Staudhammer, C. 2018. Human and biophysical legacies shape contemporary urban forests: A literature synthesis. Urban For. Urban Green. **31**(December 2017): 157–168. Elsevier. doi:10.1016/j.ufug.2018.03.004.

Rothstein, R. 2017. The color of law: a forgotten history of how our government segregated America. Liveright, New York, N.Y.

Russell, R., Guerry, A.D., Balvanera, P., Gould, R.K., Basurto, X., Chan, K.M. a., Klain, S., Levine, J., and Tam, J. 2013. Humans and Nature: How Knowing and Experiencing Nature Affect Well-Being. Annu. Rev. Environ. Resour. **38**(1): 473–502. doi:10.1146/annurev-environ-012312-110838.

Schell, C.J., Dyson, K., Fuentes, T.L., Des Roches, S., Harris, N.C., Miller, D.S., Woelfle-Erskine, C.A., and Lambert, M.R. 2020. The ecological and evolutionary consequences of systemic racism in urban environments. Science. **4497**(August): 1–19. doi:10.1126/science.aay4497.

Schmidt, C., Domaratzki, M., Kinnunen, R.P., Bowman, J., and Garroway, C.J. 2020. Continent-wide effects of urbanization on bird and mammal genetic diversity. Proc. R. Soc. B Biol. Sci. **287**(1920): 20192497. doi:10.1098/rspb.2019.2497.

Schmidt, C., and Garroway, C.J. 2020. Inconsistent effects of urbanization on amphibian genetic diversity. bioRxiv: 1–30. doi:10.1101/2020.08.16.253104.

U.S. Census Bureau. 2016. TIGER/Line Shapefiles: Urban Areas (machinereadable data files). Available from https://www.census.gov/geo/maps-data/data/tiger-line.html.

Watkins, S.L., and Gerrish, E. 2018. The relationship between urban forests and race: A meta-analysis. J. Environ. Manage. **209**: 152–168. Elsevier Ltd. doi:10.1016/j.jenvman.2017.12.021.

WCS, and CIESIN. 2005. Last of the Wild Project, Version 2, 2005 (LWP-2): Global Human Footprint Dataset (Geographic). NASA Socioeconomic Data and Applications Center (SEDAC), Palisades, NY. doi:10.7927/H4M61H5F.

Weir, B.S., and Goudet, J. 2017. A unified characterization of population structure. Genetics **206**: 2085–2103.

Table 4.1. Effects of racial segregation and environmental disturbance (the Human Footprint Index) on genetic composition. Coefficient estimates are given with 95% credible intervals. Adjusted R^2 (R^2_{Adj}) is an estimate of variation explained taking into account model complexity.

Variable	Covariate	Coefficient (95% CI)	R^2_{Adj}
allelic richness	Racial segregation (% white residents)	0.08 (0.03, 0.14)	0.75
n = 202 sites	Human Footprint Index	-0.10 (-0.16, -0.04)	0.76
	both	--	0.75
gene diversity	Racial segregation (% white residents)	0.07 (0.02, 0.13)	0.85
n = 202 sites	Human Footprint Index	-0.12 (-0.18, -0.05)	0.86
	both	--	0.86
effective population size	Racial segregation (% white residents)	0.16 (0.03, 0.30)	0.24
n = 202 sites	Human Footprint Index	-0.16 (-0.30, -0.03)	0.25
	both	--	0.24
F_{ST}	Racial segregation (% white residents)	-0.12 (-0.21, -0.04)	0.46
n = 198 sites	Human Footprint Index	0.15 (0.06, 0.25)	0.46
	both	--	0.43

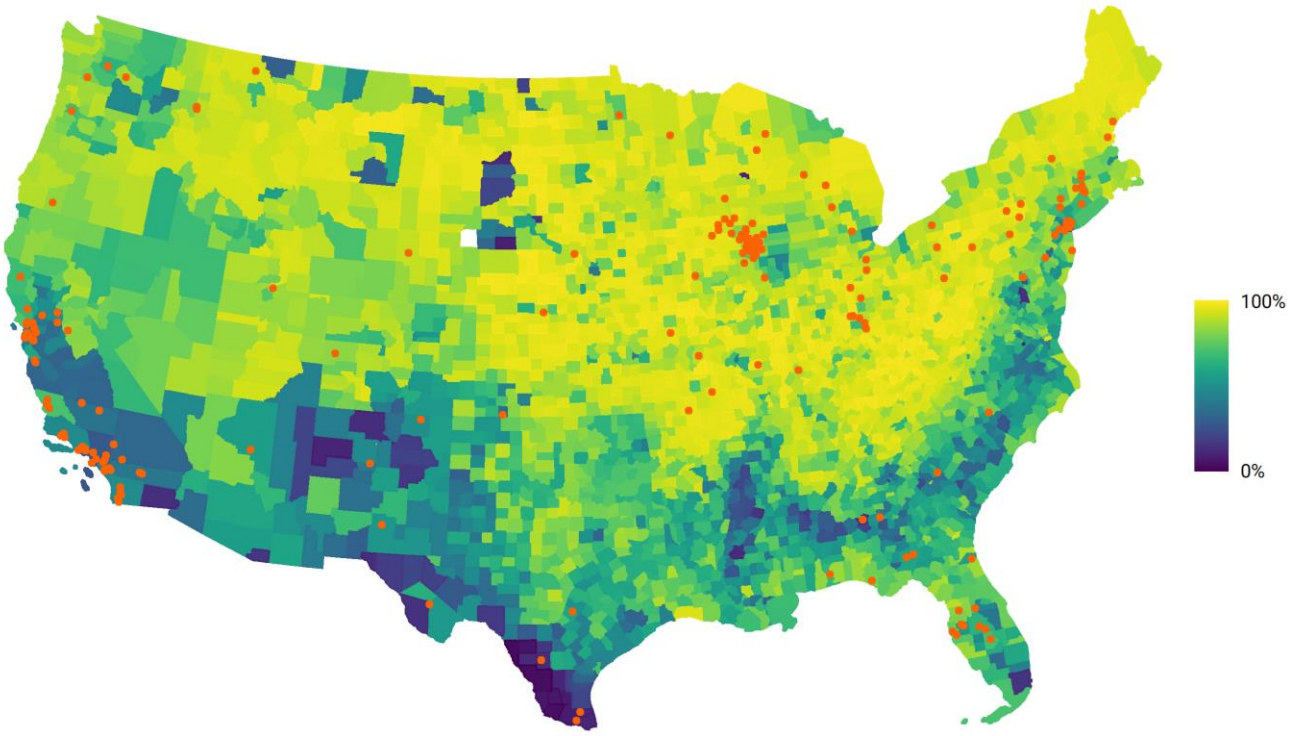


Figure 4.1. Map of 202 sample sites for 35 species of amphibian, bird, mammal, and reptile located in urban areas in the continental United States (points). Racial composition, measured by the proportion of the population identifying as white according to 2010 US census data, is depicted at the county level.

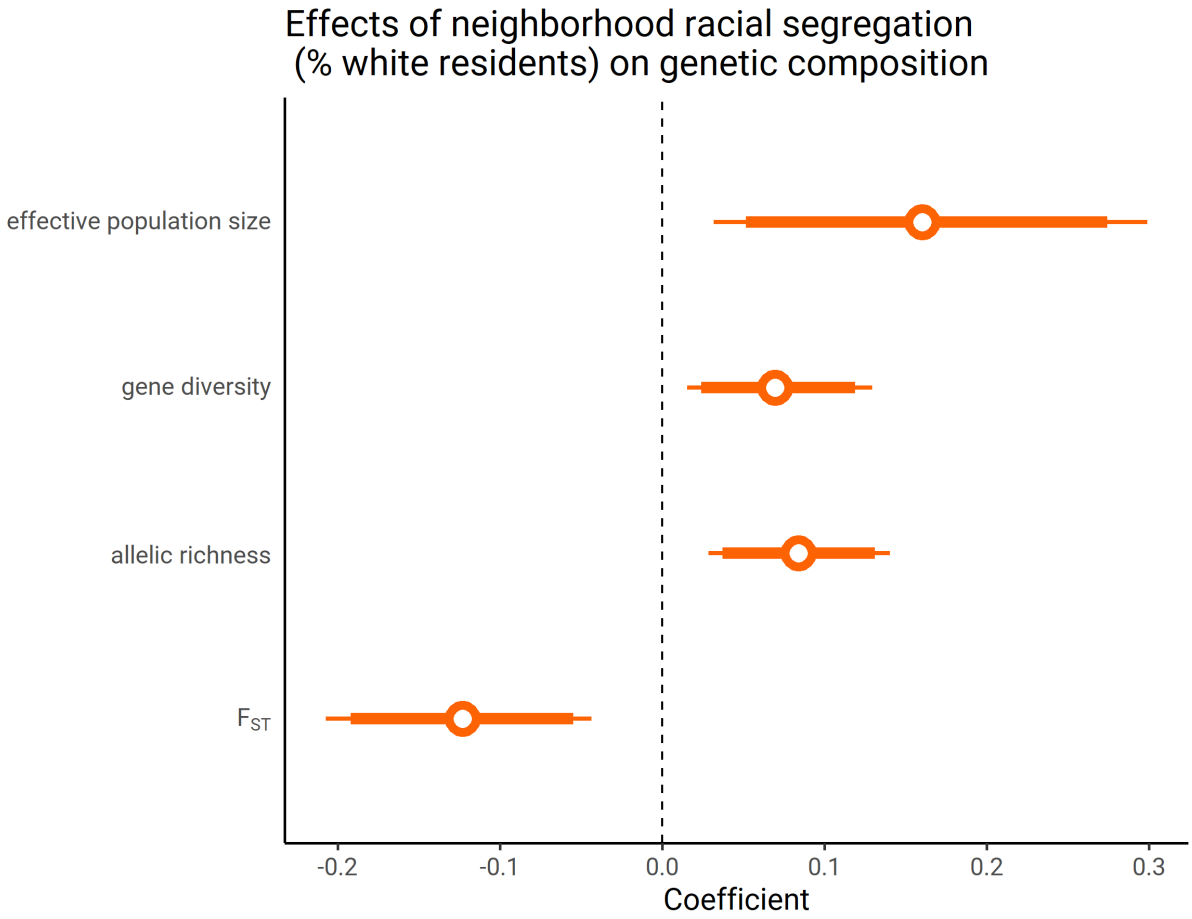


Figure 4.2. Bayesian GLMM coefficients for the effect of racial segregation, measured as percent of white residents in a neighborhood, on genetic composition. Coefficient estimates (open circles) are shown with 90% (bold lines) and 95% (narrow lines) credible intervals.

Chapter 5: Genetic and species-level biodiversity patterns are linked by demography and ecological opportunity

Chloé Schmidt[1], Stéphane Dray[2], Colin J. Garroway[1]

[1]Department of Biological Sciences, 50 Sifton Rd, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada

[2]Univ Lyon, Université Claude Bernard Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive, F-69100, Villeurbanne, France

Status: revision requested from Evolution *(preprint DOI: 10.1101/2020.06.03.132092)*

Abstract: Species richness and genetic diversity are the two most fundamental products of evolution. Both are important conservation targets—species richness contributes to ecosystem functioning and human wellbeing, while genetic diversity allows those species to respond to changes in their environment and persist in the long-term. Biogeographic patterns of species richness are well-described, but we know little about patterns of genome-wide genetic diversity at similar spatial scales. Further, despite considerable attention to latitudinal trends in species richness, we still do not have a solid empirical understanding of the various processes that produce them, how they interact, or how they affect genetic diversity. Here we show that genome-wide genetic diversity and species richness share spatial structure, however, species richness hotspots tend to harbor low levels of within-species genetic variation. A single model encompassing eco-evolutionary processes related to environmental energy availability, niche availability, and proximity to humans explained 75% of variation in gene diversity and 90% of the variation in species richness. Our empirical model of both levels of biodiversity supports theory and demonstrates the importance of carrying capacity and ecological opportunity at individual and species levels for generating continent-wide genetic and species diversity gradients.

Keywords: more individuals hypothesis, heterogeneity, Anthropocene, latitudinal diversity gradient, carrying capacity, macroecology

Introduction

Biodiversity patterns at the genetic and species levels form the foundation upon which higher-level diversity patterns emerge with the processes that generate diversity across these two base levels likely so entangled that they should be considered inseparable (Lowe et al. 2017; Pontarp et al. 2019). Biogeographic-scale variations in species-level diversity are among the best-described patterns in nature (Pontarp et al. 2019). The exploration of biogeographic patterns in genetic diversity across species has had to wait for technological advances in molecular genetics and the accumulation of data (Miraldo et al. 2016; Manel et al. 2020; Theodoridis et al. 2020). Regardless of research effort, our empirical understanding of the causes of diversity patterns remains underdeveloped (Pontarp et al. 2019), likely in part due to a lack of integrated analyses of the causes of diversity at both levels. Here we produce a continent-scale map of nuclear genetic diversity for North American mammals and show that genetic diversity and species-level diversity are spatially correlated and likely have common environmental causes.

Existing hypotheses for species diversity patterns (Lomolino et al. 2016) generally fall into three broad categories: those related to evolutionary time for diversification, different diversification rates, and ecological limits on the number of species a region can support. Evolutionary time hypotheses predict that regions that have been colonized for the longest times should tend to have higher species richness than elsewhere due to diversification having taken place for longer periods (e.g., greater time for speciation in the tropics). Diversification rate hypotheses suggest that spatial variation in speciation or extinction rates (e.g., due variation in environmental conditions, mutation rates, and generation times) explain species richness patterns. Finally, ecological limits hypotheses posit that variation in resource availability sets a species-level carrying capacity that limits the number of species able to coexist in a particular area. Here speciation, extinction, and colonization dynamics of species are analogous to the birth, death, and immigration dynamics that set population-level carrying capacities. There are at least 26 specific hypotheses that fall under these umbrella categories – detailed reviews can be found in (Mittelbach et al. 2007; Stein et al. 2014; Worm and Tittensor 2018; Pontarp et al. 2019).

Evolutionary time, evolutionary rates, and ecological limits hypotheses are often implicitly treated as competing ideas but speciation can clearly simultaneously be a product of both ecological and evolutionary processes (Pontarp and Wiens 2017). Indeed, recent modelling exercises suggest all categories of hypothesis can produce species richness gradients (Etienne et al. 2019). That said, the preponderance of theory suggests that carrying capacities limiting the supportable number of species in an environment produces the strongest and most stable species richness gradients (Vellend 2005; Worm and Tittensor 2018; Etienne et al. 2019; Brodie 2019). Etienne et al. (2019) used simulations to compare diversification rate, evolutionary time, and ecological limits hypotheses. Their models suggested that ecological limits on carrying capacity present the most parsimonious explanation for the latitudinal diversity gradient. There is also considerable empirical evidence in support of this theoretical work suggesting the likely importance of ecological limits in the formation of species richness patterns (Storch and Okie 2019; Brodie 2019). Taken together, there is good reason to consider ecological limits as a null expectation when exploring the causes of species richness patterns (Etienne et al. 2019).

We extended the consequences of processes related to ecological limits to explain multispecies population-level patterns of genetic diversity. If environments limit the number of species they can support, they must also limit the population sizes of those species and thus the strength of genetic drift. Thus, demographic processes acting at the individual and species levels could simultaneously shape genetic and species-level biodiversity (Fig. 5.1). We focused on two prominent ecological limits hypotheses for species richness—the more individuals and environmental heterogeneity hypotheses. The more individuals hypothesis posits that energy availability imposes an upper limit on the number of individuals, and as a consequence, the number of species an area can support (Storch et al. 2018). According to the neutral theory of molecular evolution (Kimura 1983) and the neutral theory of biodiversity and biogeography (Hubbell 2001), diversity tends to increase with the number of individuals in an assemblage both in terms of genetic diversity within populations and the number of species in a community. We thus predicted positive

relationships among genetic diversity, species richness, and energy availability. The habitat heterogeneity hypothesis suggests that environmental heterogeneity equates to niche availability, with heterogeneous areas able to support more specialized species, albeit at smaller population sizes because resources are divided (Kadmon and Allouche 2007; Allouche et al. 2012; Stein et al. 2014). As increasingly specialized populations diverge, genetic variation would be partitioned among locally adapted populations that may eventually no longer interbreed. These smaller populations will also lose genetic diversity due to genetic drift faster than large populations. We thus predicted that habitat heterogeneity would be positively associated with species richness and negatively associated with genetic diversity.

In addition to carrying capacity limits set by climatic factors and habitat complexity, a major contemporary environmental limitation on diversity is land transformation by humans. Habitat loss, fragmentation, and homogenization due to human activities such as urbanization reduce the amount of habitat available to wild populations (McKinney 2006; Grimm et al. 2008) with consequences at genetic and species levels. Estimates suggest that within the last century, over 400 vertebrate species have gone extinct (Ceballos et al. 2020), vertebrate population sizes worldwide have shrunk by an average of 60% (WWF 2018), and intraspecific genetic diversity across taxa has declined by approximately 6% (Leigh et al. 2019). Contemporary rapid environmental change contributes to biodiversity patterns in addition to long-term processes. Because humans are known to influence both levels of biodiversity, our effects should be examined alongside natural factors. By reducing habitable area and environmental heterogeneity, we predicted that the effects of urbanization should also cause species richness and genetic diversity to decrease in more heavily disturbed areas.

Our objectives in this study were twofold. Biogeographic-scale correlations between nuclear genetic and species-level diversity patterns have not yet been established, so we first tested for shared spatial patterns at both levels of biodiversity. Having established shared patterns of variation we then tested for common environmental causes of genetic

and species-level diversity using structural equation modelling (SEM). Structural equation modelling fits hypothesis networks that can accommodate multiple predictor and response variables within a hierarchical modelling framework. This allows the relative importance of multiple hypotheses to be assessed while accounting for species-level variation. Our data were repurposed publicly archived raw neutral nuclear genetic data for North American mammals spanning 801 sample sites, 38 species, and 34,841 individuals.

Methods

Data assembly

Genetic diversity database. We used the database of genetic metrics in North America compiled by Schmidt et al. (Schmidt et al. 2020a, 2020b). This database repurposed raw microsatellite data from 34,841 individuals across 38 mammalian species sampled at 801 sites in the United States and Canada, and includes consistently calculated measures of gene diversity (Nei 1973) and population-specific F_{ST} (Weir and Goudet 2017). See Table S5.2 for a summary of the dataset. Microsatellite markers estimate genome-wide diversity well (Mittell et al. 2015). They are commonly used in wildlife population genetic studies because they are cost-effective and do not require a reference genome, which allowed us to maximize sample size. We chose to focus on North America to control for regional history. Detailed methods for assembling this dataset can be found in (Schmidt et al. 2020b) (Chapter 2). Briefly, we performed a systematic search for species names of native North American mammals with keywords "microsat*", "single tandem*", "short tandem*", and "str" using the 'dataone' R package, which interfaces with the DataONE platform to search online open data repositories (Jones et al. 2017). We discarded search results that did not meet our criteria for inclusion and removed results where study design may have influenced genetic diversity. For example we excluded non-neutral data and samples taken after a recent bottleneck, translocations, managed or captive populations, or island populations. We additionally removed populations with fewer than 5 individuals sampled. Gene diversity estimates the richness and evenness of alleles in a population, and we used it here as our metric for genetic diversity because it is minimally affected by sample size

(Charlesworth and Charlesworth 2010)(Fig. S5.1). Sample sites are treated as point locations.

Population size. Because species-level censuses are not generally available, we used body size as a proxy for species-level population size. The inverse relationship between body size and species population size is well documented and is especially reliable in mammals (Damuth 1981, 1987). Neutral genome-wide genetic diversity is also negatively correlated with body size (Frankham 1996; Romiguier et al. 2014), the most likely explanation being strong links between body size and effective population size (Frankham 1996). We recorded mean adult body mass (g) for each species using data from the PanTHERIA database (Jones et al. 2009). Mass was log-transformed before analysis. There were no obvious outliers in these data.

Species richness. We downloaded range maps for terrestrial mammals native to North America from the IUCN Red List database (IUCN 2019). We filtered these maps to retain ranges for extant, native, resident, mainland species in ArcMap Desktop 10.3.1 (ESRI, Redlands, CA). To generate a map of species richness coincident with genetic sample sites, we estimated species richness at each site within a 10 km buffer. For the range-wide measure of species richness used in our hierarchical structural equation models, we summed the number of ranges that overlapped each of our 38 focal species' ranges. To correct for potential biases due to differences in range size (e.g. species with large ranges tending to have more overlapping ranges), we divided the number of overlapping ranges by the species' range area (km^2), giving us species richness per square kilometer for each species.

Environmental variables. We used potential evapotranspiration as our measure of energy availability (Currie 1991). Specifically, potential evapotranspiration measures the atmosphere's ability to remove water from the Earth's surface and is an indicator of atmospheric energy availability. Potential evapotranspiration is one of the strongest environmental correlates of species richness in mammals (Currie 1991; Kreft and Jetz 2007; Fisher et al. 2011; Jiménez-Alfaro et al. 2016). We estimated mean potential

evapotranspiration (mm/yr) across each species' range using annual potential evapotranspiration data from 1970-2000 available via the CGIAR Consortium for Spatial Information (Trabucco and Zomer 2019). We used a global topography map (NOAA and U.S. National Geophysical Data Center) to record the range in elevation across focal species ranges to quantify environmental heterogeneity (Stein et al. 2015). As with species richness, we corrected elevation range for potential biases introduced by species range area, because larger ranges tended to encompass greater topographical heterogeneity. Finally, human influence was a site level variable estimated using the human population density within a 10 km zone around each site, following Schmidt et al. 2020a finding its strong effect on mammalian genetic diversity.

Analysis

Genetic diversity and species richness maps. All analyses were conducted in R version 3.6.1 (R Core Team 2019). Our first step was to identify spatial patterns in genetic diversity. We accomplished this using distance-based Moran's eigenvector maps (MEMs) in the R package 'adespatial' (Dray et al. 2017). MEMs detect spatial patterns in data from a modified matrix of distances between sites—a neighbor matrix—whose eigenvalues are proportional to Moran's I index of spatial autocorrelation (Borcard and Legendre 2002; Borcard et al. 2004; Dray et al. 2006). MEMs are spatial eigenvectors that represent relationships between sites at all spatial scales detectable by the sampling scheme and can be included in linear models because they are orthogonal. A total of 199 positive MEMs were detected. Next, we used the forward selection procedure described in (Blanchet et al. 2008) to select two sets of MEMs: one describing site-level spatial patterns in genetic diversity and the other describing site-level species richness. Thirteen MEMs explained important spatial variation in gene diversity. In order of increasingly fine spatial scales, significant patterns were MEMs 2, 3, 4, 5, 22, 27, 30, 31, 47, 49, 101, 145, 152. Forty-three MEMs were important predictors of species richness, and 8 of these patterns were shared by genetic diversity (significant MEMs are listed in Fig. S5.3).

We then subset MEMs based on Moran's I to retain only those explaining broad-scale spatial patterns (MEMs with Moran's I > 0.25). The cut-off for broad-scale MEMs was MEM 5 for genetic diversity and MEM 11 for species richness. We then fit individual linear regression models for species richness and genetic diversity with the broad-scale MEMs, and plotted the predicted values on a map of North America.

Variation partitioning. We next quantified the extent to which genetic diversity and species richness covary spatially. Because MEMs for species richness and genetic diversity were computed from the same set of coordinates, they were directly comparable. This allowed us to identify shared spatial MEMs that might be related to a common environmental cause. We used linear regressions and variance partitioning to determine what fraction of the total variation in species richness and genetic diversity could be attributed to: (1) non-spatial variation, (2) non-shared spatial variation, and (3) shared spatial variation. We partitioned variation as follows:

$$y_{SR} \sim \alpha + \beta_{1S}(\mathrm{MEM}_{1S}) + \beta_{2S}(\mathrm{MEM}_{2S}) + \cdots + \beta_{iS}(\mathrm{MEM}_{iS}) + \epsilon$$

$$y_{GD} \sim \alpha + \beta_{1G}(\mathrm{MEM}_{1G}) + \beta_{2G}(\mathrm{MEM}_{2G}) + \cdots + \beta_{iG}(\mathrm{MEM}_{iG}) + \epsilon$$

Where α is the grand mean, and y_{SR} and y_{GD} are site-level metrics of species richness and genetic diversity. MEM_{iS} and MEM_{iG} refer to the set of MEMs explaining spatial variation in species richness and genetic diversity, respectively, and βs are their slopes. The coefficients of variation (R^2) for these models gave us the total proportion of variation in each response variable attributable to spatial variation. Subtracting these values from 1 gives the amount of non-spatial variation.

To determine the amount of shared variation, we used the set of MEMs shared between species richness and genetic diversity (MEM_{SG}) as predictors in the regressions below:

$$y_{SR} \sim \alpha + \beta_{1SG}(\mathrm{MEM}_{1SG}) + \beta_{2SG}(\mathrm{MEM}_{2SG}) + \cdots + \beta_{iSG}(\mathrm{MEM}_{iSG}) + \epsilon$$

$$y_{GD} \sim \alpha + \beta_{1SG}(\mathrm{MEM}_{1SG}) + \beta_{2SG}(\mathrm{MEM}_{2SG}) + \cdots + \beta_{iSG}(\mathrm{MEM}_{iSG}) + \epsilon$$

R^2 values from these models yielded the proportion of variation in genetic diversity and species richness explained by shared spatial variation. Subtracting these values from the total spatial variation in species richness and genetic diversity gives the proportion of non-shared spatial variation.

Structural equation modeling. Next, we tested the hypothesis that differential carrying capacities and human disturbance simultaneously shape biodiversity patterns on genetic and species levels. To explore the common causes of genetic and species-level diversity, we fit our conceptual model integrating population genetics and ecological limits (Fig. 5.3a) to data using structural equation modelling. Using this approach we can examine cause-effect relationships within hypothesis networks that accommodate multiple predictor and response variables in a hierarchical modeling framework. Multiple hypotheses can be retained in a final model. Structural equation modeling is an extension of multivariate multiple regression where variables can be thought of as nodes in a network, and directional paths connecting nodes represent causal relationships. The strengths of paths are equal to regression coefficients (Shipley 2016). In addition to direct effects, you can quantify indirect effects between variables by multiplying direct effects over paths. Using standardized coefficients, we can compare the strength of relationships and the relative support for retained hypotheses both within and across levels of biodiversity. The appropriateness of links in the hypothesis network can be tested using tests of directed separation (Shipley 2016), where the null hypothesis is that the two variables are independent conditional on other predictors of either variable. This means that although we start with a focus on ecological limits, the data can suggest the addition or removal of links representing alternative hypotheses.

We have primarily focused on modeling broad-scale effects of the environment on continental patterns of species richness and genetic diversity. We therefore focus here on hierarchical modeling of patterns at the population and species level. Additionally, because the spatial coverage of genetic sample sites in the data was not evenly distributed, some species ranges could be oversampled if we considered site-level environmental variation,

and thus overrepresented compared to species ranges that contain fewer sampled populations. To capture the broad spatial patterns depicted in Figure 5.2, and to avoid biasing our model as a result of uneven sample site locations, we considered species richness, energy availability, and heterogeneity at the species level in this analysis.

We implemented SEMs using the piecewiseSEM package (Lefcheck 2016; Lefcheck et al. 2019). PiecewiseSEM offers greater flexibility than other SEM software because it uses a local estimation approach where each model is assessed individually (Lefcheck 2016). All variables were scaled and centered before analysis.

We translated our conceptual model (Fig. 5.3a) into a series of 3 linear models with a single model for each response variable (gene diversity, population size/body mass, and species richness). We accounted for species-level differences in gene diversity using a linear mixed-effects model controlling for species as a random effect within our structural equation model network. Hierarchical models in piecewiseSEM were fit using the lme4 package (Bates et al. 2015). Conceptually, a hierarchical model is a model of models—here, we are modelling gene diversity within species and summarizing effects across species. Multiple linear regression models are fit in base R.

Goodness-of-fit in SEM is determined by evaluating whether there are any missing links in the causal structure, i.e. whether adding paths between pairs of variables would be more consistent with the data. In piecewiseSEM missing links are tested using tests of directed separation (Shipley 2016), where the null hypothesis is that the two variables are independent conditional on other predictors of either variable. Starting with our conceptual model (Fig. 5.3a), we iteratively updated models by adding links according to tests of directed separation until no further biologically sensible links were suggested. We assessed model fit using the *p*-value for the model network, where the null hypothesis is that the model is consistent with the data. Thus, models with $p > 0.05$ are considered acceptable—we fail to reject our causal structure. We also assessed fit using R^2 values for each response variable in the model network. For genetic diversity, we used marginal (R^2_m) and conditional R^2 (R^2_c) values which respectively measure the total variation explained by

fixed effects, and the variation explained by both fixed and random effects. We tested the residuals from component models for spatial autocorrelation using Moran's tests and spatial correlograms.

Effect of heterogeneity on population divergence. After detecting a negative effect of heterogeneity on intraspecific genetic diversity in our SEM, we performed a post hoc analysis to test whether topographic heterogeneity also caused greater population differentiation within species. A positive correlation between F_{ST} and heterogeneity, while controlling for distance, would suggest that individuals move less between local environments, possibly due to niche specialization. To test for differentiation we used population-specific F_{ST} (Weir and Goudet 2017) as a measure of genetic divergence, which was included in the genetic diversity database (Schmidt et al. 2020b) where it was calculated in R using the 'hierfstat' package (Goudet and Jombart 2015). Population-specific F_{ST} can be interpreted as a relative estimate of the time since a population has diverged from a common ancestor. This metric requires at least 2 sampled populations within a study to estimate, and due to this constraint 16 sites were excluded from this analysis (n = 785). We controlled for isolation-by-distance by including MEMs significantly related to F_{ST} to account for spatial structure. We scaled and centered all variables, then used a linear mixed model controlling for species differences by including it as a random effect.

Results

Spatial patterns in genetic diversity and species richness

We detected spatial patterns at genetic and species levels of diversity. Sixty-five percent of the total variation in species richness and 24% of variation in genetic diversity was spatially structured (Fig. S5.2). Variance partitioning suggested that 85% of the total spatial variation in genetic diversity, and 32% of spatial variation in species richness was accounted for by spatial patterns shared at both levels of diversity (Fig. S5.2). We found no obvious relationship between latitude and nuclear genetic diversity. Similar to patterns of species richness, a longitudinal gradient in genetic diversity is the dominant pattern for North American mammals—however, diversity gradients at the two levels trend in

opposite directions. Nuclear genetic diversity appears markedly lower in regions with high species richness, such as on the west and mid-Atlantic coasts, where there is high energy availability and topographic relief (Fig. 5.2).

Joint environmental causes of genetic diversity and species richness

Our conceptual model, updated according to tests of conditional independence among variables (directed separation), fit the data well (SEM p= 0.23, Fisher's C= 2.92; Fig. 5.3b, Table S5.1). Note that for structural equation models, $p > 0.05$ indicates that we fail to reject our model. There was no spatial autocorrelation in the body size model residuals, but genetic diversity and species richness models had statistically significant spatially autocorrelated residuals at very local scales (genetic diversity Moran's I = 0.025, species richness Moran's I = 0.029). These Moran's I values do not indicate strong spatial structure in the data, and we decided not to integrate it into our model. Positive spatial autocorrelation at such short distances is likely an artifact of irregular site locations and the hierarchical nature of the data. A lack of strong spatial autocorrelation in the model residuals suggests that the spatial structure of the diversity data was well captured by our model's environmental covariates (Fig. S5.3).

All predicted links in our conceptual model were supported (Fig 3a, b). Tests of directed separation suggested additional direct links from energy availability to species richness, genetic diversity to species richness, and heterogeneity to genetic diversity (Fig. 5.3b). Energy availability, niche heterogeneity, and human population density, acting both directly, and indirectly through species population size, explained 32% of the variation in genetic diversity. The species-level variation explained by the random effect for species brought the total variation in genetic diversity explained by our model to 75%. The same model explained 90% of the variation in species richness. The strength of effects related to the more individuals hypothesis was most prominent at the genetic level of diversity. The strength of the indirect effect of energy on genetic diversity acting via population size was 0.13 compared to 0.02 for species richness (Fig. 5.3b, Table S5.1). Environmental heterogeneity, however, was the strongest single predictor of species richness (path

coefficient = 0.70 ± 0.01 SE), and a good predictor of genetic diversity (path coefficient = -0.30 ± 0.07 SE). Directions of effects were as expected if greater niche availability reduces population sizes, leading to increased genetic drift (Fig. 5.3, Table S5.1). Gene diversity is not a measure of divergence so we tested whether environmental heterogeneity predicted evolutionary divergence at the population level. Divergence increased in heterogeneous environments (β = 0.13 ± 0.06 SE). Finally, human population density both directly and indirectly (via body mass/population size) affected species richness and genetic diversity (Fig. 5.3b). Human population density had the strongest effect on population size/body mass (path coefficient = -0.15 ± 0.03 SE), and relatively weaker direct effects on genetic diversity and species richness (Fig. 5.3b, Table S5.1).

Discussion

We found striking content-wide spatial gradients in nuclear genetic diversity and show that these patterns are negatively correlated with well-described biogeographic patterns in species richness (Simpson 1964) (Fig. 5.2). Controlling for species-level variation, a considerable portion of the variation in both genetic diversity and species richness patterns could be explained by just three environmental factors – these were environmental energy availability, niche availability, and human disturbance. Our model was consistent with the hypothesis that environmentally set species-level carrying capacities simultaneously limit species population sizes, and consequently genetic diversity through their effects on the strength of genetic drift. Niche availability was the strongest contributor to broad-scale patterns at both levels of diversity, followed by energy availability, and then human disturbance. This is strong empirical evidence suggesting that genetic diversity and species richness patterns emerge from the same processes thus jointly forming the base of the biodiversity hierarchy.

In support of the more individuals hypothesis (solid lines in Fig. 5.3b), our data indicated that low energy environments supported fewer species and smaller population sizes with lower genetic diversity. High energy areas had greater species richness and larger, more genetically diverse populations. However, effects related to the more individuals

hypothesis were weaker than those of environmental heterogeneity (dashed lines in Fig. 5.3b). Heterogeneity appeared to increase species richness and facilitate coexistence through greater niche availability, however partitioning resources among niches seemed to support smaller numbers of individuals from those species, creating a negative relationship between species richness and genetic diversity. At the genetic level, greater population divergence in more heterogeneous environments suggests that genetic drift is strong and gene flow limited in these areas.

Selection is more spatially varying in heterogeneous environments, and coupled with low gene flow, this could create sufficient conditions for local adaptation—which can happen even under relatively high levels of genetic drift (Hämälä et al. 2018). At lower latitudes where small-bodied species with large effective population sizes dominate, heterogeneity and spatially varying selection could be efficient drivers of ecological speciation. These results lend support to the idea that there are higher diversification rates in more complex environments because there are more opportunities for speciation. We additionally speculate that the direct effect of energy on species richness we detected even after accounting for population size and heterogeneity (Fig. 5.3b) may be related to niche availability as well. This relationship has been noted elsewhere and has sometimes been interpreted as refuting the more individuals hypothesis (Storch et al. 2018). Vegetation structure may drive the link between species richness and temperature (Pautasso and Gaston 2005; Jiménez-Alfaro et al. 2016), as complex, vegetation-rich habitats in warmer environments also have greater niche availability. Because both links are retained in our model it seems clear that this additional link does not negate the more individuals hypothesis, but rather is additive and indeed more important in determining species richness than the more individuals effect.

The specific ways environments shape nuclear genetic- and species-level diversity will likely differ across taxa. This carrying capacity-based interpretation of our results assumes that an environmentally set equilibrium between speciation, immigration and extinction has been reached. There is good evidence for this in North American mammals, where

diversification rates have slowed as diversity increased (Brodie 2019). It seems likely that processes other than ecological limits will be more important for the diversity dynamics of taxa that may not have reached or have been displaced from equilibrium at the genetic or species levels. Speciation is a product of both ecological and evolutionary processes, and it is unlikely ecological limits act in isolation. Indeed, the underlying causes of species richness gradients—be they ecological limits, evolutionary time, or diversification rates—have likely been debated for so long precisely because several processes operating with different importance across the timeline of diversification are capable of producing gradients (Etienne et al. 2019). Recent thinking (Pontarp and Wiens 2017) advocates a more interconnected view, suggesting that time for speciation should be most detectable more immediately following broad-scale environmental change. When all locales are colonized, habitats that provide more opportunities for speciation should over time become the most diverse. As diversity increases, diversification rates slow as regions approach equilibrium (Brodie 2019). It follows that evolutionary time and diversification rates may have each at different periods of history been the dominant driver of biodiversity, but both are ultimately affected by variation in carrying capacity (Pontarp and Wiens 2017).

Contemporary drivers of biodiversity patterns are rarely modeled in a way that makes them comparable to evolutionary scale causes. Understanding the ecological processes generating gradients in genetic diversity and species richness has important implications for understanding how biodiversity responds to human-caused environmental transformation. Cities are the world's newest and most rapidly expanding biome, and it is clear that they have already had profound effects on biodiversity patterns (Palumbi 2001; WWF 2018; Schmidt et al. 2020b). The negative effect of human population density we detected on body size is consistent with previous findings showing that urban communities tend to be made up of smaller species (Merckx et al. 2018). Although it seems human presence and heterogeneity both have negative effects on genetic diversity in our model, species richness was reduced in more urban environments (Fig. 5.3b). This result suggests that cities reduce population sizes and gene flow (Schmidt et al. 2020b), but currently do

not support diverse communities. Because cities are relatively new habitat types and they are still in the initial phase of colonization, we would not expect them to be in equilibrium. At this stage processes related to evolutionary time will likely predominate until all available niches are occupied. Indeed, there is some evidence that following an initial extinction debt after rapid urbanization, older cities support more biodiversity (Aronson et al. 2014; Norton et al. 2016). Presently, a subset of species do well in cities (McKinney 2006), but the broader effects of habitat transformation remain to be seen in the long term.

It is notable that the negative correlation we find between species richness and nuclear genetic diversity contradicts relatively consistent positive correlations found between species richness and mitochondrial genetic diversity (Miraldo et al. 2016; Manel et al. 2020; Millette et al. 2020; Theodoridis et al. 2020). However, mitochondrial DNA has several idiosyncrasies associated with the specific biology of mitochondria that distinguish it from genetic diversity measured with neutral nuclear DNA. It is inherited as a single non-recombining locus, has highly variable mutation rates which can vary 100-fold across species (Nabholz et al. 2008), and is not clearly related to life history, ecological traits, or census and effective population sizes (Bazin et al. 2006; Nabholz et al. 2008; James and Eyre-Walker 2020). Thus it is not certain whether genetic diversity patterns measured using neutral nuclear and mitochondrial markers should be positively correlated. Indeed, the most commonly used markers in mtDNA studies are the protein-coding genes *cytochrome oxidase I* and *cytochrome b*, which are involved in cellular respiration and very likely do not evolve under neutrality (Galtier et al. 2009). Genetic diversity patterns at these loci may thus reflect patterns of adaptive genetic diversity related to metabolism. By using genetic diversity metrics estimated from neutral nuclear DNA, we can more clearly link environments to species richness and genetic diversity through demography, population size, and by extension, species life history traits which partly set the effective population size.

Ecosystem sustainability given environmental perturbations occurring more frequently due to human causes, depends on the resiliency of landscapes, communities, and

populations (Oliver et al. 2015). Genetic diversity is crucial to a population's adaptive potential because the efficiency with which selection can act is determined by the effective population size which sets the rate of genetic drift. Yet genetic diversity is not equally distributed in space and indeed, in mammals, appears to be lower in heterogeneous environments which exert greater spatially varying selection. Knowledge of how natural environments shape population genetic composition is fundamental to understanding how these natural patterns will shift with continued land transformation by humans. Mammals are one of the best-studied taxa, however, rules applicable to them may not generalize well across other groups. For instance, the relevance of the more individuals hypothesis for ectotherms has been questioned because their energy usage is well below that of endotherms (Buckley et al. 2008). Indeed, continental patterns of species richness differ across taxa, which may stem from life history or physiology differences (Currie 1991). It will be necessary to test the hypothesis developed here on other taxonomic groups and in different regions to gain a more holistic understanding of the causes of biodiversity. The intimate connections between the environment, species richness, and genetic diversity we find here suggest that changes on one level can cascade throughout the system and profoundly reshape biodiversity patterns across multiple biological levels in ways we do not yet fully grasp.

Acknowledgements: We would like to thank the Population Ecology and Evolutionary Genetics group for their feedback on this manuscript. We are also grateful to the authors whose work provided the raw data for this synthesis. C.S. and C.J.G. were supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to C.J.G. C.S. was also supported by a U. Manitoba Graduate Fellowship, and a U. Manitoba Graduate Enhancement of Tri-council funding grant to C.J.G.

References

Allouche, O., Kalyuzhny, M., Moreno-Rueda, G., Pizarro, M., and Kadmon, R. 2012. Area-heterogeneity tradeoff and the diversity of ecological communities. Proc. Natl. Acad. Sci. U. S. A. **109**(43): 17495–17500. doi:10.1073/pnas.1208652109.

Aronson, M.F.J., La Sorte, F.A., Nilon, C.H., Katti, M., Goddard, M.A., Lepczyk, C.A., Warren, P.S., Williams, N.S.G., Cilliers, S., Clarkson, B., Dobbs, C., Dolan, R., Hedblom, M., Klotz, S., Kooijmans, J.L., Kühn, I., MacGregor-Fors, I., McDonnell, M., Mörtberg, U., Pyšek, P., Siebert, S., Sushinsky, J., Werner, P., and Winter, M. 2014. A global analysis of the impacts of urbanization on bird and plant diversity reveals key anthropogenic drivers. Proc. R. Soc. B Biol. Sci. **281**(1780): 20133330. doi:10.1098/rspb.2013.3330.

Bates, D., Mächler, M., Bolker, B., and Walker, S. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. **67**(1). doi:10.18637/jss.v067.i01.

Bazin, E., Glémin, S., and Galtier, N. 2006. Population size does not influence mitochondrial genetic diversity in animals. Science. **312**(5773): 570–572. doi:10.1126/science.1122033.

Blanchet, G.F., Legendre, P., and Borcard. 2008. Forward selection of explanatory variables. Ecology **89**(9): 2623–2632.

Borcard, D., and Legendre, P. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecol. Modell. **153**(1–2): 51–68. doi:10.1016/S0304-3800(01)00501-4.

Borcard, D., Legendre, P., Avois-Jacquet, C., and Tuomisto, H. 2004. Dissecting the spatial structure of ecological data at multiple scales. Ecology **85**(7): 1826–1832.

Brodie, J.F. 2019. Environmental limits to mammal diversity vary with latitude and global temperature. Ecol. Lett. **22**(3): 480–485. doi:10.1111/ele.13206.

Buckley, L.B., Rodda, G.H., and Jetz, W. 2008. Thermal and energetic constraints on ectotherm abundance: A global test using lizards. Ecology **89**(1): 48–55. doi:10.1890/07-0845.1.

Ceballos, G., Ehrlich, P.R., and Raven, P.H. 2020. Vertebrates on the brink as indicators of biological annihilation and the sixth mass extinction. Proc. Natl. Acad. Sci. U. S. A. **117**(24): 13596–13602. doi:10.1073/pnas.1922686117.

Charlesworth, B., and Charlesworth, D. 2010. Elements of evolutionary genetics. Roberts & Company Publishers, Greenwood Village, Colorado, USA.

Currie, D.J. 1991. Energy and large-scale patterns of animal- and plant-species richness. Am. Nat. **137**(1): 27–49.

Damuth, J. 1981. Population density and body size in mammals. Nature **290**(April): 1980–1981.

Damuth, J. 1987. Interspecific allometry of population density in mammals and other animals: the independence of body mass and population energy-use. Biol. J. Linn. Soc. **31**(3): 193–246. doi:10.1111/j.1095-8312.1987.tb01990.x.

Dray, S., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., and Wagner, H.H. 2017. adespatial: Multivariate Multiscale Spatial Analysis. Available from https://cran.r-project.org/package=adespatial.

Dray, S., Legendre, P., and Peres-Neto, P.R. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol. Modell. **196**(3–4): 483–493. doi:10.1016/j.ecolmodel.2006.02.015.

Etienne, R.S., Cabral, J.S., Hagen, O., Hartig, F., Hurlbert, A.H., Pellissier, L., Pontarp, M., and Storch, D. 2019. A minimal model for the latitudinal diversity gradient suggests a dominant role for ecological limits. Am. Nat. **194**(5): E122–E133. doi:10.1086/705243.

Fisher, J.B., Whittaker, R.J., and Malhi, Y. 2011. ET come home: Potential evapotranspiration in geographical ecology. Glob. Ecol. Biogeogr. **20**(1): 1–18. doi:10.1111/j.1466-8238.2010.00578.x.

Frankham, R. 1996. Relationship of Genetic Variation to Population Size in Wildlife. Conserv. Biol. **10**(6): 1500–1508. doi:10.1046/j.1523-1739.1996.10061500.x.

Galtier, N., Nabholz, B., Glémin, S., and Hurst, G.D.D. 2009. Mitochondrial DNA as a marker of molecular diversity: A reappraisal. Mol. Ecol. **18**(22): 4541–4550. doi:10.1111/j.1365-

294X.2009.04380.x.

Goudet, J., and Jombart, T. 2015. hierfstat: Estimation and Tests of Hierarchical F-Statistics. Available from https://cran.r-project.org/package=hierfstat.

Grimm, N.B., Faeth, S.H., Golubiewski, N.E., Redman, C.L., Wu, J., Bai, X., and Briggs, J.M. 2008. Global Change and the Ecology of Cities. Science. **319**: 756–760. doi:10.1126/science.1150195.

Hämälä, T., Mattila, T.M., and Savolainen, O. 2018. Local adaptation and ecological differentiation under selection, migration, and drift in *Arabidopsis lyrata*. Evolution. **72**(7): 1373–1386. doi:10.1111/evo.13502.

Hubbell, S.P. 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton NJ.

IUCN. 2019. The IUCN Red List of Threatened Species. Version 2019-1. Available from https://www.iucnredlist.org.

James, J., and Eyre-Walker, A. 2020. Mitochondrial DNA sequence diversity in mammals: a correlation between the effective and census population sizes. Genome Biol. Evol. doi:10.1093/gbe/evaa222.

Jiménez-Alfaro, B., Chytrý, M., Mucina, L., Grace, J.B., and Rejmánek, M. 2016. Disentangling vegetation diversity from climate-energy and habitat heterogeneity for explaining animal geographic patterns. Ecol. Evol. **6**(5): 1515–1526. doi:10.1002/ece3.1972.

Jones, K.E., Bielby, J., Cardillo, M., Fritz, S.A., O'Dell, J., Orme, C.D.L., Safi, K., Sechrest, W., Boakes, E.H., Carbone, C., Connolly, C., Cutts, M.J., Foster, J.K., Grenyer, R., Habib, M., Plaster, C.A., Price, S.A., Rigby, E.A., Rist, J., Teacher, A., Bininda-Emonds, O.R.P., Gittleman, J.L., Mace, G.M., and Purvis, A. 2009. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. Ecology **90**(9): 2648–2648. doi:10.1890/08-1494.1.

Jones, M.B., Slaughter, P., Nahf, R., Boettiger, C., Jones, C., Read, J., Walker, L., Hart, E., and

Chamberlain, S. 2017. dataone: R Interface to the DataONE REST API. Available from https://cran.r-project.org/package=dataone.

Kadmon, R., and Allouche, O. 2007. Integrating the effects of area, isolation, and habitat heterogeneity on species diversity: A unification of island biogeography and niche theory. Am. Nat. **170**(3): 443–454. doi:10.1086/519853.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge.

Kreft, H., and Jetz, W. 2007. Global patterns and determinants of vascular plant diversity. Proc. Natl. Acad. Sci. **104**(14): 5925–5930. doi:10.1073/pnas.0608361104.

Lefcheck, J., Byrnes, J., and Grace, J. 2019. piecewiseSEM: Piecewise Structural Equation Modeling. Available from https://cran.r-project.org/package=piecewiseSEM.

Lefcheck, J.S. 2016. piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol. Evol. **7**(5): 573–579. doi:10.1111/2041-210X.12512.

Leigh, D.M., Hendry, A.P., Vázquez-Domínguez, E., and Friesen, V.L. 2019. Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. Evol. Appl. **12**(8): 1505–1512. doi:10.1111/eva.12810.

Lomolino, M., Riddle, B., and Whittaker, R. 2016. Biogeography: biological diversity across space and time. *In* 5th edition. Oxford University Press.

Lowe, W.H., Kovach, R.P., and Allendorf, F.W. 2017. Population genetics and demography unite ecology and evolution. Trends Ecol. Evol. **32**(2): 141–152. doi:10.1016/j.tree.2016.12.002.

Manel, S., Guerin, P.E., Mouillot, D., Blanchet, S., Velez, L., Albouy, C., and Pellissier, L. 2020. Global determinants of freshwater and marine fish genetic diversity. Nat. Commun. **11**(1): 1–9. Springer US. doi:10.1038/s41467-020-14409-7.

McKinney, M.L. 2006. Urbanization as a major cause of biotic homogenization. Biol.

Conserv. **127**(3): 247–260. doi:10.1016/j.biocon.2005.09.005.

Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L.F., Backeljau, T., Bonte, D., Brans, K.I., Cours, M., Dahirel, M., Debortoli, N., De Wolf, K., Engelen, J.M.T., Fontaneto, D., Gianuca, A.T., Govaert, L., Hendrickx, F., Higuti, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E., Sablon, R., Schön, I., Van Doninck, K., De Meester, L., and Van Dyck, H. 2018. Body-size shifts in aquatic and terrestrial urban communities. Nature **558**(7708): 113–116. doi:10.1038/s41586-018-0140-0.

Millette, K.L., Fugère, V., Debyser, C., Greiner, A., Chain, F.J.J., and Gonzalez, A. 2020. No consistent effects of humans on animal genetic diversity worldwide. Ecol. Lett. **23**(1): 55–67. doi:10.1111/ele.13394.

Miraldo, A., Li, S., Borregaard, M.K., Florez-Rodriguez, A., Gopalakrishnan, S., Rizvanovic, M., Wang, Z., Rahbek, C., Marske, K.A., and Nogues-Bravo, D. 2016. An Anthropocene map of genetic diversity. Science. **353**(6307): 1532–1535. doi:10.1126/science.aaf4381.

Mittelbach, G.G., Schemske, D.W., Cornell, H. V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., McCain, C.M., McCune, A.R., McDade, L.A., McPeek, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M., and Turelli, M. 2007. Evolution and the latitudinal diversity gradient: Speciation, extinction and biogeography. Ecol. Lett. **10**(4): 315–331. doi:10.1111/j.1461-0248.2007.01020.x.

Mittell, E.A., Nakagawa, S., and Hadfield, J.D. 2015. Are molecular markers useful predictors of adaptive potential? Ecol. Lett. **18**(8): 772–778. doi:10.1111/ele.12454.

Nabholz, B., Mauffrey, J.F., Bazin, E., Galtier, N., and Glemin, S. 2008. Determination of mitochondrial genetic diversity in mammals. Genetics **178**(1): 351–361. doi:10.1534/genetics.107.073346.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. **70**(12): 3321–3323. doi:10.1073/pnas.70.12.3321.

NOAA, and U.S. National Geophysical Data Center. (n.d.). TerrainBase, release 1.0. Boulder

CO. Available from https://nelson.wisc.edu/sage/data-and-models/atlas/maps.php?datasetid=28&includerelatedlinks=1&dataset=28.

Norton, B.A., Evans, K.L., and Warren, P.H. 2016. Urban biodiversity and landscape ecology: patterns, processes and planning. Curr. Landsc. Ecol. Reports **1**(4): 178–192. Current Landscape Ecology Reports. doi:10.1007/s40823-016-0018-5.

Oliver, T.H., Heard, M.S., Isaac, N.J.B., Roy, D.B., Procter, D., Eigenbrod, F., Freckleton, R., Hector, A., Orme, C.D.L., Petchey, O.L., Proença, V., Raffaelli, D., Suttle, K.B., Mace, G.M., Martín-López, B., Woodcock, B.A., and Bullock, J.M. 2015. Biodiversity and resilience of ecosystem functions. Trends Ecol. Evol. **30**(11): 673–684. doi:10.1016/j.tree.2015.08.009.

Palumbi, S.R. 2001. Humans as the world's greatest evolutionary force. Science. **293**(5536): 1786–1790. doi:10.1017/CBO9781107415324.004.

Pautasso, M., and Gaston, K.J. 2005. Resources and global avian assemblage structure in forests. Ecol. Lett. **8**(3): 282–289. doi:10.1111/j.1461-0248.2005.00724.x.

Pontarp, M., Bunnefeld, L., Cabral, J.S., Etienne, R.S., Fritz, S.A., Gillespie, R., Graham, C.H., Hagen, O., Hartig, F., Huang, S., Jansson, R., Maliet, O., Münkemüller, T., Pellissier, L., Rangel, T.F., Storch, D., Wiegand, T., and Hurlbert, A.H. 2019. The latitudinal diversity gradient: novel understanding through mechanistic eco-evolutionary models. Trends Ecol. Evol. **34**(3): 211–223. doi:10.1016/j.tree.2018.11.009.

Pontarp, M., and Wiens, J.J. 2017. The origin of species richness patterns along environmental gradients: uniting explanations based on time, diversification rate and carrying capacity. J. Biogeogr. **44**(4): 722–735. doi:10.1111/jbi.12896.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. Vienna, Austria. Available from http://www.r-project.org/.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., Chiari, Y., Dernat, R., Duret, L., Faivre, N., Loire, E., Lourenco, J.M., Nabholz, B., Roux, C., Tsagkogeorga, G., Weber, A.A.T., Weinert, L.A., Belkhir, K., Bierne, N., Glémin, S., and

Galtier, N. 2014. Comparative population genomics in animals uncovers the determinants of genetic diversity. Nature **515**(7526): 261–263. doi:10.1038/nature13685.

Schmidt, C., Domaratzki, M., Kinnunen, R.P., Bowman, J., and Garroway, C.J. 2020a. Data from: Continent-wide effects of urbanization on bird and mammal genetic diversity. Dryad Data Repository. doi:10.5061/dryad.cz8w9gj0c.

Schmidt, C., Domaratzki, M., Kinnunen, R.P., Bowman, J., and Garroway, C.J. 2020b. Continent-wide effects of urbanization on bird and mammal genetic diversity. Proc. R. Soc. B Biol. Sci. **287**(1920): 20192497. doi:10.1098/rspb.2019.2497.

Shipley, B. 2016. Cause and correlation in biology. *In* Cause and Correlation in Biology, 2nd edition. Cambridge University Press, Cambridge.

Simpson, G.G. 1964. Species density of North American recent mammals. Syst. Zool. **13**(2): 57–73.

Stein, A., Beck, J., Meyer, C., Waldmann, E., Weigelt, P., and Kreft, H. 2015. Differential effects of environmental heterogeneity on global mammal species richness. Glob. Ecol. Biogeogr. **24**(9): 1072–1083. doi:10.1111/geb.12337.

Stein, A., Gerstner, K., and Kreft, H. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. Ecol. Lett. **17**(7): 866–880. doi:10.1111/ele.12277.

Storch, D., Bohdalková, E., and Okie, J. 2018. The more-individuals hypothesis revisited: the role of community abundance in species richness regulation and the productivity–diversity relationship. Ecol. Lett. **21**(6): 920–937. doi:10.1111/ele.12941.

Storch, D., and Okie, J.G. 2019. The carrying capacity for species richness. Glob. Ecol. Biogeogr. **28**(10): 1519–1532. doi:10.1111/geb.12987.

Theodoridis, S., Fordham, D.A., Brown, S.C., Li, S., Rahbek, C., and Nogues-Bravo, D. 2020. Evolutionary history and past climate change shape the distribution of genetic

diversity in terrestrial mammals. Nat. Commun. **11**(1): 2557. doi:10.1038/s41467-020-16449-5.

Trabucco, A., and Zomer, R. 2019. Global Aridity Index and Potential Evapotranspiration (ET0) Climate Database v2. doi:10.6084/m9.figshare.7504448.v3.

Vellend, M. 2005. Species diversity and genetic diversity: parallel processes and correlated patterns. Am. Nat. **166**(2): 199–215. doi:10.1086/431318.

Weir, B.S., and Goudet, J. 2017. A unified characterization of population structure. Genetics **206**: 2085–2103.

Worm, B., and Tittensor, D.P. 2018. A theory of global biodiversity. Princeton University Press, Princeton, New Jersey.

WWF. 2018. Living Planet Report - 2018: Aiming higher. *Edited By*M. Grooten and R.E.A. Almond. WWF, Gland, Switzerland. Available from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:LIVING+PLANET+REPORT+2004#0.

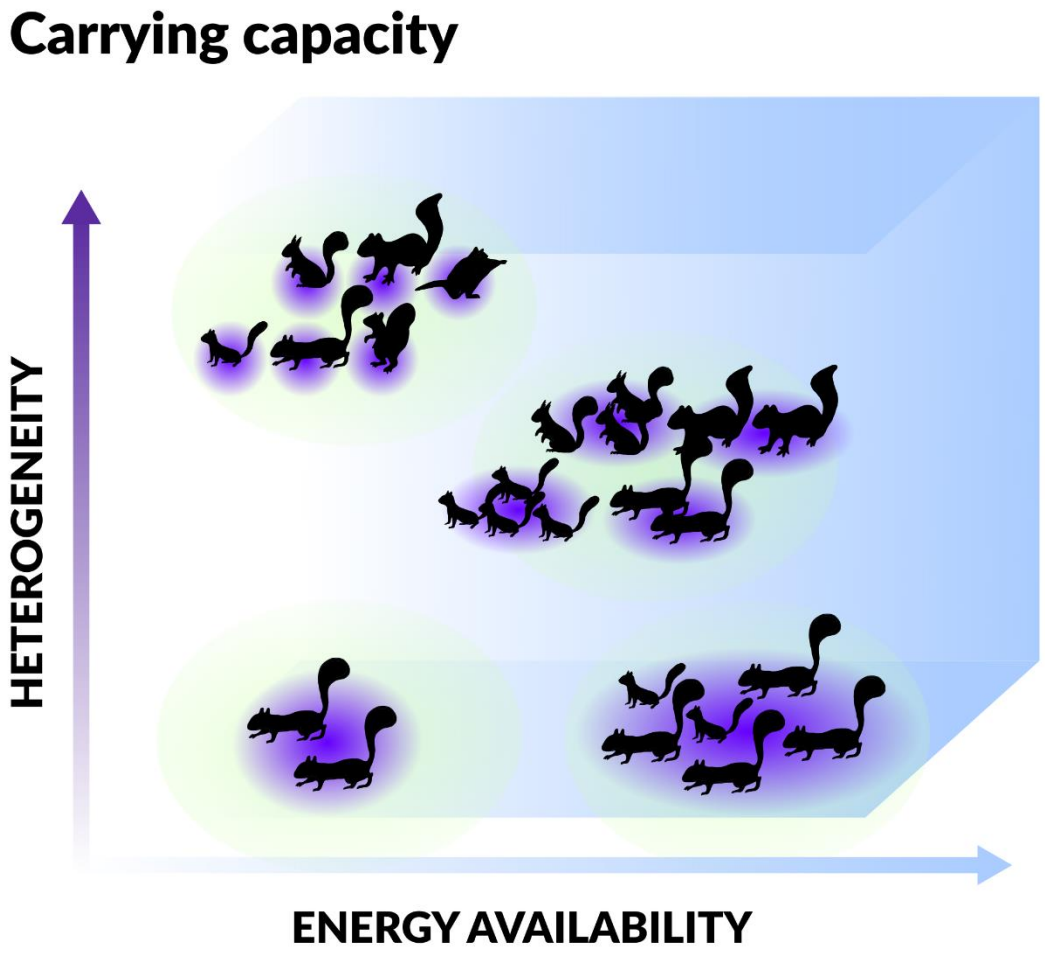


Figure. 5.1. Carrying capacities at population and species levels. Green areas represent total habitat area, and are all equal in size. Purple areas are niches, which increase in number with increasing heterogeneity (y axis), and increase in area with higher energy availability (x axis). In general, as energy availability increases, individual carrying capacities are higher, resulting in greater diversity at species and genetic levels (the more individuals hypothesis). As heterogeneity increases, species richness is higher due to the increased availability of niches. However, population sizes are reduced because niche area is smaller in more heterogeneous areas, generating a negative relationship between species richness and genetic diversity (heterogeneity hypothesis).

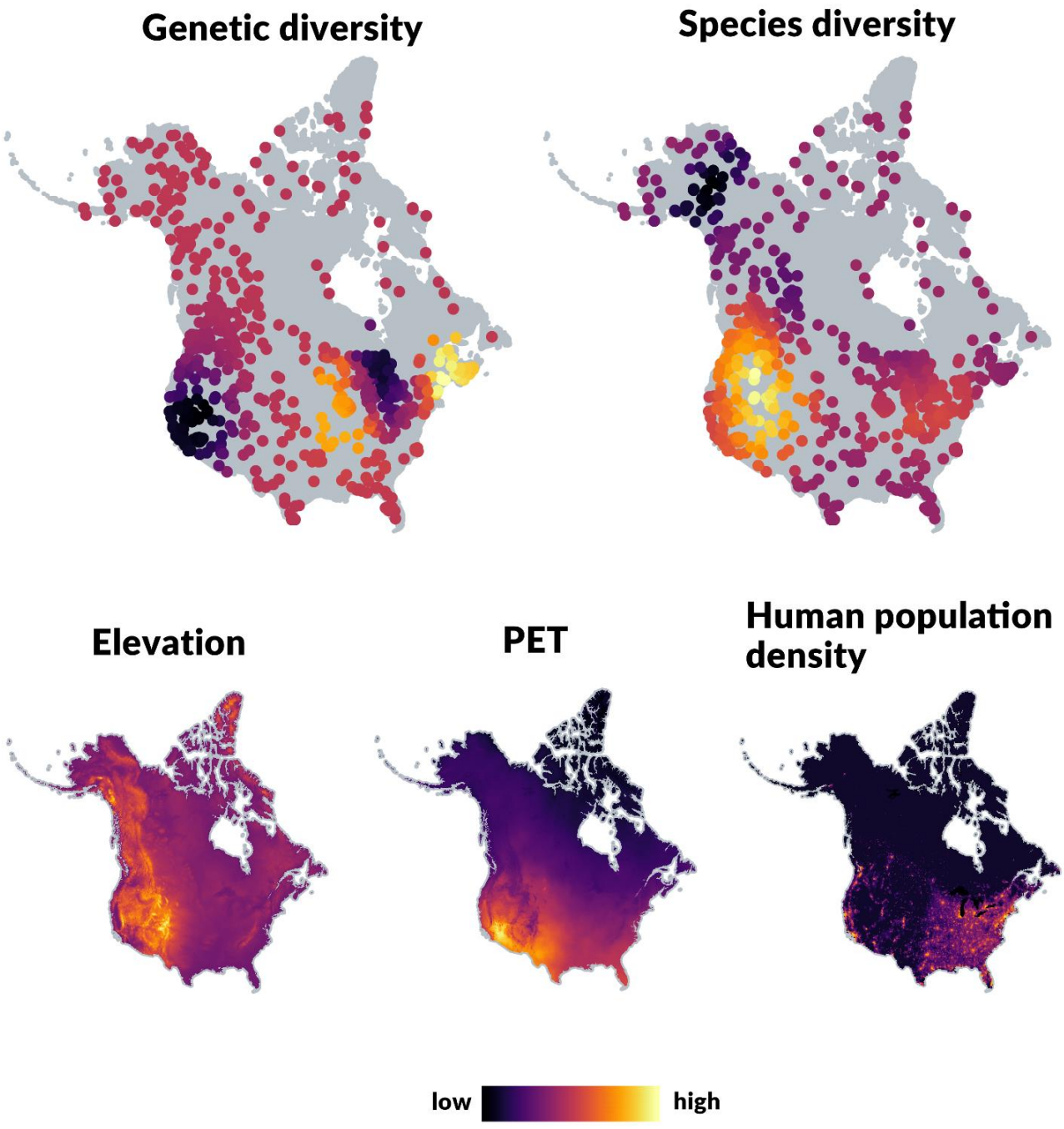


Figure 5.2. Maps depicting spatial patterns of biodiversity and environmental factors. *(Top row)* Points are the locations of 801 North American mammal populations for which raw microsatellite data was available in public repositories. Point color indicates predicted values of genetic diversity and species richness based on spatial patterns detected in the

data. (*Bottom row*) Maps showing the three environmental variables which we tested for simultaneous effects on genetic diversity and species richness.

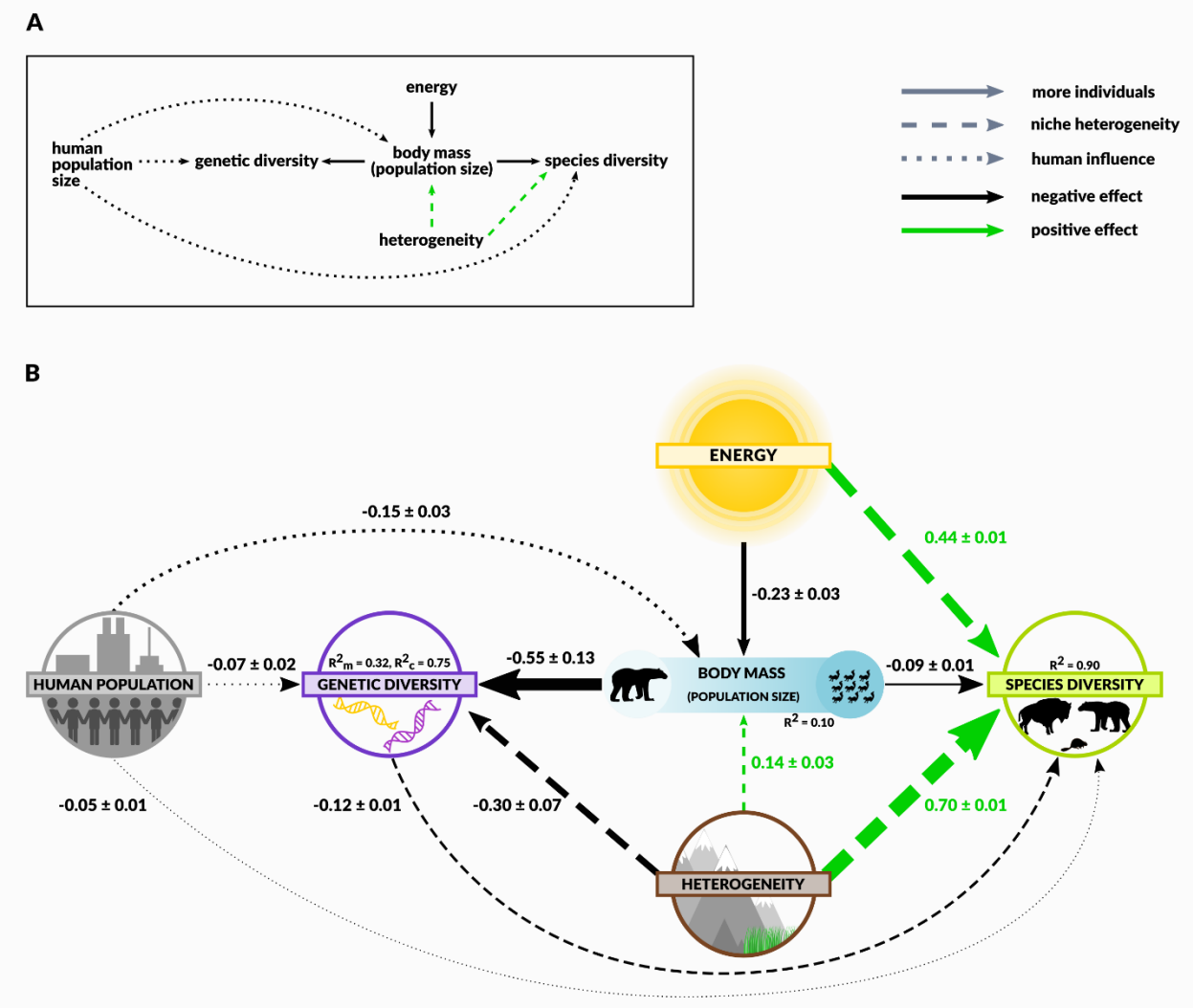


Figure 5.3. Structural equation models. (a) Our conceptual hypothesis network combining the more individuals hypothesis (solid lines) with the effects of environmental heterogeneity (dashed lines) and human presence (dotted lines). Arrows represent unidirectional relationships between variables. (b) Structural equation model results. Green and black lines positive and negative relationships, respectively. Line widths reflect coefficient estimates, which are listed above each path with standard errors. R^2 values are the amount of variation explained for each response variable. Mass and species richness were measured at the species level, and genetic diversity was measured at the population

level and fit with a random effect for species: R^2_m is the variation explained by fixed effects only, and R^2_c is the variation explained by fixed and random effects.

Chapter 6: Determinants of genetic diversity and species richness of North American amphibians

Chloé Schmidt[1], Jason Munshi-South[2], Colin J Garroway[1]

[1]Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

[2]Department of Biological Sciences and Louis Calder Center, Fordham University, Armonk, NY, USA

Status: unsubmitted (preprint DOI: 10.1101/2021.01.04.425301)

Abstract: We know little about the general links between broad-scale biodiversity patterns at the nuclear genetic and species levels. Recent work in mammals suggests environmental carrying capacity and ecological opportunity link these two base levels of biodiversity. Energy- and resource-rich environments are thought to support larger populations with higher genetic diversity and species richness. Niche availability is expected to limit population size causing drift while increasing genetic differentiation due to environmental specialization. Several of the processes underlying these links are temperature-dependent, so we might expect different patterns for endotherms and ectotherms. We use a database comprised of raw microsatellite genotypes for 13616 individuals of 18 species sampled at 548 locations in the United States and Canada. We analyzed salamander and frog species separately and simultaneously fit our hypotheses with structural equation models. Similar to mammals, niche availability was the primary contributor to diversity at both the genetic and species levels in frogs, and energy availability was an important predictor of species richness for both taxa. Different than mammals, environmental energy availability was not linked to genetic diversity. There are shared underlying mechanisms linking genetic and species-level diversity but the processes are not entirely general across these species groups.

Keywords: latitudinal diversity gradient, biogeography, heterozygosity, frogs, salamanders, more individuals hypothesis

Introduction

Although species richness is higher in the tropics for most taxa, spatial patterns differ between species groups. In North America for instance, vertebrate richness generally increases with energy availability, but mammals and birds tend to have higher species richness in dry, mountainous areas, and reptiles and amphibians are more diverse in wet, low-elevation regions (Currie 1991). This finding suggests that while richness increases with greater energy availability, taxon-specific traits may cause richness patterns to diverge from a strictly latitudinal gradient. Because several hypotheses link species richness gradients to temperature-dependent processes (Currie et al. 2004), different patterns may exist for endotherms and ectotherms. Similar broad-scale patterns for genetic diversity have only recently been identified due to the accumulation of open data in public repositories (Miraldo et al. 2016; Manel et al. 2020; Schmidt et al. 2020c; Theodoridis et al. 2020). Genetic diversity is typically thought of as the most fundamental level of biodiversity because it influences the potential for adaptive evolution in response to environmental change (Frankham 1995b). Recent analyses of mammals suggest that environments simultaneously shape species richness and genetic diversity on continental scales (Schmidt et al. 2020c). Whether this is also true in ectothermic taxa is unknown. Understanding common processes underlying variation in biogeographic patterns across taxa with different environmental requirements can help us move toward a general understanding of the drivers of biodiversity.

Recent empirical tests incorporating estimates of genome-wide diversity from mammals (Schmidt et al. 2020c) demonstrate the importance of carrying capacity and ecological opportunity in shaping broad-scale patterns of genetic diversity and species richness. Energy- and resource-rich environments supported larger populations with higher genetic diversity and species richness, while niche availability in heterogeneous habitats reduced population sizes, increased genetic differentiation, and promoted species coexistence because specialization reduces available resources. These processes are related to two prominent hypotheses for the latitudinal species richness gradient: the more-individuals hypothesis (Wright 1983) and the heterogeneity hypothesis (Allouche et al. 2012). The

more individuals hypothesis posits that resource-rich regions near the equator are capable of supporting larger populations and communities, and thus more species than more temperate regions. The heterogeneity hypothesis suggests that greater niche availability in more complex heterogeneous environments allow more species to coexist, but with smaller population sizes because resources are partitioned.

The effects of environmental heterogeneity are generally applicable across taxa (Stein et al. 2014); however, the relevance of the more-individuals hypothesis for ectotherms is unclear (Buckley and Jetz 2010). Compared to endotherms, ectotherms have lower energy requirements and can behaviorally thermoregulate—meaning their abundances are less likely to be limited by energy-related carrying capacities (Buckley and Jetz 2010). Instead, ectotherm distributions, and therefore species richness, appear to be more directly constrained by environmental temperature because fewer species have evolved thermal adaptations required for expanding into cooler regions (Buckley and Jetz 2010). Further, the evolution of traits associated with better survival in temperate regions may have additional effects on speciation dynamics. For example, species turnover tends to be higher among viviparous squamate reptiles (Pyron and Burbrink 2014). Species richness gradients in ectotherms may thus be less strongly governed by energy limits on population size than mammals, whose temperature independence allows them to colonize a wider range of habitats. If true, then we might expect the effects of habitat heterogeneity to be more pronounced in ectotherms. In mammals, the effects of energy availability on species richness appear to dominate in low-energy regions, but once a minimum energy threshold is reached (1000 mm/yr potential evapotranspiration), habitat heterogeneity becomes the main cause of richness (Kerr and Packer 1997). The effects of heterogeneity may thus be more pronounced in ectotherms because fewer species inhabit low-energy regions.

The major determinants of richness across all terrestrial vertebrates are generally shown to be related to energy (potential evapotranspiration, primary productivity), water-energy balance (actual evapotranspiration, precipitation), and environmental heterogeneity (elevation variability, land cover) (Currie 1991; Kerr and Packer 1997; Hawkins et al. 2003; Rodríguez et al. 2005; Buckley and Jetz 2007; Stein et al. 2014; Jiménez-Alfaro et al. 2016).

Amphibians are an interesting case because they are doubly constrained by water availability and temperature. Water availability is consistently identified as an important driver of diversity in amphibians (Rodríguez et al. 2005; Buckley and Jetz 2007). Indeed in Europe, the best predictors of species richness in mammals and birds shifted from energy to water availability at decreasing latitudes, but amphibian richness was strongly related to water-energy balance regardless of latitude (Whittaker et al. 2007).

The causes of population genetic diversity are rarely studied at the same time or scale as patterns of species richness (but see Marshall and Camp 2006; Schmidt et al. 2020c), yet the presumed mechanisms related to more-individuals and environmental heterogeneity hypotheses are intricately related to carrying capacity and population-level processes. The more-individuals mechanism predicts a positive relationship between species richness and population genetic diversity because bigger populations and communities tend to have higher levels of genetic and species diversity (Kimura 1983; Hubbell 2001). With higher population-level carrying capacities, more species persist because they can reach minimal viable population sizes. On the other hand, heterogeneity causes negative correlations between genetic diversity and species richness by increasing the number of species a given area can support which in turn reduces population size and limits gene flow due to increased niche specialization. Heterogeneous environments also facilitate population divergence due to spatially varying selection. In mammals, evolutionary processes acting on the population level scaled up and interacted with environmental factors to produce previously identified species richness patterns (Schmidt et al. 2020c).

Whether carrying capacity mechanisms related to energy and niche availability predict patterns of species richness in ectotherms is unclear. To test this idea, we repurposed raw microsatellite data from 18 North American amphibian species (8 frogs, 10 salamanders), with >13000 individuals sampled at 548 sites. Our first objective was to identify spatial patterns in genetic diversity and quantify the extent to which genetic diversity and species richness covary spatially. We then tested whether limits on energy and niche availability jointly determined genetic diversity and species richness using structural equation models, which allowed us to evaluate and assess the relative importance of both hypotheses at both

levels of biodiversity simultaneously. Finally, we compare our results to previous results in mammals (Schmidt et al. 2020c) to infer whether similar environmental features contribute to diversity gradients across endothermic and ectothermic taxa in North America.

Methods

Data

Genetic diversity. We used a database of genetic metrics in North American amphibians compiled by Schmidt and Garroway 2020 (Chapter 3). This database was assembled by calculating metrics of genetic diversity and differentiation from raw microsatellite datasets publicly archived in the Dryad repository. To build the database we conducted a systematic search of the Dryad data repository with the following keywords: species name (e.g., *Plethodon cinereus*), "microsat*", "short tandem*", and "single tandem*". We used the IUCN Red List database to obtain a list of amphibian species native to North America for the search. We excluded datasets that lacked spatial reference, were not located in North America, did not sample neutral microsatellite loci, or had study designs that may have affected genetic diversity (including sampling island populations, or captive or managed populations). The database includes data from 13616 individuals of 18 species sampled at 548 locations in the contiguous United States and Canada. Here, we used gene diversity (Nei 1973) as a measure of genetic diversity because it is minimally affected by sample size (Charlesworth and Charlesworth 2010). Gene diversity is a measure of heterozygosity which measures the evenness of alleles in a population (Nei 1973).

Population size. Because species censuses are not widely available, we used body size as a proxy for species population size. Abundance typically scales negatively with body size (Peters and Wassenberg 1983; Damuth 1987). Body size is also correlated with several life history traits which partly determine the effective population size, and is generally negatively related to neutral genetic variation across diverse taxa (Romiguier et al. 2014;

Brüniche-Olsen et al. 2018; Mackintosh et al. 2019). We used body length (mm) as our metric of body size, obtained from the AmphiBIO v1 database (Oliveira et al. 2017).

Species richness. We estimated species richness using amphibian range extent data from the IUCN RedList (IUCN 2019), applying filters for native, extant species ranges. For each species included in our dataset, we counted the number of overlapping species ranges as a measure of species richness at the species level. We took this approach because we were interested in environmental factors operating at broad spatial scales, and to avoid over- or under-sampling areas due to irregular site placement in the aggregated dataset. To account for biases due to range size, species with larger ranges having more overlaps, we divided the number of overlapping ranges by range area (km^2). To generate the maps in Figure 6.1, we used a site-level measure of species richness calculated by summing the number of species ranges overlapping each genetic sample site.

Environmental variables. Amphibians are habitat-limited by both temperature and water availability. Water availability can be measured by evapotranspiration, or the amount of water removed from the Earth's surface through soil or open water evaporation and plant transpiration processes. Potential evapotranspiration (PET) measures the atmospheric demand for water, depending on factors such as temperature and wind (Peng et al. 2019). It is strongly correlated with temperature. PET is the maximum amount of water that would be removed in the absence of biophysical limitations (Peng et al. 2019). The amount of water actually removed, actual evapotranspiration (AET), reflects water availability and soil moisture levels. Actual evapotranspiration has also been shown to be one of the strongest predictors of amphibian species richness (Buckley and Jetz 2007). PET can be viewed as a measure of energy availability, and AET one of water-energy balance (see (Currie 1991; Buckley and Jetz 2007; Kreft and Jetz 2007). We measured mean PET and AET (mm/yr) values across each species' range using data from the CGIAR Consortium for Spatial Information (Trabucco and Zomer 2019).

Finally, we measured habitat heterogeneity by calculating the range (m) in elevation across each species range using a topography map obtained from NOAA (National Oceanic and Atmospheric Administration (NOAA) and U.S. National Geophysical Data Center). Larger

ranges generally have greater topographical variation, thus we corrected for species range area to avoid potential biases.

Analysis

Detecting shared spatial structure in genetic diversity and species richness. We used distance-based Moran's eigenvector maps (MEMs) to detect spatial patterns in genetic diversity and compare these to patterns of species richness. MEMs are orthogonal spatial eigenvectors with eigenvalues that are directly proportional to Moran's *I*. They measure spatial autocorrelation at all scales present in the data. We computed dbMEMs in the R package adespatial (Dray et al. 2017). We used the forward selection procedure described in (Blanchet et al. 2008) to select two sets of MEMs describing important patterns in genetic diversity and species richness. To create maps of genetic diversity and species richness (Fig. 6.1), we used the predicted values for gene diversity and species richness regressed on selected MEMs. Next, we determined the extent to which spatial patterns in genetic diversity and species richness were shared using variation partitioning. Because our dbMEM analysis for both levels of biodiversity had the same input distance matrix, the resulting spatial MEMs were directly comparable. We determined the fraction of total variation explained by spatial structure, shared spatial structure, and non-spatial variation using variation partitioning as follows. We ran a series of linear regressions with either species richness (y_{SR}) or gene diversity (y_{GD}) as the response variable using all MEMs selected for that variable (Equations 1 and 2), or only MEMs shared by both variables as predictors (Equations 3 and 4):

$$\mathrm{y}_{SR} \sim \alpha + \beta_{1S}(\mathrm{MEM}_{1S}) + \beta_{2S}(\mathrm{MEM}_{2S}) + \cdots + \beta_{iS}(\mathrm{MEM}_{iS}) + \epsilon \qquad \text{Eq. 1}$$

$$\mathrm{y}_{GD} \sim \alpha + \beta_{1G}(\mathrm{MEM}_{1G}) + \beta_{2G}(\mathrm{MEM}_{2G}) + \cdots + \beta_{iG}(\mathrm{MEM}_{iG}) + \epsilon \qquad \text{Eq. 2}$$

$$\mathrm{y}_{SR} \sim \alpha + \beta_{1SG}(\mathrm{MEM}_{1SG}) + \beta_{2SG}(\mathrm{MEM}_{2SG}) + \cdots + \beta_{iSG}(\mathrm{MEM}_{iSG}) + \epsilon \qquad \text{Eq. 3}$$

$$\mathrm{y}_{GD} \sim \alpha + \beta_{1SG}(\mathrm{MEM}_{1SG}) + \beta_{2SG}(\mathrm{MEM}_{2SG}) + \cdots + \beta_{iSG}(\mathrm{MEM}_{iSG}) + \epsilon \qquad \text{Eq. 4}$$

where α is the grand mean, and MEM_{iS} and MEM_{iG} are the set of MEMs selected for species richness and genetic diversity, respectively. The coefficients of variation (R^2) from Eqs. 1 and 2 give the total amount of variation explained by spatial patterns for species richness and genetic diversity. Subtracting these values from 1 gives the amount of non-spatial variation. MEM_{iSG} represents the set of MEMs shared by both species richness and genetic diversity. R^2 values from Eqs. 3 and 4 tell us the amount of variation in each response variable which can be explained by spatial variation shared at both levels of diversity. When subtracted from the total spatial variation in genetic diversity or species richness (Eqs. 1 and 2), we get the proportion of non-shared spatial variation.

Identifying environmental determinants of spatial patterns in genetic diversity and species richness. Our next aim was to determine whether genetic diversity and species richness are shaped by differential environmental carrying capacities due to limits on energy and niche availability. To do this we used structural equation modeling (SEM). Hypotheses in structural equation models are envisioned as a hypothesis network representing a conceptual model, where paths between variables represent predicted causal relationships (Fig 2a). In SEM, the effects of multiple predictors are simultaneously assessed for multiple response variables (Shipley 2016). We implemented structural equation models using the piecewiseSEM package (version 2.0.2), which uses a local estimation approach for models in the hypothesis network allowing for the incorporation of more complex model types (Lefcheck et al. 2019). Model fit is evaluated using tests of directed separation (Shipley 2016), which determine whether an association exists between two variables in the network conditional on each of their causes. If two variables are not conditionally independent, the model is updated by adding a path between them to make the model more consistent with the data. In general, using causal diagrams and examining independence relationships between variables considerably helps to reduce confounder and collider bias in statistical models (McElreath 2015). *P*-values from tests of directed separation are used to calculate Fisher's *C* which follows a chi-squared distribution. Models are a good fit to the data when $p > 0.05$, indicating the null hypothesis—the proposed hypothesis network—is not rejected.

We based our conceptual model on previous findings in mammals (Schmidt et al. 2020c). This model supposes that limitations on energy (mean potential evapotranspiration) and niche availability (elevation range) set limits on supportable population sizes (body size), and therefore also the number of species that can coexist in a given habitat (Fig 6.2a). We amended this conceptual model for amphibians. First, we excluded human presence because previous investigation shows it did not have a clear effect on amphibian genetic diversity (Schmidt and Garroway 2020). Second, we included mean actual evapotranspiration as an additional measure of energy, because water availability is an important environmental constraint on amphibian ranges and site occupancy. All variables except genetic diversity were measured at the species level because we are primarily interested in the processes underlying diversity gradients at broad spatial scales. We log-transformed elevation range, and scaled and centered all variables before analysis so path coefficients could be compared across models. We tested our conceptual model using the entire dataset with frogs and salamanders combined, and also analyzed frogs and salamanders separately. There is some evidence that body size, our measure of population size, in these orders may have different relationships to temperature (Olalla-Tárraga and Rodríguez 2007), and there appears to be little overlap in body size between orders (Fig. S6.1). We tested residuals from frog and salamander SEMs for spatial autocorrelation using Moran tests.

Following results from SEM analysis, we tested for effects of heterogeneity on population differentiation in frogs. We measured differentiation using a population-specific F_{ST} metric (Weir and Goudet 2017) included in the genetic database. Population-specific F_{ST} differs from pairwise F_{ST} (Weir and Cockerham 1984) in that it measures how far single populations in a sample have diverged from a common ancestor. We tested for the effects of heterogeneity on population differentiation with a mixed effects model controlling for spatial structure using MEMs and including species as a random effect allowing intercepts to vary.

Results

Spatial patterns in genetic diversity. We found previously identified patterns of amphibian species richness with MEMs, where richness was highest in the high-energy, moist, low elevation region in the southeastern United States (Fig. 5.1; (Currie 1991). Western USA, which is hotter and drier, had a comparatively low number of species. As in mammals, the major species richness gradient in North American amphibians is longitudinal rather than latitudinal. We found no broad spatial trends in genetic diversity. Spatial patterns of genetic diversity were more complex in the east, while western populations all had similarly high levels of genetic diversity (Fig. 6.1). In general, species richness was more spatially structured than genetic diversity, with 85% and 56% of variation explained by spatial patterns, respectively (Fig. S6.2). We detected shared spatial patterns between both levels of biodiversity, however, while shared patterns explained 98% of the spatial variation in genetic diversity, they explained very little of the variation in species richness (Fig. S6.2).

Common causes of genetic diversity and species richness. Our conceptual model (Fig 2a) fit the data well with no additional links suggested (combined: Fisher's C = 3.56, $p = 0.47$, $n = 548$, Table S6.1; frogs: Fisher's C = 2.46, $p = 0.65$, $n = 288$; salamanders: Fisher's C = 2.05, $p = 0.73$, $n = 260$, Table S6.2). Note that for SEM, $p > 0.05$ means our null hypothesis is not rejected. Here we discuss results from the separate frog and salamander analyses; results from the combined model are given in Table S6.1. In both frogs and salamanders, species richness was well explained (frog $R^2 = 0.86$; salamander $R^2 = 0.95$), and increased with water availability, environmental heterogeneity, and species body size (Fig 6.2, Table S6.2). Heterogeneity was the most important determinant of amphibian species richness (frog $\beta = 0.59 \pm 0.04$ SE; salamander $\beta = 1.16 \pm 0.03$ SE; Fig 6.2, Table S6.2). Frog species richness was unrelated to energy availability and inversely related to genetic diversity (Fig 6.2b). In salamanders, species richness increased with energy availability and genetic diversity (Fig 6.2c). Across both groups body size was inversely related to water availability and environmental heterogeneity, and in salamanders energy availability additionally had a negative effect on body size. We note that in the combined model, water availability had a positive effect on body size (Table S6.1) which may be due to general size differences

between frogs and salamanders, the latter tending to be larger (Fig. S6.1). In frogs, heterogeneity had a strong negative effect on genetic diversity (β = -0.77 ± 0.28 SE), however genetic diversity in salamanders was not well predicted by any variables in our model (Fig. 6.2). There was no residual spatial autocorrelation in the salamander model. In frogs, body size and species richness residuals were spatially autocorrelated at very local scales (body size Moran's *I* = 0.01, species richness Moran's *I* = 0.03). In general, the environmental covariates in our models captured broad spatial patterns well, and we did not incorporate fine-scale spatial structure into our model for frogs as this was likely due to the hierarchical structure of the data.

Environmental heterogeneity had a negative effect on genetic diversity in frogs; however, genetic diversity is not an indicator of population divergence. We tested the idea that heterogeneity also increased divergence using population-specific F_{ST}. Frog populations indeed tended to be more genetically differentiated in heterogeneous environments (β = 0.99 ± 0.40 SE).

Discussion

Patterns of amphibian biodiversity

The clearest effects in our models suggest that heterogeneity is a prominent determinant of biodiversity in both frogs and salamanders. We did not detect an obvious gradient in genetic diversity in North American amphibians, and it appears the relationships between intraspecific genetic diversity and species richness in these taxa are different than they are in mammals (Schmidt et al. 2020c). Notably, our variation partitioning analysis suggests that the spatial patterns shared between genetic diversity and species richness explain most of the variation in genetic diversity, but almost none of the variation in species richness (Fig. S6.2). This finding suggests that the processes affecting intraspecific genetic diversity and population structure contribute little to broader gradients in species richness.

We suspect the general disconnect between genetic diversity and species richness and climate we find may come down to amphibian population dynamics. Carrying capacity

hypotheses assume that communities are in equilibrium between speciation, colonization, and extinction (Storch et al. 2018)—by extending this to the genetic level, we also assume populations are in an equilibrium state with regard to gene flow, mutation and genetic drift, as is often assumed by neutral population genetic models. However, amphibians have highly variable local population sizes which can sometimes fluctuate between orders of magnitude from year to year (Collins et al. 2009). Frequent bottlenecks and founder effects due to recolonization from nearby areas in sampled populations could obscure a general relationship of population genetic diversity with population size, species richness, and the climatic factors we explore here.

Interestingly, genetic diversity had opposite effects on species richness in frogs and salamanders. In frogs, populations in more heterogeneous environments tended to be less genetically diverse and more differentiated. This pattern, in turn, was associated with greater species richness. These are the predictions of the heterogeneity hypothesis, where increased niche availability reduces population sizes and facilitates population divergence and species coexistence. However, heterogeneity had no detectable effect on salamander genetic diversity, which was positively related to species richness. These relationships suggest that more diverse populations, which are presumably larger, lead to an increase in species richness in line with predictions for the more individuals hypothesis. It thus appears that broad-scale heterogeneity seems to have different effects on salamander and frog population genetic diversity. In general, frogs have better dispersal capabilities than salamanders (Smith and Green 2005). Our dataset included several widely distribted, northern-adapted generalist species, including the wood frog (*Lithobates sylvaticus*), northern leopard frog (*L. pipiens*), and spring peeper (*Pseudacris crucifer*). Such species have greater potential for population isolation and divergence. The spotted salamander (*Ambystoma maculatum*) is likewise a northern-adapted species with a broad distribution, however its range is comparatively smaller (Fig. S6.3). In salamanders the importance of niche conservatism—the tendency for closely related species to occupy similar niches—for speciation declines with latitude (Kozak and Wiens 2006). Thus, speciation in temperate North American salamanders may primarily have been a product of geographic isolation

and niche conservatism rather than ecological divergence (Kozak and Wiens 2006). However, the same pattern is not true for frogs, where niche conservatism does not appear to be generally important for allopatric speciation (Hua and Wiens 2010).

Previous evidence in support of the more individuals hypothesis was reported in Plethodontid salamanders (Marshall and Camp 2006), where the authors found a positive correlation between genetic diversity, species richness, and water and energy availability. Topographic heterogeneity made a secondary contribution to diversity (Marshall and Camp 2006). Salamanders' limited dispersal capability relative to frogs may mean population genetic diversity is affected by environmental heterogeneity at finer scales not captured by our model, but that broad-scale heterogeneity is sufficient to resolve diversity patterns at the species level. Bringing this idea back to spatial variation partitioning, it could be that while broad- and fine-scale processes both contribute to species richness, fine-scale processes contribute less. The same fine-scale processes, however, are the primary drivers of spatial genetic diversity patterns. The prominence of heterogeneity in our model contrasts with previous studies reporting species richness most strongly varies with water availability, with heterogeneity being a secondary cause of diversity (Rodríguez et al. 2005; Marshall and Camp 2006; Buckley and Jetz 2007).

The relevance of temperature-based carrying capacity limits for amphibians and ectotherms more generally is debatable due to their low energy usage relative to endotherms. Given the caveats noted above, body size may not be a good proxy for population size in amphibians. It has also been suggested elsewhere that habitat availability does not have an effect on body size in frogs (Olalla-Tárraga et al. 2009). Opposite to our expectations under the more individuals hypothesis, our models suggest that species richness in amphibians is positively correlated with body size, but greater resource availability favors smaller body sizes. In contrast to mammals, body size in amphibians may be more directly related to environmental conditions because it affects thermoregulation and moisture balance. Amphibians can behaviorally thermoregulate and avoid desiccation to an extent by selecting microhabitats with suitable temperature and humidity levels, and by changing their posture (Pough et al. 1983; Olalla-Tárraga et al.

2009; Rozen-Rechels et al. 2019). However, smaller species desiccate faster than large species due to evaporative water loss and higher surface-to-volume ratios (Olalla-Tárraga et al. 2009; Levy and Heald 2016), meaning larger body sizes are favored in drier climates.

Although we did not detect any consistent latitudinal or longitudinal gradients in genetic diversity, previous findings suggest mitochondrial genetic diversity in amphibians and other ectotherms varies latitudinally and mirrors species richness patterns (Miraldo et al. 2016; Manel et al. 2020). Amphibian mitochondrial genetic diversity in North America was highest in the species-rich southeastern United States, supporting the evolutionary speed hypothesis, where high environmental temperature increases rates of population divergence and speciation through its effects on mutation rate and generation time (Miraldo et al. 2016). However, we detected no effect of temperature on nuclear genetic diversity in our SEM, casting doubt on this hypothesis for amphibian nuclear genetic diversity. Furthermore, a lack of latitudinal gradient indicates that nuclear genetic diversity is not related to temperature in a straightforward way, likely due to strong temporal instability in amphibian population sizes. Our nuclear genetic data suggests heterogeneity is a major determinant of genetic diversity at broad scales in frogs, but mitochondrial DNA alone is not a reliable marker for detecting intraspecific patterns of population structure (Galtier et al. 2009b). Disagreement between biogeographic patterns of mitochondrial and nuclear DNA diversity is relatively common, and often arises from demographic disparities and sex-biased dispersal (Toews and Brelsford 2012). Thus, marker choice is very likely responsible for the divergent patterns we find here. This also appears to be true in mammals, where patterns of genetic diversity measured using mitochondrial DNA and nuclear DNA trended in opposite directions (Schmidt et al. 2020c).

Generality of causal mechanisms

Despite limitations modeling population size in amphibians, it appears that genetic diversity and species richness in frogs are driven by processes similar to mammals. Although Schmidt et al. (2020) found support for both the more individuals hypothesis and

the role of heterogeneity in mammals, heterogeneity was the main contributor to diversity across both genetic and species levels. Heterogeneity has previously been put forth as a universal driver of species richness (Stein et al. 2014). Our findings combining genetic diversity and species richness in amphibians and previous findings in mammals (Schmidt et al. 2020c) indeed suggest that heterogeneity is a major determinant of biodiversity on species and genetic levels in both endothermic and ectothermic vertebrates. However, the underlying mechanisms are not universal, and appear to vary depending on species groups. While in mammals shared spatial patterns between genetic diversity and species richness pointed to common causes, patterns of species richness in amphibians were not well predicted by spatial patterns shared with genetic diversity. Thus, the role of population-level processes in determining amphibian species richness is unclear. Energy and water limitations likely act directly on species richness, and not genetic diversity, by imposing physiological limits on amphibian distributions (Buckley and Jetz 2007). The demographic effects of carrying capacity and niche availability may then only be borne out within regions with suitable environmental conditions. It appears that similar environmental factors are capable of generating an overall latitudinal species richness gradient across taxa, but slight deviations from this general pattern are mediated by differential interactions between environments, species traits, and population processes.

Genetic diversity and species richness are two important metrics for biodiversity conservation because they contribute to the resilience of populations and communities in rapidly changing environments (Oliver et al. 2015). Genetic diversity in particular helps ensure population viability, fitness, and capacity to respond to environmental change through adaptation (Frankham 1995b). Amphibians are among the most imperiled vertebrates (Stuart et al. 2004) and are especially susceptible to environmental change. Macrogenetics approaches to mapping multispecies patterns of genetic diversity at broad scales have great potential for incorporation into conservation policies targeting regional conservation of genetic diversity. However, complex ecophysiological requirements, life histories, and population dynamics may render this approach impractical for amphibians. Finer-scale measures of environmental heterogeneity, energy availability, and habitat

suitability may prove to be more reliable predictors of genetic diversity but may do less well at the species level. Population size fluctuation in amphibians could pose a significant roadblock for macrogenetics studies that do not take into account temporal sampling, whether due to study design or lack of available data for this purpose. We are only beginning to explore broad-scale patterns of intraspecific nuclear genetic diversity across several species, but it is already apparent that they are not as consistently clear as gradients in species richness (Miraldo et al. 2016; Manel et al. 2020; Schmidt et al. 2020c; Theodoridis et al. 2020). We look forward to the continued exploration of these patterns in other taxonomic groups to build a comprehensive picture of the distribution of genetic biodiversity across the globe.

Acknowledgements: We would like to thank Mitchell Green for assistance with data acquisition, as well as the authors of the original datasets for making their data public. C.S. and C.J.G. were supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to C.J.G. C.S. was also supported by a U. Manitoba Graduate Fellowship, and a U. Manitoba Graduate Enhancement of Tri-council funding grant to C.J.G.

References

Allouche, O., Kalyuzhny, M., Moreno-Rueda, G., Pizarro, M., and Kadmon, R. 2012. Area-heterogeneity tradeoff and the diversity of ecological communities. Proc. Natl. Acad. Sci. U. S. A. **109**(43): 17495–17500. doi:10.1073/pnas.1208652109.

Blanchet, G.F., Legendre, P., and Borcard. 2008. Forward selection of explanatory variables. Ecology **89**(9): 2623–2632.

Brüniche-Olsen, A., Kellner, K.F., Anderson, C.J., and DeWoody, J.A. 2018. Runs of homozygosity have utility in mammalian conservation and evolutionary studies. Conserv. Genet. **19**(6): 1295–1307. doi:10.1007/s10592-018-1099-y.

Buckley, L.B., and Jetz, W. 2007. Environmental and historical constraints on global patterns of amphibian richness. Proc. R. Soc. B Biol. Sci. **274**(1614): 1167–1173.

doi:10.1098/rspb.2006.0436.

Buckley, L.B., and Jetz, W. 2010. Lizard community structure along environmental gradients. J. Anim. Ecol. **79**(2): 358–365. doi:10.1111/j.1365-2656.2009.01612.x.

Charlesworth, B., and Charlesworth, D. 2010. Elements of evolutionary genetics. Roberts & Company Publishers, Greenwood Village, Colorado, USA.

Collins, J.P., Crump, M.L., and Lovejoy III, T.E. 2009. Extinction in Our Times: Global Amphibian Decline. Oxford University Press, Oxford.

Currie, D.J. 1991. Energy and large-scale patterns of animal- and plant-species richness. Am. Nat. **137**(1): 27–49.

Currie, D.J., Mittelbach, G.G., Cornell, H. V., Field, R., Guégan, J.F., Hawkins, B.A., Kaufman, D.M., Kerr, J.T., Oberdorff, T., O'Brien, E., and Turner, J.R.G. 2004. Predictions and tests of climate-based hypotheses of broad-scale variation in taxonomic richness. Ecol. Lett. **7**(12): 1121–1134. doi:10.1111/j.1461-0248.2004.00671.x.

Damuth, J. 1987. Interspecific allometry of population density in mammals and other animals: the independence of body mass and population energy-use. Biol. J. Linn. Soc. **31**(3): 193–246. doi:10.1111/j.1095-8312.1987.tb01990.x.

Dray, S., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., and Wagner, H.H. 2017. adespatial: Multivariate Multiscale Spatial Analysis. Available from https://cran.r-project.org/package=adespatial.

Frankham, R. 1995. Conservation Genetics. Annu. Rev. Genet. **29**: 305–332. Available from www.annualreviews.org.

Galtier, N., Nabholz, B., Glémin, S., and Hurst, G.D.D. 2009. Mitochondrial DNA as a marker of molecular diversity: A reappraisal. Mol. Ecol. **18**(22): 4541–4550. doi:10.1111/j.1365-294X.2009.04380.x.

Hawkins, B.A., Field, R., Cornell, H. V., Currie, D.J., Guégan, J.-F., Kaufman, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., Porter, E.E., and Turner, J.R.G. 2003.

Energy, water, and broad-scale geographic patterns of species richness. Ecology **84**(12): 3105–3117. doi:10.1890/03-8006.

Hua, X., and Wiens, J.J. 2010. Latitudinal variation in speciation mechanisms in frogs. Evolution. **64**(2): 429–443. doi:10.1111/j.1558-5646.2009.00836.x.

Hubbell, S.P. 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press, Princeton NJ.

IUCN. 2019. The IUCN Red List of Threatened Species. Version 2019-1. Available from https://www.iucnredlist.org.

Jiménez-Alfaro, B., Chytrý, M., Mucina, L., Grace, J.B., and Rejmánek, M. 2016. Disentangling vegetation diversity from climate-energy and habitat heterogeneity for explaining animal geographic patterns. Ecol. Evol. **6**(5): 1515–1526. doi:10.1002/ece3.1972.

Kerr, J.T., and Packer, L. 1997. Habitat heterogeneity as a determinant of mammal species richness. Nature **385**: 253–254.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge University Press, Cambridge.

Kozak, K.H., and Wiens, J.J. 2006. Does Niche Conservatism Promote Speciation? a Case Study in North American Salamanders. Evolution. **60**(12): 2604. doi:10.1554/06-334.1.

Kreft, H., and Jetz, W. 2007. Global patterns and determinants of vascular plant diversity. Proc. Natl. Acad. Sci. **104**(14): 5925–5930. doi:10.1073/pnas.0608361104.

Lefcheck, J., Byrnes, J., and Grace, J. 2019. piecewiseSEM: Piecewise Structural Equation Modeling. Available from https://cran.r-project.org/package=piecewiseSEM.

Levy, D.L., and Heald, R. 2016. Biological scaling problems and solutions in amphibians. Cold Spring Harb. Perspect. Biol. **8**(1): 1–16. doi:10.1101/cshperspect.a019166.

Mackintosh, A., Laetsch, D.R., Hayward, A., Charlesworth, B., Waterfall, M., Vila, R., and Lohse, K. 2019. The determinants of genetic diversity in butterflies. Nat. Commun.

10(1): 1–9. doi:10.1038/s41467-019-11308-4.

Manel, S., Guerin, P.E., Mouillot, D., Blanchet, S., Velez, L., Albouy, C., and Pellissier, L. 2020. Global determinants of freshwater and marine fish genetic diversity. Nat. Commun. **11**(1): 1–9. Springer US. doi:10.1038/s41467-020-14409-7.

Marshall, J.L., and Camp, C.D. 2006. Environmental correlates of species and genetic richness in lungless salamanders (family plethodontidae). Acta Oecologica **29**(1): 33–44. doi:10.1016/j.actao.2005.07.008.

McElreath, R. 2015. Statistical rethinking: a bayesian course with examples in R and Stan. CRC press.

Miraldo, A., Li, S., Borregaard, M.K., Florez-Rodriguez, A., Gopalakrishnan, S., Rizvanovic, M., Wang, Z., Rahbek, C., Marske, K.A., and Nogues-Bravo, D. 2016. An Anthropocene map of genetic diversity. Science. **353**(6307): 1532–1535. doi:10.1126/science.aaf4381.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. **70**(12): 3321–3323. doi:10.1073/pnas.70.12.3321.

NOAA, and U.S. National Geophysical Data Center. (n.d.). TerrainBase, release 1.0. Boulder CO. Available from https://nelson.wisc.edu/sage/data-and-models/atlas/maps.php?datasetid=28&includerelatedlinks=1&dataset=28.

Olalla-Tárraga, M.Á., Diniz-Filho, J.A.F., Bastos, R.P., and Rodríguez, M.Á. 2009. Geographic body size gradients in tropical regions: Water deficit and anuran body size in the Brazilian Cerrado. Ecography (Cop.). **32**(4): 581–590. doi:10.1111/j.1600-0587.2008.05632.x.

Olalla-Tárraga, M.Á., and Rodríguez, M.Á. 2007. Energy and interspecific body size patterns of amphibian faunas in Europe and North America: anurans follow Bergmann's rule, urodeles its converse. Glob. Ecol. Biogeogr. **16**(5): 606–617. doi:10.1111/j.1466-8238.2007.00309.x.

Oliveira, B.F., São-Pedro, V.A., Santos-Barrera, G., Penone, C., and Costa, G.C. 2017.

AmphiBIO, a global database for amphibian ecological traits. Sci. Data **4**: 1–7. The Author(s). doi:10.1038/sdata.2017.123.

Oliver, T.H., Heard, M.S., Isaac, N.J.B., Roy, D.B., Procter, D., Eigenbrod, F., Freckleton, R., Hector, A., Orme, C.D.L., Petchey, O.L., Proença, V., Raffaelli, D., Suttle, K.B., Mace, G.M., Martín-López, B., Woodcock, B.A., and Bullock, J.M. 2015. Biodiversity and resilience of ecosystem functions. Trends Ecol. Evol. **30**(11): 673–684. doi:10.1016/j.tree.2015.08.009.

Peng, L., Zeng, Z., Wei, Z., Chen, A., Wood, E.F., and Sheffield, J. 2019. Determinants of the ratio of actual to potential evapotranspiration. Glob. Chang. Biol. **25**(4): 1326–1343. doi:10.1111/gcb.14577.

Peters, R.H., and Wassenberg, K. 1983. The effect of body size on animal abundance. Oecologia **60**(1): 89–96. doi:10.1007/BF00379325.

Pough, F.H., Taigen, T.L., Stewart, M.M., and Brussard, P.F. 1983. Behavioral modification of evaporative water loss by a Puerto Rican frog (*Eleutherodactylus coqui*). Ecology **64**(2): 244–252. doi:10.2307/1937072.

Pyron, R.A., and Burbrink, F.T. 2014. Early origin of viviparity and multiple reversions to oviparity in squamate reptiles. Ecol. Lett. **17**(1): 13–21. doi:10.1111/ele.12168.

Rodríguez, M.Á., Belmontes, J.A., and Hawkins, B.A. 2005. Energy, water and large-scale patterns of reptile and amphibian species richness in Europe. Acta Oecologica **28**(1): 65–70. doi:10.1016/j.actao.2005.02.006.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., Chiari, Y., Dernat, R., Duret, L., Faivre, N., Loire, E., Lourenco, J.M., Nabholz, B., Roux, C., Tsagkogeorga, G., Weber, A.A.T., Weinert, L.A., Belkhir, K., Bierne, N., Glémin, S., and Galtier, N. 2014. Comparative population genomics in animals uncovers the determinants of genetic diversity. Nature **515**(7526): 261–263. doi:10.1038/nature13685.

Rozen-Rechels, D., Dupoué, A., Lourdais, O., Chamaillé-Jammes, S., Meylan, S., Clobert, J., and

Le Galliard, J.F. 2019. When water interacts with temperature: Ecological and evolutionary implications of thermo-hydroregulation in terrestrial ectotherms. Ecol. Evol. **9**(17): 10029–10043. doi:10.1002/ece3.5440.

Schmidt, C., Dray, S., and Garroway, C.J. 2020. Genetic and species-level biodiversity patterns are linked by demography and ecological opportunity. bioRxiv. doi:10.1101/2020.06.03.132092.

Schmidt, C., and Garroway, C.J. 2020. Inconsistent effects of urbanization on amphibian genetic diversity. bioRxiv: 1–30. doi:10.1101/2020.08.16.253104.

Shipley, B. 2016. Cause and correlation in biology. *In* Cause and Correlation in Biology, 2nd edition. Cambridge University Press, Cambridge.

Smith, M.A., and Green, D.M. 2005. Dispersal and the Metapopulation Paradigm in Amphibian Ecology and Conservatio : Are All Amphibian Populations Metapopulations? Ecography (Cop.). **28**(1): 110–128.

Stein, A., Gerstner, K., and Kreft, H. 2014. Environmental heterogeneity as a universal driver of species richness across taxa, biomes and spatial scales. Ecol. Lett. **17**(7): 866–880. doi:10.1111/ele.12277.

Storch, D., Bohdalková, E., and Okie, J. 2018. The more-individuals hypothesis revisited: the role of community abundance in species richness regulation and the productivity–diversity relationship. Ecol. Lett. **21**(6): 920–937. doi:10.1111/ele.12941.

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., and Waller, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. Science. **306**(5702): 1783–1786. doi:10.1126/science.1103538.

Theodoridis, S., Fordham, D.A., Brown, S.C., Li, S., Rahbek, C., and Nogues-Bravo, D. 2020. Evolutionary history and past climate change shape the distribution of genetic diversity in terrestrial mammals. Nat. Commun. **11**(1): 2557. doi:10.1038/s41467-020-16449-5.

Toews, D.P.L., and Brelsford, A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol. Ecol. **21**(16): 3907–3930. doi:10.1111/j.1365-294X.2012.05664.x.

Trabucco, A., and Zomer, R. 2019. Global Aridity Index and Potential Evapotranspiration (ET0) Climate Database v2. doi:10.6084/m9.figshare.7504448.v3.

Weir, B.S., and Cockerham, C.C. 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution. **38**(6): 1358–1370. doi:10.2307/2408641.

Weir, B.S., and Goudet, J. 2017. A unified characterization of population structure. Genetics **206**: 2085–2103.

Whittaker, R.J., Nogués-Bravo, D., and Araújo, M.B. 2007. Geographical gradients of species richness: a test of the water-energy conjecture of Hawkins et al. (2003) using European data for five taxa. Glob. Ecol. Biogeogr. **16**: 76–89. doi:10.1111/j.1466-822x.2006.00268.x.

Wright, D.H. 1983. Species-Energy Theory: An Extension of Species-Area Theory. Oikos **41**(3): 496–506.

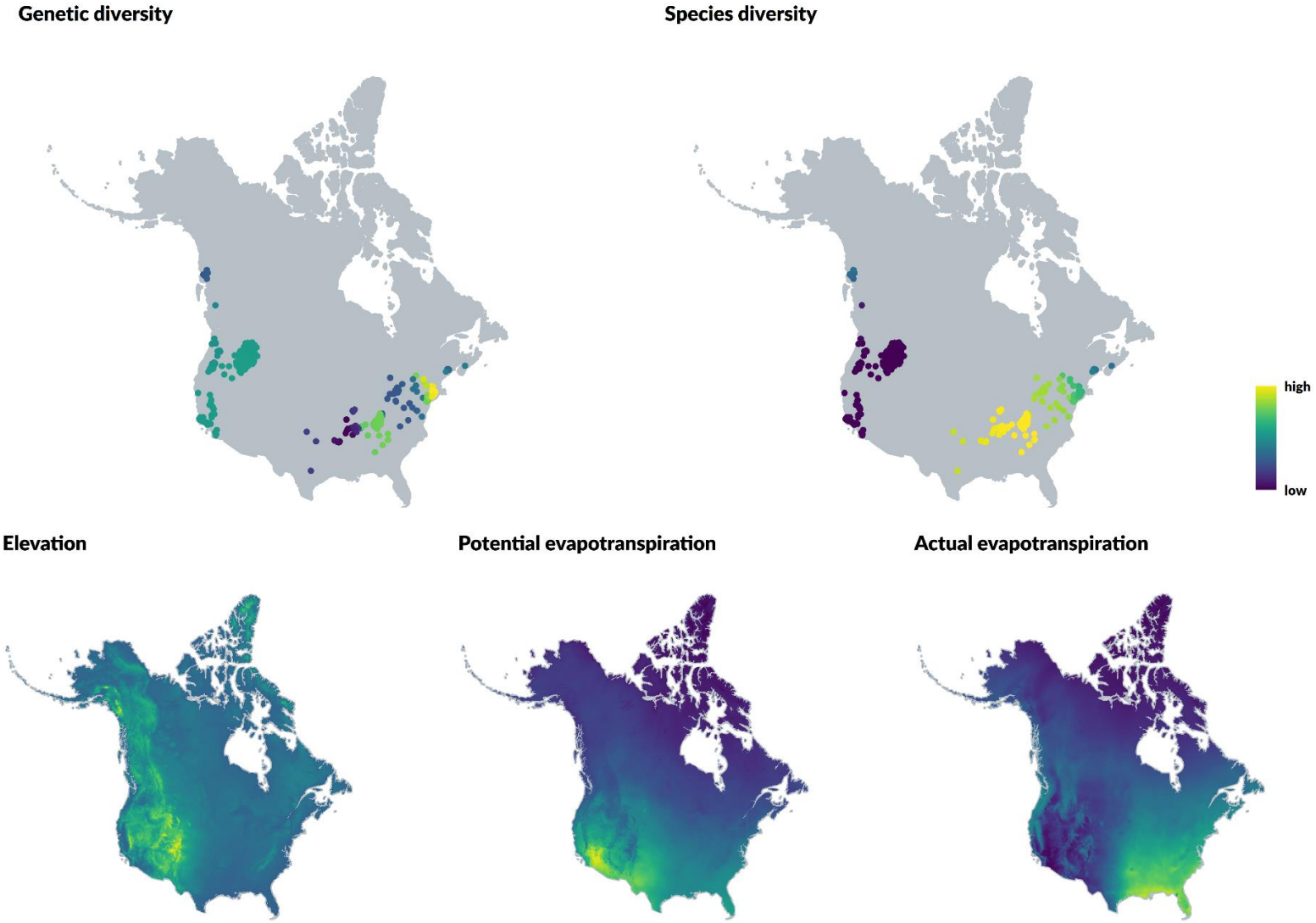


Figure 6.1. *(Top row)* Maps of predicted genetic diversity and species richness at genetic sample sites (points) based on spatial MEMs for frogs and salamanders combined. No obvious pattern was detected in genetic diversity, but MEMs were able

to recover known patterns of species richness. *(Bottom row)* Maps depicting the environmental variables predicted to have simultaneous effects on genetic diversity and species richness.

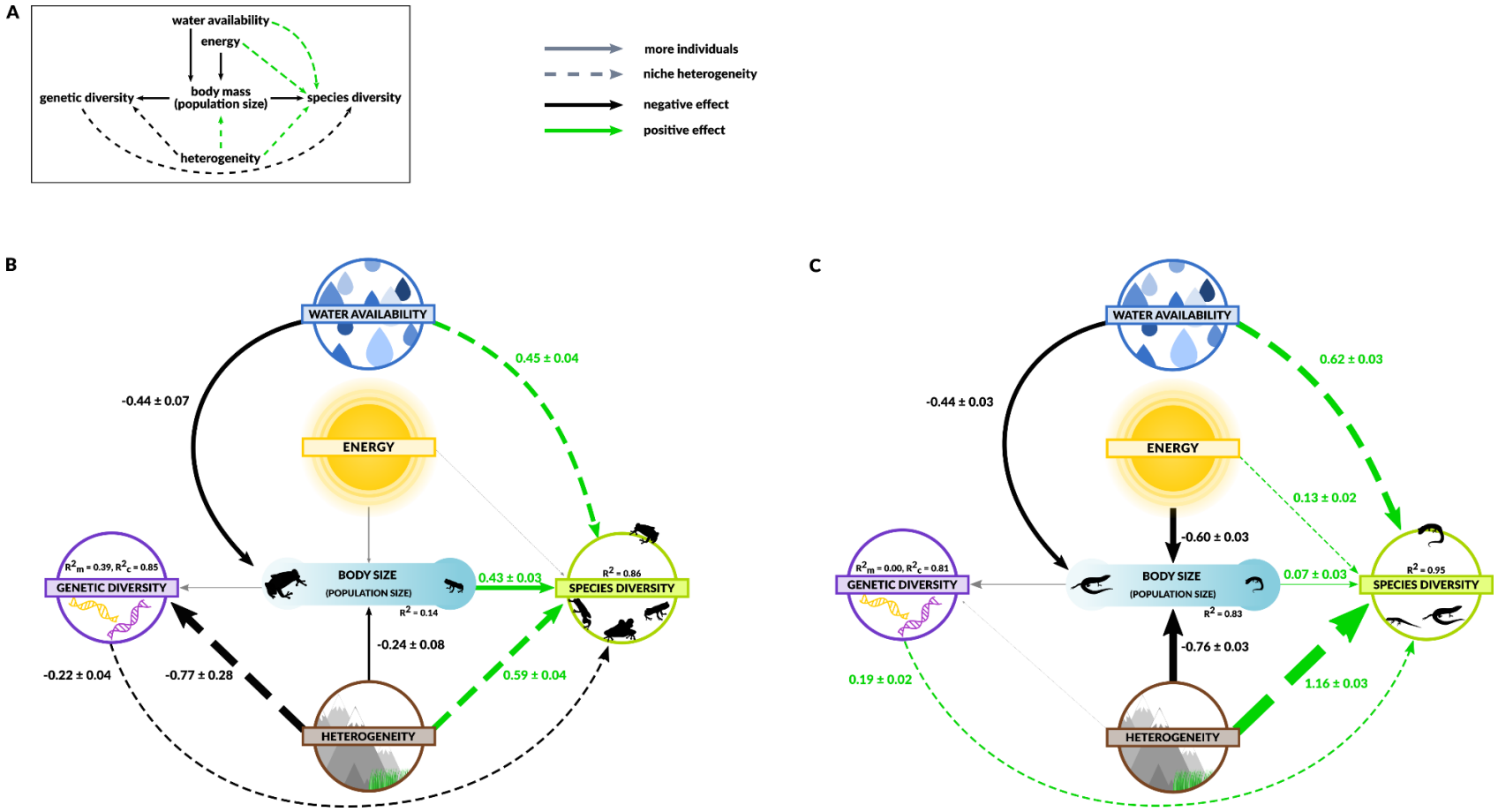


Figure 6.2. Structural equation models. (A) Our hypothesized conceptual model based on findings in mammals; solid and dashed lines correspond to processes related to the more individuals and heterogeneity hypotheses, respectively. Line width is proportional to path coefficients. Model results are shown for (B) frogs, and (C) salamanders. Results from the overall model with both orders are in Table S1. Regression coefficients with standard errors are shown along each path. Paths between

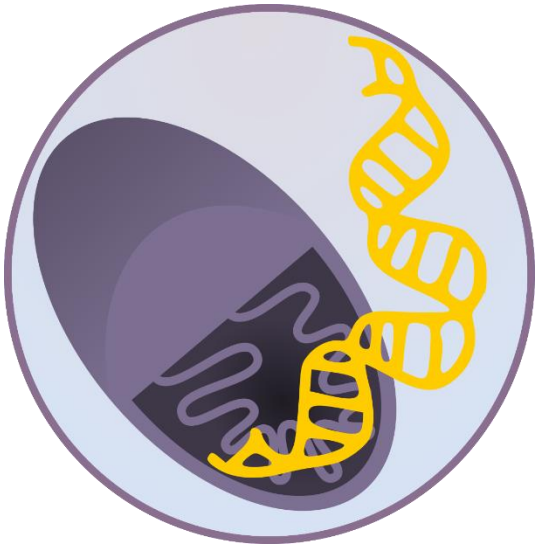
variables where no effect was detected are colored in gray (see Table S2 for a complete summary of all paths). The proportion of variation explained (R^2) is given for all dependent variables. For genetic diversity, R^2_m is the variation explained by fixed effects and R^2_c is the variation explained by both fixed effects and the random species effect.

Chapter 7: The conservation utility of mitochondrial genetic diversity in macrogenetic research

Chloé Schmidt, Colin J. Garroway

Department of Biological Sciences, University of Manitoba, Winnipeg, Canada

Status: published in Conservation Genetics *(DOI: 10.1007/s10592-021-01333-6)*



Introduction

As soon as molecular genetic data began to accumulate, population geneticists started to address questions about the nature of genetic variation across species (Soulé 1976). Early approaches to multi-species population genetics relied on harvesting population genetic information from the literature and merging it with other data to address multi-species scale questions (Soulé 1976; Loveless and Hamrick 1984; Nevo et al. 1984; Frankham 1996). The recent accumulation of open molecular genetic data in repositories such as GenBank and DRYAD has vastly increased the power, scope, and types of multi-species population genetic questions we can ask because raw data can be used for new purposes. Perhaps unsurprisingly then, we have seen an increased interest in this area of research (Miraldo et al. 2016; Lawrence and Fraser 2020; Manel et al. 2020; Millette et al. 2020; Schmidt et al. 2020b; Theodoridis et al. 2020). This new work has led to a coalescence of ideas around the emerging subdiscipline of *macrogenetics* (Blanchet et al. 2017). *Macrogenetics* has come to encompass population genetic research that repurposes genetic data, whether collected from the literature or calculated from raw data, to address questions about the ecological and evolutionary causes and consequences of genetic variation across multiple species.

Having recently identified macrogenetic processes as a subject matter worth dedicated study, we are only beginning to identify the phenomena that fall under its purview. A recent focus has been the mapping of broad-scale patterns of genetic diversity and the exploration of its relationships with environments and species richness (Miraldo et al. 2016; Manel et al. 2020; Theodoridis et al. 2020). This line of inquiry is exciting with important implications for our understanding of biodiversity and its conservation. Miraldo et al. (2016) were the first to explore global patterns of genetic diversity by harvesting georeferenced publicly available mitochondrial DNA (mtDNA) sequences for mammals and amphibians. They detected a latitudinal gradient in mtDNA diversity in mammals and amphibians that mirrored species richness patterns. Manel et al. (2020) and Theodoridis et al. (2020) used similar methodological approaches focusing on fish and mammals

respectively, also finding that latitudinal gradients in mtDNA diversity reflect species richness patterns.

Each of these papers highlights the need for the multi-layered conservation of biodiversity at the genetic and species levels and recognize that describing broad-scale patterns in genetic diversity will be necessary for this. Each paper also notes that our understanding of the processes underlying biogeographic scale genetic diversity patterns would be greatly enhanced by incorporating analyses of nuclear genetic markers. This is easier said than done. Raw nuclear genetic data is not programmatically accessible in centralized data repositories; however, mtDNA is – hence the early emphasis on mtDNA diversity patterns. Our goal is to delve further into the caveats associated with the use of mtDNA markers for macrogenetics studies as noted by the authors of Miraldo et al. (2016), Manel et al. (2020), and Theodoridis et al. (2020). We expand on the potential drawbacks of mtDNA sequence data for macrogenetic studies and its interpretation for conservation decision-making within that context. The evolution of mitochondrial genomes across species is notably "capricious" (Galtier et al. 2009a). This makes linking mtDNA diversity patterns to population-level processes (Zink and Barrowclough 2008; Edwards and Bensch 2009; Bohonak and Vandergast 2011) and thus the conservation utility of mtDNA diversity gradients, fraught. We first describe the disconnect between mtDNA variation and adaptive potential—the quantity of interest for conservation. We then discuss the mismatch between patterns and population-level processes due to idiosyncrasies in mtDNA evolution. We conclude with potential future directions for the continued study of mtDNA patterns in macrogenetics.

Measuring genetic diversity that is relevant for conservation

The target when conserving genetic diversity is "genetic material of actual or potential value" (CBD and UNEP 2010). Genetic material of potential value refers to the genetic variation underlying a population's capacity to adapt—that is, the additive genetic variance in fitness in that population (Fisher 1930). Quantifying additive genetic variation requires

the direct measurement of fitness across a large number of relatives for multiple generations, which is difficult for wild animal populations. Theory predicts that neutral estimates of genome-wide diversity should be proportional to additive genetic variation (Falconer and Mackay 1996) and empirical evidence suggests there is indeed a weak positive correlation (Mittell et al. 2015). It is thus notable that the first macrogenetic analysis of neutral nuclear genetic diversity found that it was negatively correlated with species richness (Schmidt et al. 2020c). This contrasts with the consistent positive correlation between mtDNA diversity and species richness. If patterns of nuclear DNA diversity—which is positively correlated with adaptive potential—trend opposite those of mtDNA, multi-species gradients in mtDNA diversity are not capturing genetic diversity of conservation value in a straightforward way. This is not to say that mtDNA does not have conservation value for some species-specific applications. For example, it can be useful for revealing glacial refugia or identifying cryptic lineages, and high levels of mtDNA diversity are rarely found in highly inbred populations.

mtDNA diversity patterns

Typical approaches for identifying mtDNA diversity patterns divide the globe into grid cells, then summarize diversity within cells by calculating the average nucleotide diversity for each species and finally averaging nucleotide diversity across species (Miraldo et al. 2016; Manel et al. 2020; Theodoridis et al. 2020). However, this diversity metric is hard to interpret because mtDNA mutation rates are highly variable across taxa (Nabholz et al. 2008b; Allio et al. 2017). For example, in mammals mitochondrial mutation rates can vary 100-fold across species (Nabholz et al. 2008b). Furthermore, not all grid cells contain the same species. Multi-species cell-wise averages thus seem likely to strongly depend on what species are in the cell, making comparisons of diversity across cells difficult to interpret. We suspect averages of mtDNA diversity taken across species likely obscures intraspecific spatial variation. We note that this is not a criticism of mtDNA *per se,* as the biological meaning of multi-species averages of nuclear genetic diversity would also be unclear. Mutation rate variation can be accounted for by treating species as a random effect

in multilevel models (as in Millette et al. 2020; Schmidt et al. 2020b). We are uncertain of the extent to which multi-species averages can precisely capture patterns of mtDNA diversity.

Inferring processes

There has been considerable debate surrounding the use of mtDNA as a sole marker for inferring population and species-level pattern and process in other areas (Ballard and Whitlock 2004; Rubinoff and Holland 2005; Zink and Barrowclough 2008; Edwards and Bensch 2009; Bohonak and Vandergast 2011). There is now a general consensus among advocates and detractors of the various uses of mtDNA that it is most useful for inferring patterns (e.g., phylogenies), but alone it is often not sufficient for inferring processes shaping population history (Zink and Barrowclough 2008; Edwards and Bensch 2009). This is because the bulk of evidence suggests mtDNA diversity is not systematically or strongly related to ecology, demography, or genome-wide diversity (Bazin et al. 2006; Nabholz et al. 2008b; Galtier et al. 2009b; James and Eyre-Walker 2020). In practice maintaining a disconnect between pattern and process when interpreting our analyses is difficult because we are inherently interested in process (Edwards and Bensch 2009).

Identifying the common causes of biodiversity at species and genetic levels would considerably advance our basic evolutionary knowledge in addition to laying important groundwork for the joint conservation of species and genetic diversity. To varying extents, Miraldo et al., Manel et al., and Theodoridis et al. each interpret mtDNA diversity patterns in terms of processes related to ecology and demography. As noted above, the link between pattern and process in these cases is tenuous. The authors interpret their mtDNA diversity gradients in terms of established hypotheses for the origins of the species richness gradient. Hypotheses with mechanisms that might produce genetic diversity gradients positively correlated with species richness include evolutionary speed, climatic stability, and energy availability. Evolutionary speed hypotheses suggest that higher temperatures in the tropics cause higher metabolic rates and shorter generation times, leading to increased

mutation rates and faster rates of population divergence and speciation. The climate stability hypothesis posits that environmental instability causes recurring bottlenecks that limit both species and genetic diversity. Energy availability hypotheses suggest that high energy regions support larger populations and communities with high genetic diversity and species richness due to greater chances of population persistence. Reviews of these hypotheses can be found in (Currie 1991; Mittelbach et al. 2007; Pontarp et al. 2019). These hypotheses hinge on ecological and demographic processes.

With respect to the evolutionary speed hypothesis, mutation rates in mtDNA are not strongly correlated with nuclear mutation rates—indeed, there is some evidence that mtDNA nucleotide diversity measured at silent sites (approximately neutral) is correlated with nuclear diversity only after applying corrections for differences in mutation rate (Allio et al. 2017). Furthermore, the relationship between metabolic rate and mtDNA mutation rates are complex and appear to not be consistent across taxa (Lanfear et al. 2007; Galtier et al. 2009a). One idea is that mutagenesis is driven by the increased production of reactive oxygen species in the mitochondria when metabolic rates are high, but oxidative damage is likely not the primary contributor to high mtDNA mutation rates (DeBalsi et al. 2017). Regardless, reactive oxygen species produced in the mitochondria during cellular respiration do not cause oxidative damage to nuclear DNA (Hoffmann et al. 2004). Thus it is unclear whether higher mtDNA diversity towards the equator is the expected pattern under the evolutionary speed hypothesis. Climate stability and energy availability hypotheses depend on environmental limits on population size. Yet, the relationship between mtDNA diversity and population size, or ecological and life history correlates of population size, is unclear and perhaps too weak to be useful (Bazin et al. 2006; Nabholz et al. 2008b; James and Eyre-Walker 2020). Given the peculiarities of mtDNA evolution and its likely non-neutral status it is not certain whether a general positive relationship with population size is the null expectation. Even so, relationships between mtDNA diversity at silent sites and commonly used proxies of population size do not vary in consistently expected directions (James and Eyre-Walker 2020). This lack of consistent relationship between mtDNA diversity and demography makes it ill-suited for testing general

relationships between conservation relevant genetic diversity, environments, and species richness.

To illustrate this issue, we can take the well-founded prediction that human activity and urbanization should reduce genome-wide diversity by decreasing population sizes due to habitat fragmentation (Johnson and Munshi-South 2017). Synthetic analyses of genome-wide diversity of mammals through space and time consistently agree with this prediction (DiBattista 2008; Li et al. 2016; Leigh et al. 2019; Schmidt et al. 2020b). However, this relationship appears not to hold in general for mammalian mtDNA diversity (Miraldo et al. 2016; Millette et al. 2020; Theodoridis et al. 2020). Using mtDNA in this instance seems to miss declines in nuclear genetic diversity relevant for conservation.

Moving forward

The wealth of raw genetic data now available is exciting because of the new opportunities for exploring previously hidden levels of biodiversity it brings, and its value as a conservation tool. But the use of mtDNA as a metric for conservation-related decisions should be done with care. We note that Miraldo et al., Manel et al., and Theodoridis et al. do not make explicit conservation recommendations based on their findings, but the potential use of global maps of mtDNA diversity for the preservation of biodiversity is clear. For example, protected areas are a critical conservation tool, and the integration of genetic diversity patterns into protected area designation and management is needed for the maintenance of genetic diversity. Discussions about just how genetic diversity patterns could be integrated into international biodiversity conventions are underway (Hoban et al. 2020). Given our current understanding of the conservation utility of macrogenetic patterns of mtDNA diversity, decisions whether to integrate them into policy should be made carefully, with explicit presentations of the shortcomings of the marker. After discussing the caveats and nuanced interpretations of mtDNA gradients, we feel the case for its use should be strongly argued, not taken for granted. Indeed, the general targeting of regional conservation actions based on global patterns of interspecific mtDNA variation

could inadvertently capture regions of low adaptive capacity, contradicting our conservation goals (Schmidt et al. 2020c). Thus, mtDNA diversity gradients should not be used uncritically to provide general conservation guidance, nor to test general links between conservation relevant genetic diversity and ecological or environmental processes. mtDNA variation is an important element of genetic heritage, but its variation will primarily be related to cellular respiration and does not reflect genome-wide diversity well.

Beyond concerns about its relevance for conservation, we reiterate that macrogenetic patterns in mtDNA are not uninteresting and provide an opportunity to test other hypotheses. The longevity hypothesis, for example, posits that selection in long-lived species acts to lower mtDNA mutation rates and reduce oxidative damage to the mitochondrial genome which may contribute to ageing (Nabholz et al. 2008a). The clear spatial relationships between body size and environmental temperature (Bergmann's Rule), and life history correlations between body size and longevity (Stearns 1992) suggest a possible mechanism capable of producing the consistently identified broad-scale gradients in mtDNA diversity that positively correlate with species richness. It would also be worthwhile to more directly test purported links between temperature, metabolic rate, and mitochondrial mutation rate. For instance, whereas metabolic rates in ectotherms increase with environmental temperature, this relationship is more complicated for endotherms. If a causal connection between temperature, metabolism, mutation rate, and mtDNA diversity exists, we would expect it to be more apparent in ectothermic species. Importantly, both of these ideas require focusing on those species which have enough data for intraspecific tests to identify and compare patterns.

mtDNA can clearly inform conservation decision-making in some species-specific contexts. It is however, unclear that multi-species macrogenetic patterns of mtDNA variation are useful conservation tools. We echo the calls of Miraldo et al, Manel et al., and Theodoridis et al. to continue exploring these patterns with multiple marker types. In the meantime, we call for a very careful presentation of just what mtDNA data can tell us about the type of genetic biodiversity we want to conserve.

References

Allio, R., Donega, S., Galtier, N., and Nabholz, B. 2017. Large variation in the ratio of mitochondrial to nuclear mutation rate across animals: Implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. Mol. Biol. Evol. **34**(11): 2762–2772. doi:10.1093/molbev/msx197.

Ballard, J.W.O., and Whitlock, M.C. 2004. The incomplete natural history of mitochondria. Mol. Ecol. **13**(4): 729–744. doi:10.1046/j.1365-294X.2003.02063.x.

Bazin, E., Glémin, S., and Galtier, N. 2006. Population size does not influence mitochondrial genetic diversity in animals. Science. **312**(5773): 570–572. doi:10.1126/science.1122033.

Blanchet, S., Prunier, J.G., and De Kort, H. 2017. Time to Go Bigger: Emerging Patterns in Macrogenetics. Trends Genet. **33**(9): 579–580. doi:10.1016/j.tig.2017.06.007.

Bohonak, A.J., and Vandergast, A.G. 2011. The value of DNA sequence data for studying landscape genetics. Mol. Ecol. **20**(12): 2477–2479. doi:10.1111/j.1365-294X.2011.05122.x.

CBD, and UNEP. 2010. Strategic plan for biodiversity 2011–2020 and the Aichi targets.

Currie, D.J. 1991. Energy and large-scale patterns of animal- and plant-species richness. Am. Nat. **137**(1): 27–49.

DeBalsi, K.L., Hoff, K.E., and Copeland, W.C. 2017. Role of the mitochondrial DNA replication machinery in mitochondrial DNA mutagenesis, aging and age-related diseases. Ageing Res. Rev. **33**: 89–104. doi:10.1016/j.arr.2016.04.006.

DiBattista, J.D. 2008. Patterns of genetic variation in anthropogenically impacted populations. Conserv. Genet. **9**(1): 141–156. doi:10.1007/s10592-007-9317-z.

Edwards, S., and Bensch, S. 2009. Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. Mol. Ecol. **18**: 2930–2933.

Falconer, D.S., and Mackay, T.F.C. 1996. Introduction to Quantitative Genetics. *In* 4th edition. Longman, Harlow, Essex, UK.

Fisher, R.A. 1930. The Genetical Theory of Natural Selection. Oxford University Press, Oxford.

Frankham, R. 1996. Relationship of Genetic Variation to Population Size in Wildlife. Conserv. Biol. **10**(6): 1500–1508. doi:10.1046/j.1523-1739.1996.10061500.x.

Galtier, N., Jobson, R.W., Nabholz, B., Glémin, S., and Blier, P.U. 2009a. Mitochondrial whims: Metabolic rate, longevity and the rate of molecular evolution. Biol. Lett. **5**(3): 413–416. doi:10.1098/rsbl.2008.0662.

Galtier, N., Nabholz, B., Glémin, S., and Hurst, G.D.D. 2009b. Mitochondrial DNA as a marker of molecular diversity: A reappraisal. Mol. Ecol. **18**(22): 4541–4550. doi:10.1111/j.1365-294X.2009.04380.x.

Hoban, S., Bruford, M., D'Urban Jackson, J., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P.A., Paz-Vinas, I., Sjögren-Gulve, P., Segelbacher, G., Vernesi, C., Aitken, S., Bertola, L.D., Bloomer, P., Breed, M., Rodríguez-Correa, H., Funk, W.C., Grueber, C.E., Hunter, M.E., Jaffe, R., Liggins, L., Mergeay, J., Moharrek, F., O'Brien, D., Ogden, R., Palma-Silva, C., Pierson, J., Ramakrishnan, U., Simo-Droissart, M., Tani, N., Waits, L., and Laikre, L. 2020. Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. Biol. Conserv. **248**: 108654. doi:10.1016/j.biocon.2020.108654.

Hoffmann, S., Spitkovsky, D., Radicella, J.P., Epe, B., and Wiesner, R.J. 2004. Reactive oxygen species derived from the mitochondrial respiratory chain are not responsible for the basal levels of oxidative base modifications observed in nuclear DNA of mammalian cells. Free Radic. Biol. Med. **36**(6): 765–773. doi:10.1016/j.freeradbiomed.2003.12.019.

James, J., and Eyre-Walker, A. 2020. Mitochondrial DNA sequence diversity in mammals: a correlation between the effective and census population sizes. Genome Biol. Evol.

doi:10.1093/gbe/evaa222.

Johnson, M.T.J., and Munshi-South, J. 2017. Evolution of life in urban environments. Science. **358**(6363): eaam8327. doi:10.1126/science.aam8327.

Lanfear, R., Thomas, J.A., Welch, J.J., Brey, T., and Bromham, L. 2007. Metabolic rate does not calibrate the molecular clock. Proc. Natl. Acad. Sci. U. S. A. **104**(39): 15388–15393. doi:10.1073/pnas.0703359104.

Lawrence, E.R., and Fraser, D.J. 2020. Latitudinal biodiversity gradients at three levels: Linking species richness , population richness and genetic diversity. Glob. Ecol. Biogeogr. (May 2019): 1–19. doi:10.1111/geb.13075.

Leigh, D.M., Hendry, A.P., Vázquez-Domínguez, E., and Friesen, V.L. 2019. Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. Evol. Appl. **12**(8): 1505–1512. doi:10.1111/eva.12810.

Li, H., Xiang-Yu, J., Dai, G., Gu, Z., Ming, C., Yang, Z., Ryder, O.A., Li, W.H., Fu, Y.X., and Zhang, Y.P. 2016. Large numbers of vertebrates began rapid population decline in the late 19th century. Proc. Natl. Acad. Sci. U. S. A. **113**(49): 14079–14084. doi:10.1073/pnas.1616804113.

Loveless, M.., and Hamrick, J.. 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. **51**: 65–95.

Manel, S., Guerin, P.E., Mouillot, D., Blanchet, S., Velez, L., Albouy, C., and Pellissier, L. 2020. Global determinants of freshwater and marine fish genetic diversity. Nat. Commun. **11**(1): 1–9. Springer US. doi:10.1038/s41467-020-14409-7.

Millette, K.L., Fugère, V., Debyser, C., Greiner, A., Chain, F.J.J., and Gonzalez, A. 2020. No consistent effects of humans on animal genetic diversity worldwide. Ecol. Lett. **23**(1): 55–67. doi:10.1111/ele.13394.

Miraldo, A., Li, S., Borregaard, M.K., Florez-Rodriguez, A., Gopalakrishnan, S., Rizvanovic, M., Wang, Z., Rahbek, C., Marske, K.A., and Nogues-Bravo, D. 2016. An Anthropocene map

of genetic diversity. Science. **353**(6307): 1532–1535. doi:10.1126/science.aaf4381.

Mittelbach, G.G., Schemske, D.W., Cornell, H. V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., McCain, C.M., McCune, A.R., McDade, L.A., McPeek, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M., and Turelli, M. 2007. Evolution and the latitudinal diversity gradient: Speciation, extinction and biogeography. Ecol. Lett. **10**(4): 315–331. doi:10.1111/j.1461-0248.2007.01020.x.

Mittell, E.A., Nakagawa, S., and Hadfield, J.D. 2015. Are molecular markers useful predictors of adaptive potential? Ecol. Lett. **18**(8): 772–778. doi:10.1111/ele.12454.

Nabholz, B., Glémin, S., and Galtier, N. 2008a. Strong variations of mitochondrial mutation rate across mammals - The longevity hypothesis. Mol. Biol. Evol. **25**(1): 120–130. doi:10.1093/molbev/msm248.

Nabholz, B., Mauffrey, J.F., Bazin, E., Galtier, N., and Glemin, S. 2008b. Determination of mitochondrial genetic diversity in mammals. Genetics **178**(1): 351–361. doi:10.1534/genetics.107.073346.

Nevo, E., Beiles, A., and Ben-Shlomo, R. 1984. The evolutionary significance of genetic diversity: ecological, demographic, and life history correlates. *In* Lecture Notes in Biomathematics vol 53, Evolutionary Dynamics of Genetic Diversity. *Edited by* G.S. Mani. Springer-Verlag Berlin Heidelberg. pp. 13–213. doi:10.1007/978-3-642-51588-0.

Pontarp, M., Bunnefeld, L., Cabral, J.S., Etienne, R.S., Fritz, S.A., Gillespie, R., Graham, C.H., Hagen, O., Hartig, F., Huang, S., Jansson, R., Maliet, O., Münkemüller, T., Pellissier, L., Rangel, T.F., Storch, D., Wiegand, T., and Hurlbert, A.H. 2019. The latitudinal diversity gradient: novel understanding through mechanistic eco-evolutionary models. Trends Ecol. Evol. **34**(3): 211–223. doi:10.1016/j.tree.2018.11.009.

Rubinoff, D., and Holland, B.S. 2005. Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Syst. Biol.

54(6): 952–961. doi:10.1080/10635150500234674.

Schmidt, C., Domaratzki, M., Kinnunen, R.P., Bowman, J., and Garroway, C.J. 2020a. Continent-wide effects of urbanization on bird and mammal genetic diversity. Proc. R. Soc. B Biol. Sci. **287**(1920): 20192497. doi:10.1098/rspb.2019.2497.

Schmidt, C., Dray, S., and Garroway, C.J. 2020b. Genetic and species-level biodiversity patterns are linked by demography and ecological opportunity. bioRxiv. doi:10.1101/2020.06.03.132092.

Soulé, M.E. 1976. Allozyme variation, its determinants in space and time. *Edited by* F.J. Ayala. Sinauer Associates, Sunderland, Massachusetts. pp. 60–77.

Stearns, S.C. 1992. The Evolution of Life Histories. Oxford University Press, New York.

Theodoridis, S., Fordham, D.A., Brown, S.C., Li, S., Rahbek, C., and Nogues-Bravo, D. 2020. Evolutionary history and past climate change shape the distribution of genetic diversity in terrestrial mammals. Nat. Commun. **11**(1): 2557. doi:10.1038/s41467-020-16449-5.

Zink, R.M., and Barrowclough, G.F. 2008. Mitochondrial DNA under siege in avian phylogeography. Mol. Ecol. **17**(9): 2107–2121. doi:10.1111/j.1365-294X.2008.03737.x.

Chapter 8: Discussion

The study of population genetic responses to human land transformation provides insights into evolutionary responses to rapid environmental change in a conservation-relevant context. By integrating population-level genetic data with publicly available environmental, census, and biological datasets, I have taken a generalizable approach to test the effects of human activity on genetic diversity and species richness across terrestrial vertebrates. My thesis contributes to a cohesive understanding of how human-caused environmental change drives evolutionary change across multiple biological scales. This work suggests that although genetic diversity exhibits broad spatial structure, it is not as consistent latitudinally or across taxonomic groups as the species richness gradient (Worm and Tittensor 2018). In addition, it is clear that contemporary environmental change, notably human land use, is altering historical patterns of genetic diversity.

Broad-scale patterns of neutral nuclear genetic diversity in North American mammals are surprisingly well-predicted by body size and just two environmental factors: energy availability and heterogeneity (Chapter 5). These results underscore the central importance of demography in determining both genetic diversity and species richness in communities. The roles of population processes are not often considered at genetic and species levels (Lowe et al. 2017; Pontarp et al. 2019), despite the existence of a conceptual framework for considering how the four evolutionary processes (genetic drift, gene flow, selection, and mutation) can similarly be applied at the community level (Vellend and Geber 2005). Indeed, the disconnect between these disciplines is long-known: “the fusion of population genetics with population ecology can be compared to a prearranged marriage between partners who speak different languages” (Roughgarden 1979). Integrating population genetics and evolutionary perspectives with macroecology has the potential to substantially enhance our mechanistic understanding of processes underlying biogeographic patterns (Lowe et al. 2017; Pontarp et al. 2019).

Conservation of genetic diversity

Species richness and genetic diversity appear to be jointly shaped by environmental factors in mammals, which is starting ground on which to build conservation and management policies to conserve both of these fundamental levels of biodiversity at once. Indeed, it is likely that setting protected areas in species diversity hotspots will also conserve groups of small, but genetically differentiated populations. Thus this type of action is beneficial for the conservation of beta—but not alpha—genetic diversity. Beta diversity, or variation in species or genetic composition between sites, is important for regional conservation consideration because it represents the accumulation of diversity across different landscapes (Socolar et al. 2016). Beta diversity can inform the design of protected area networks with complementary genetic or species compositions, which ensures that the biodiversity within an area is more than the sum of its alpha diversities (Bush et al. 2016). However, as noted in Chapter 7, if the aim is to preserve additive genetic variation to maximize adaptive potential, alternative management options will be needed such as captive breeding programs or targeted gene flow (Macdonald et al. 2017). In these ways, broad-scale maps of genetic diversity have great potential for wider integration into conservation policy. Understanding the relationships between species richness and genetic diversity and how they are affected by environments can inform management practices in data-poor regions where genetic monitoring is not widespread. Such maps also provide opportunities to consider genetic diversity more deeply for a wider variety of species than is currently done at present (Hoban et al. 2020).

Macrogenetics beyond conservation

Macrogenetics and data synthesis present an opportunity to test general principles in ecology and evolution. Oftentimes, results from single-species studies undertaken at limited spatial scales may not be generalizable, or weak effects may undetectable. Macrogenetics approaches address both of these issues. By combining data from several species, detectable effects are more likely to be generalizable across a given taxonomic group (mammals, for example). Furthermore, using hierarchical models allowing random slopes to vary is a particularly powerful statistical approach in this context. The typical

benefit of including random effects in regression models is shrinkage (McElreath 2015; Harrison et al. 2018)—which allows the levels of a random effect to share information and borrow strength from one another. For example: in contrast to fitting independent regressions per species in a dataset, as a random effect, we assume that species were sampled from a common statistical population. Thus, the distribution of species-specific effects in a random slope model shrink towards the overall mean across species, which makes parameter estimates more robust. It is considered best practice to always model both random slopes and intercepts in regressions (Harrison et al. 2018), but this is often not possible because such models require large sample sizes within and across levels of a random factor. This is less of a concern for macrogenetics studies (Blanchet et al. 2017; Lawrence et al. 2019; Schmidt et al. 2020a)—increasing power to detect weak effects. Macrogenetics is therefore a useful tool to explore research questions at broad taxonomic and spatial scales and produce generalizable inferences. That being said, the strength of macrogenetics relies on data from small-scale or species-specific studies, and this approach is less applicable for questions addressing local, fine-scale processes.

Macrogenetics has added to our understanding of how life history and ecological traits (such as body size or trophic level) contribute to different levels of genetic variation across species (Soulé 1976; Loveless and Hamrick 1984; Nevo et al. 1984; Romiguier et al. 2014); how movement ability affects population structure in animals (Hillman et al. 2014; Medina et al. 2018); the relationship between genetic diversity and population size in natural populations (nuclear: Frankham 1995a, 1996; mitochondrial: Bazin et al. 2006); and the relationships between genetic diversity, conservation status, and adaptive potential (Spielman et al. 2004; Doyle et al. 2015; Mittell et al. 2015). More recently, efforts to improve metadata associated with archived datasets (e.g., including sample coordinates) has opened the door to asking spatially and temporally explicit macrogenetics questions such as those addressed here, including the effects of urbanization on genetic composition (Miraldo et al. 2016; Leigh et al. 2019; Millette et al. 2020; Schmidt et al. 2020a; Theodoridis et al. 2020) and its relationships with climate and species richness (Miraldo et al. 2016; Manel et al. 2020; Millette et al. 2020; Schmidt et al. 2020b; Theodoridis et al.

2020). Given difficulty defining and measuring adaptive genetic variation (Ellegren and Galtier 2016), thus far macrogenetics has largely focused on patterns of putatively neutral genetic diversity. However, attention is turning towards diversity within functional genes as well (Yiming et al. 2020).

The importance of open data

Evolutionary biology was among the first disciplines to embrace open data, particularly due to the recognized potential for genetic data to be reused for new purposes (Whitlock et al. 2010). GenBank, for example, was launched in 1982 and is currently home to $>4^{10}$ mitochondrial DNA sequences. Data sharing is now mandatory in many leading ecology and evolution journals since the Joint Data Archiving Policy was adopted in 2011 (Whitlock et al. 2010; Whitlock 2011). Yet, data available in public repositories are often incomplete or poorly documented (Michener 2015; Pope et al. 2015; Roche et al. 2015; Culina et al. 2020), rendering a not insignificant portion of datasets unusable. For example, in building the database used in this work, ~11% of datasets were excluded only due to missing metadata. Over 85% of sequences archived in GenBank are not associated with coordinates (Miraldo et al. 2016). There are distinct advantages of repurposing raw data compared to more traditional meta-analyses techniques (e.g., the ability to consistently calculate values of interest, or avoid merging results from different marker types). With the now considerable interest in repurposing raw data, comprehensive metadata and open code are necessary to ensure their longevity. Improving data stewardship will push the door wide open for macrogenetics and data synthesis in other fields, giving us unprecedented opportunities to test big-picture questions, at broad spatiotemporal scales, with better resolution.

References

Bazin, E., Glémin, S., and Galtier, N. 2006. Population size does not influence mitochondrial genetic diversity in animals. Science. **312**(5773): 570–572. doi:10.1126/science.1122033.

Blanchet, S., Prunier, J.G., and De Kort, H. 2017. Time to Go Bigger: Emerging Patterns in Macrogenetics. Trends Genet. **33**(9): 579–580. doi:10.1016/j.tig.2017.06.007.

Bush, A., Harwood, T., Hoskins, A.J., Mokany, K., and Ferrier, S. 2016. Current Uses of Beta-Diversity in Biodiversity Conservation: A response to Socolar et al. Trends Ecol. Evol. **31**(5): 337–338. doi:10.1016/j.tree.2016.02.020.

Culina, A., van den Berg, I., Evans, S., and Sánchez-Tójar, A. 2020. Low availability of code in ecology: A call for urgent action. PLoS Biol. **18**(7): 1–9. doi:10.1371/journal.pbio.3000763.

Doyle, J.M., Hacking, C.C., Willoughby, J.R., Sundaram, M., and DeWoody, J.A. 2015. Mammalian Genetic Diversity as a Function of Habitat, Body Size, Trophic Class, and Conservation Status. J. Mammal. **96**(3): 564–572. doi:10.1093/jmammal/gyv061.

Ellegren, H., and Galtier, N. 2016. Determinants of genetic diversity. Nat. Rev. Genet. **17**(7): 422–433. doi:10.1038/nrg.2016.58.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: A review. Genet. Res. **66**: 95–107. doi:10.1017/S0016672308009695.

Frankham, R. 1996. Relationship of Genetic Variation to Population Size in Wildlife. Conserv. Biol. **10**(6): 1500–1508. doi:10.1046/j.1523-1739.1996.10061500.x.

Harrison, X.A., Donaldson, L., Correa-Cano, M.E., Evans, J., Fisher, D.N., Goodwin, C.E.D., Robinson, B.S., Hodgson, D.J., and Inger, R. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ **6**: e4794. doi:10.7717/peerj.4794.

Hillman, S.S., Drewes, R.C., Hedrick, M.S., and Hancock, T. V. 2014. Physiological vagility and its relationship to dispersal and neutral genetic heterogeneity in vertebrates. J. Exp. Biol. **217**(18): 3356–3364. doi:10.1242/jeb.105908.

Hoban, S., Bruford, M., D'Urban Jackson, J., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P.A., Paz-Vinas, I., Sjögren-Gulve, P., Segelbacher, G., Vernesi, C., Aitken, S., Bertola, L.D.,

Bloomer, P., Breed, M., Rodríguez-Correa, H., Funk, W.C., Grueber, C.E., Hunter, M.E., Jaffe, R., Liggins, L., Mergeay, J., Moharrek, F., O'Brien, D., Ogden, R., Palma-Silva, C., Pierson, J., Ramakrishnan, U., Simo-Droissart, M., Tani, N., Waits, L., and Laikre, L. 2020. Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. Biol. Conserv. **248**: 108654. doi:10.1016/j.biocon.2020.108654.

Lawrence, E.R., Benavente, J.N., Matte, J.-M., Marin, K., Wells, Z.R.R., Bernos, T.A., Krasteva, N., Habrich, A., Nessel, G.A., Koumrouyan, R.A., and Fraser, D.J. 2019. Geo-referenced population-specific microsatellite data across American continents, the MacroPopGen Database. Sci. Data **6**(1): 14. doi:10.1038/s41597-019-0024-7.

Leigh, D.M., Hendry, A.P., Vázquez-Domínguez, E., and Friesen, V.L. 2019. Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. Evol. Appl. **12**(8): 1505–1512. doi:10.1111/eva.12810.

Loveless, M., and Hamrick, J. 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. **51**: 65–95.

Lowe, W.H., Kovach, R.P., and Allendorf, F.W. 2017. Population genetics and demography unite ecology and evolution. Trends Ecol. Evol. **32**(2): 141–152. doi:10.1016/j.tree.2016.12.002.

Macdonald, S.L., Llewelyn, J., Moritz, C., and Phillips, B.L. 2017. Peripheral Isolates as Sources of Adaptive Diversity under Climate Change. Front. Ecol. Evol. **5**: 1–10. doi:10.3389/fevo.2017.00088.

Manel, S., Guerin, P.E., Mouillot, D., Blanchet, S., Velez, L., Albouy, C., and Pellissier, L. 2020. Global determinants of freshwater and marine fish genetic diversity. Nat. Commun. **11**(1): 1–9. Springer US. doi:10.1038/s41467-020-14409-7.

McElreath, R. 2015. Statistical rethinking: a bayesian course with examples in R and Stan. CRC press.

Medina, I., Cooke, G.M., and Ord, T.J. 2018. Walk, swim or fly? Locomotor mode predicts

genetic differentiation in vertebrates. Ecol. Lett. **21**(5): 638–645. doi:10.1111/ele.12930.

Michener, W.K. 2015. Ecological data sharing. Ecol. Inform. **29**(P1): 33–44. doi:10.1016/j.ecoinf.2015.06.010.

Millette, K.L., Fugère, V., Debyser, C., Greiner, A., Chain, F.J.J., and Gonzalez, A. 2020. No consistent effects of humans on animal genetic diversity worldwide. Ecol. Lett. **23**(1): 55–67. doi:10.1111/ele.13394.

Miraldo, A., Li, S., Borregaard, M.K., Florez-Rodriguez, A., Gopalakrishnan, S., Rizvanovic, M., Wang, Z., Rahbek, C., Marske, K.A., and Nogues-Bravo, D. 2016. An Anthropocene map of genetic diversity. Science. **353**(6307): 1532–1535. doi:10.1126/science.aaf4381.

Mittell, E.A., Nakagawa, S., and Hadfield, J.D. 2015. Are molecular markers useful predictors of adaptive potential? Ecol. Lett. **18**(8): 772–778. doi:10.1111/ele.12454.

Nevo, E., Beiles, A., and Ben-Shlomo, R. 1984. The evolutionary significance of genetic diversity: ecological, demographic, and life history correlates. *In* Lecture Notes in Biomathematics vol 53, Evolutionary Dynamics of Genetic Diversity. *Edited by* G.S. Mani. Springer-Verlag Berlin Heidelberg. pp. 13–213. doi:10.1007/978-3-642-51588-0.

Pontarp, M., Bunnefeld, L., Cabral, J.S., Etienne, R.S., Fritz, S.A., Gillespie, R., Graham, C.H., Hagen, O., Hartig, F., Huang, S., Jansson, R., Maliet, O., Münkemüller, T., Pellissier, L., Rangel, T.F., Storch, D., Wiegand, T., and Hurlbert, A.H. 2019. The latitudinal diversity gradient: novel understanding through mechanistic eco-evolutionary models. Trends Ecol. Evol. **34**(3): 211–223. doi:10.1016/j.tree.2018.11.009.

Pope, L.C., Liggins, L., Keyse, J., Carvalho, S.B., and Riginos, C. 2015. Not the time or the place: The missing spatio-temporal link in publicly available genetic data. Mol. Ecol. **24**(15): 3802–3809. doi:10.1111/mec.13254.

Roche, D.G., Kruuk, L.E.B., Lanfear, R., and Binning, S.A. 2015. Public Data Archiving in Ecology and Evolution: How Well Are We Doing? PLoS Biol. **13**(11): 1–12.

doi:10.1371/journal.pbio.1002295.

Romiguier, J., Gayral, P., Ballenghien, M., Bernard, A., Cahais, V., Chenuil, A., Chiari, Y., Dernat, R., Duret, L., Faivre, N., Loire, E., Lourenco, J.M., Nabholz, B., Roux, C., Tsagkogeorga, G., Weber, A.A.T., Weinert, L.A., Belkhir, K., Bierne, N., Glémin, S., and Galtier, N. 2014. Comparative population genomics in animals uncovers the determinants of genetic diversity. Nature **515**(7526): 261–263. doi:10.1038/nature13685.

Roughgarden, J. 1979. Theory of population genetics and evolutionary ecology: an introduction. Prentice Hall, Upper Saddle River, NJ.

Schmidt, C., Domaratzki, M., Kinnunen, R.P., Bowman, J., and Garroway, C.J. 2020a. Continent-wide effects of urbanization on bird and mammal genetic diversity. Proc. R. Soc. B Biol. Sci. **287**(1920): 20192497. doi:10.1098/rspb.2019.2497.

Schmidt, C., Dray, S., and Garroway, C.J. 2020b. Genetic and species-level biodiversity patterns are linked by demography and ecological opportunity. bioRxiv. doi:10.1101/2020.06.03.132092.

Socolar, J.B., Gilroy, J.J., Kunin, W.E., and Edwards, D.P. 2016. How Should Beta-Diversity Inform Biodiversity Conservation? Trends Ecol. Evol. **31**(1): 67–80. Elsevier Ltd. doi:10.1016/j.tree.2015.11.005.

Soulé, M. 1976. Allozyme variation: its determinants in space in time. *In* Molecular Evolution. Sinauer Associates, Sunderland, MA. pp. 60–77.

Spielman, D., Brook, B.W., and Frankham, R. 2004. Most species are not driven to extinction before genetic factors impact them. Proc. Natl. Acad. Sci. U. S. A. **101**(42): 15261–15264. doi:10.1073/pnas.0403809101.

Theodoridis, S., Fordham, D.A., Brown, S.C., Li, S., Rahbek, C., and Nogues-Bravo, D. 2020. Evolutionary history and past climate change shape the distribution of genetic diversity in terrestrial mammals. Nat. Commun. **11**(1): 2557. doi:10.1038/s41467-020-16449-5.

Vellend, M., and Geber, M.A. 2005. Connections between species diversity and genetic diversity. Ecol. Lett. **8**(7): 767–781. doi:10.1111/j.1461-0248.2005.00775.x.

Whitlock, M.C. 2011. Data archiving in ecology and evolution: Best practices. Trends Ecol. Evol. **26**(2): 61–65. doi:10.1016/j.tree.2010.11.006.

Whitlock, M.C., McPeek, M.A., Rausher, M.D., Rieseberg, L., and Moore, A.J. 2010. Data Archiving. Am. Nat. **175**(2): 145–146. doi:10.1086/650340.

Worm, B., and Tittensor, D.P. 2018. A theory of global biodiversity. Princeton University Press, Princeton, New Jersey.

Yiming, L., Siqi, W., Chaoyuan, C., Jiaqi, Z., Supen, W., Xianglei, H., Xuan, L., Xuejiao, Y., and Xianping, L. 2020. Latitudinal gradients in genetic diversity and natural selection at a highly adaptive gene in terrestrial mammals. Ecography: 1–13. doi:10.1111/ecog.05082.

Appendix

Supplementary Information 2

Continent-wide effects of urbanization on bird and mammal genetic diversity

Contents:

Methods

Microsatellite data compilation

Our dataset was comprised of bird and mammal microsatellite data collected from publicly archived, previously published work (Table S2). To create this dataset, we conducted two systematic searches of online databases (Figure S3). We obtained a list of species names for 859 birds and 450 mammals native to Canada and the United States from the IUCN Red List database which includes all species regardless of Red List status. We then queried the Dryad Digital Repository in February 2018 using a python script with the following search terms: species name (e.g. "*Branta canadensis*"), "microsat*", "single tandem*", "short tandem*", and "str". This search yielded 194 unique data packages associated with papers. A second search was performed in May 2018, this time querying DataOne.org, a network which provides access to data from multiple repositories such as Dryad, the Knowledge Network for Biocomplexity (KNB), and the United States Geographic Survey (USGS). This search was conducted in R using the dataone package (Jones et al. 2017), a convenient method of querying the DataOne network. Using identical keywords, 237 unique results were generated, 121 of which overlapped with our first search (Figure S3, Data S1).
All data sets were then individually screened for suitability, ensuring: location (Canada and the United States), taxon (native birds and terrestrial mammals), data type (neutral microsatellite markers), and georeferenced sampling (coordinates, maps, or place names). Studies with other factors which may have influenced genetic diversity (e.g. island sites, genetic rescue, translocation, managed or captive populations) were excluded. In total, data from 85 studies were retained for analysis. In a final step, we assured individual sample sites within datasets adhered to our study criteria, and removed those which did not. We maintained the same sample site delineations as in the original work. Criteria for removal from a dataset included island, managed, or captive populations; sites outside of Canada and the United States; and historical samples (where identified). Sites for which we were unable to extract geographic information were also removed, as well as sites with <5 individuals. Any non-neutral microsatellite markers in the data were removed. We note that we compiled all datasets regardless of their location in urban or rural areas, because we determined the level of human disturbance for individual sites using our own criteria

which could be consistently applied across all datasets (see "Measures of urbanization and human presence" below). Finally, unique names were assigned to each site, and all datasets were formatted as either STRUCTURE or GENEPOP files and read into R version 3.4.2 (R Core Team 2013) using the adegenet package (Jombart et al. 2017).

Geographic site locations

Geographic coordinates provided by the authors were used when available (Table S2). Where spatial location was available for each individual sampled, coordinates were averaged. If site names were provided (e.g. "Yellowstone National Park") with no coordinate reference, we performed a Google Maps search and noted the resulting coordinates. Where applicable, coordinate information was obtained by searching for site names in the Geographic Names Information System (GNIS) or GeoNames database, as was the case for a few datasets from the USGS. In instances where only maps of sampling sites were available, site coordinates were extracted using a reference map in ArcMap version 10.3.1 (ESRI). When georeferencing map images, if sampling locations indicated regions rather than single points, centroid coordinates served as the site location. Centroid coordinates were also calculated as site location for data accompanied by polygon shapefiles as a spatial reference. All coordinates were recorded using the WGS84 (World Geodetic System 1984) coordinate system in decimal degrees, and transformed from other systems or map projections in ArcMap as needed. Finally, when site locations were offshore (42 sites), points were moved to the nearest terrestrial location using the Generate Near Table tool in ArcGIS. Offshore sites (those located in bodies of water) were moved to avoid generating null values for population density and the Human Footprint Index—both of which are high-resolution terrestrial maps which do not extend far past the coast. In some instances, offshore sites were recorded as thus in the original publication, while other times they were generated during the process of obtaining a single location for a site (e.g. the average location of individual coordinates, or centroid location, was in a body of water). Polar bear sites in the Arctic Archipelago constituted half of all offshore sites, while the remainder were coastal species and species sampled near lakes or oceans.

Genetic diversity estimates

We chose to measure gene diversity (Nei 1973) and allelic richness for each site as measures of genetic diversity. Gene diversity uses allele frequencies to determine the probability that pairs of alleles drawn at random from a population are different, and accounts for both the number and evenness of alleles. This measure is minimally affected by sample size and rare alleles (Charlesworth and Charlesworth 2010), and thus is convenient to use when sample sizes are variable, as is the case here. Gene diversity was calculated using the adegenet package (Jombart et al. 2017). Allelic richness, the number of alleles per locus, is strongly influenced by sample size and effective population size. To account for differences in sample size, we used rarefaction as employed in the R package hierfstat (Goudet and Jombart 2015) to standardize allele counts to the minimum sample size (n = 5 individuals) across sites (Leberg 2002). Values were then averaged across loci to obtain a single value per site.

Effective population size estimates

We estimated contemporary effective population sizes at each sites using the linkage disequilibrium method for single samples implemented in the software NeEstimator 2.1 (Do et al. 2014). The presence of rare alleles produces an upward bias when estimating effective population size which is especially apparent at small sample sizes (Waples and Do 2008). We therefore set a conservative exclusion threshold (P_{crit}) of 0.1, meaning estimates were made based only on alleles with frequencies higher than this value, which has been shown to markedly reduce bias (Waples and Do 2008). Linkage disequilibrium methods work well for estimating effective population sizes in small populations, however are less reliable for large populations (Waples and Do 2010). An estimate of infinity is returned when sampling error swamps detectable signals of genetic drift—which may be the case if too few individuals or loci were sampled to yield any useful information about effective population size. In these instances, rather than replacing infinity values with arbitrary large values, we chose to exclude all sites for which we were unable to estimate effective population size (Table S2.1).

Population-specific F_{ST}

To estimate levels of population differentiation in relation to human disturbance, we measured population-specific F_{ST} (Weir and Goudet 2017). Population-specific F_{ST} characterizes differentiation using the proportion of pairs of matching alleles within populations (the probability of identity by descent) relative to that of pairs from different populations. It can be interpreted as a measure of how far single populations have diverged from a common ancestor population. This measure differs from pairwise F_{ST} estimates (Weir and Cockerham 1984) in that it provides a measure of population differentiation for a single population, as opposed to a single value for population pairs. Using a population-specific estimator of structure allows us to make comparisons between populations of different species. Population-specific F_{ST} was calculated in R using hierfstat (Goudet and Jombart 2015), and values were averaged across loci. Because population-specific F_{ST} calculations still use comparisons of pairs of alleles between populations, it could only be measured for species with two or more sample sites. Sample size was slightly decreased when this condition was not met (Table S2.1).

Measures of urbanization and human presence

Urban-rural classification. Our next step was to define urban habitats in North America. The United States Census Bureau and Statistics Canada provide publicly available maps of urban areas and population centers, respectively (Statistics Canada 2016; U.S. Census Bureau 2016). According to the US Census Bureau, an urban area is defined as any densely developed territory with at least 2500 inhabitants. Statistics Canada defines a population center as any area with a minimum population of 1000, and a population density of 400 persons or more per square kilometer. We considered these international designations of urbanization to be comparable. Canadian and American urban area maps were downloaded as polygon GIS layers and merged into a single layer. Site coordinates were transformed from WGS84 to the same projection as the urban area maps (GCS North American 1983) in ArcMap to ensure correct alignment. A spatial join was then performed between sites and the urban area layer in order to classify sample locations as “urban” or “nonurban”. The search radius parameter was set to 10 km to encompass the entire urban

gradient, and account for sprawl. Periurban landscapes which are adjacent to cities may be less densely inhabited, however often encompass areas highly managed or disturbed by humans including farmland, parks, and golf courses; in larger cities, periurban landscapes may extend up to 10 km away from the city center (Clergeau et al. 2001). Thus, any site located in, or within 10 km of, an urban area was considered "urban" for the purposes of this study.

Human population density. Human population density was used as a proxy of urbanization and human effects on the environment. In contrast to our binary urban-rural designation of sample sites, human population reflects the continuous distribution of the effects of human presence, and thus should indicate the intensity of the effects of human activity on genetic diversity. A raster map of global population density per square kilometer was obtained for the most recent available year (2000) from NASA's Center for Near Earth Object Studies (https://neo.sci.gsfc.nasa.gov/view.php?datasetId=SEDAC_POP). Next, the raster map and shapefile containing sites as point features were read into R (package rgdal and raster; Bivand et al. 2017, Hijmans 2017). Mean population density was calculated within a 10 km buffer zone around each site.

Human Footprint Index. The Global Human Footprint Index (Sanderson et al. 2002; WCS and CIESIN 2005) quantifies human influence on a scale of 0 (most wild) to 100 (most transformed) at a 1 km^2 resolution. It provides a more comprehensive assessment of the effects of humans than urban-rural designations or population density alone because it incorporates data from multiple sources of land use. In particular, it captures human population density, human land use and infrastructure (built-up areas, nighttime lights, land use, and land cover), and human access (coastlines, roads, railways, and navigable rivers). As with the raster map of population density, the Human Footprint Index was imported to R and values per site (within a 10 km buffer zone) calculated using the same method.

Statistical analysis

We modelled birds and mammals separately because we expected them to respond to human disturbance in fundamentally different ways. Within birds, we further classified

each species as migratory or non-migratory using information from species accounts in The Birds of North America (Rodewald 2015). We then created a separate data subset comprised of only non-migratory species which was analyzed in parallel. Species with a mix of migratory and resident populations were counted as migratory and excluded, as were species with unknown migratory behavior.

Genetic diversity is also affected by regional historical contingencies which would be difficult to specifically identify without detailed knowledge of each species and region in our data set (Shafer et al. 2010). Such events will, however, produce spatial patterns in our genetic measures. These spatial patterns are detectable and can be controlled for—even if their causes are unknown—using distance-based Moran's Eigenvector Maps (dbMEMs) (Borcard et al. 2004; Dray et al. 2006; Manel et al. 2010). The dbMEM analysis we used (R package adespatial (Dray et al. 2017)) is a type of eigenanalysis based on principal coordinates analysis which produces a set of spatially explicit variables, dbMEMs, that quantify spatial trends at multiple scales. Because they are orthogonal, dbMEMs can subsequently be included in regression analyses to explicitly model spatial patterns (Dray et al. 2017). In the first steps of dbMEM analysis, a modified matrix of distances between pairs of sites is calculated from site coordinates. The eigenvalues of this matrix are proportional to Moran's I coefficients of spatial autocorrelation (Moran 1948; Dray et al. 2006). Importantly, only positive eigenvalues are considered because negative eigenvalues generate complex principal coordinate axes (Borcard and Legendre 2002). dbMEMs therefore correspond to positive values of Moran's I, and can account for positive spatial autocorrelation present in the data. Positive spatial autocorrelation occurs when sites nearer to each other are more similar than sites further away, and violates the assumption of independence in our statistical tests. Before undertaking dbMEM, any linear trends in the response variables were removed. Although dbMEM analysis is capable of detecting linear spatial gradients, dbMEMs used to model such trends then cannot be used to recover other, potentially more interesting spatial patterns (Borcard et al. 2004). dbMEM analyses were run in parallel for measures of genetic diversity (gene diversity and allelic richness), population-specific F_{ST}, and effective population size. We were able to calculate gene

diversity and allelic richness for all sites, however, removed sites where effective population size was infinite and sites where population-specific F_{ST} could not be computed. To capitalize on available data, we created subsets for genetic diversity, population-specific F_{ST}, and effective population size, omitting rows where the focal variable(s) had null values. For each taxon we thus had 3 data subsets: one for gene diversity and allelic richness, which included all sites; population-specific F_{ST}; and effective population size (Table S2.1). To select dbMEMs for inclusion in regression analyses, we used forward selection with a p-value criterion (alpha = 0.05) in the SignifReg package (Kim and Zambom 2017).

Testing effects of human presence on genetic diversity. To test for the effects of human disturbance on genetic diversity, and to determine whether alternate proxies of urbanization would yield similar results, we constructed four linear mixed models per response variable (effective population size, gene diversity, allelic richness, and population-specific F_{ST}). Three of these models included spatial dbMEMs and a measure of human presence as explanatory variables: (1) urban-rural category, (2) human population density, and (3) Human Footprint Index. The fourth model consisted of dbMEMs only, or, where no dbMEMs were significant, was a null model (Table 2.1). Species was included as a random effect in all models to account for species-level variation in genetic diversity, effective population size, and population-specific F_{ST}. The random species effect also accommodated potential variation in the level of species' responses to human-caused environmental degradation (random slope models). Random effects account for non-independence of samples within groups and increase the accuracy of parameter estimation (Harrison et al. 2018). We fit these models in a Bayesian framework using the R package brms (Bürkner 2019) which fits models using Stan. We used default priors (uniform distribution over all real numbers) for parameter estimates with 4000 iterations after discarding warm-up runs (1000 iterations). In cases where models did not converge, we first increased the number of iterations or warmup period (*mammals:* allelic richness ~ population density: 5000 iterations, 5000 warmup; *birds:* F_{ST} ~ population density, 5000 iterations, 4000 warmup; *non-migratory birds:* allelic richness ~ urban category, 4000 iterations, 2000 warmup; allelic richness ~ population density, 4000 iterations, 4000

warmup; F_{ST} ~ population density, 4000 iterations, 2000 warmup). If convergence issues persisted we restricted priors to a uniform distribution bounded at -10 and 10 (birds F_{ST} ~ population density and non-migratory, and birds allelic richness ~ population density). Lastly, we computed marginal and conditional Bayesian R^2 to evaluate and compare model fits using the performance package (Lüdecke et al. 2019).

Results

Data Summary

Our final dataset included 1,008 sites consisting of 66 species (41 mammals and 25 birds; Fig. 2.2, Table S2.1). There were 812 mammal sample sites, and 196 bird sample sites (129 non-migratory), with more rural than urban sites (Table S2.1). The data included samples from a total of 41,023 individuals. Minimum group size for both classes was set to 5 individuals, and there was a maximum of 2444 individuals from a single sample site for mammals (median = 26 individuals), 602 individuals (median = 19 individuals) from a single sample site among all birds, and 141 individuals (median = 19 individuals) for the non-migratory bird subset.

The number of loci sampled ranging between 5 – 210 loci with a median of 13. For all birds (both migratory and non-migratory), the median number of loci sampled was 11 with a range of 6 – 30; non-migratory birds also had a median number of 11 loci with a range between 6 – 20 loci. Gene diversity (mean ± SD) was slightly higher in mammals (0.72 ± 0.11), compared to birds (all: 0.63 ± 0.13; non-migratory: 0.67 ± 0.09). Allelic richness (mean ± SD, max) was similar between mammals (4.79 ± 1.28, 13.70) and birds (all: 4.59 ± 2.19, 22.64; non-migratory: 5.01 ± 2.41, 22.64).

We obtained estimates of effective population size for 639 mammal sites, 125 sites across all birds, and for 87 non-migratory bird sites (Table S2.1). Effective population sizes (mean ± SD) were on average lower in mammals (614.52 ± 8275.46) compared to birds (all: 980.30 ± 9400.30; non-migratory: 1314.92 ± 11267.02). This corresponded to higher average population-specific F_{ST} among mammals (0.06 ± 0.09) relative to birds (all: 0.04 ±

0.06; non-migratory: 0.04 ± 0.04). F_{ST} was estimated at 796 sites for mammals, 190 for all birds, and 128 for non-migratory birds.

Effective population size sample sizes

Mammals. We were able to obtain estimates of effective population size for 639 out of 812 mammal sites, with all 41 species represented except for one (moose, *Alces alces*, 2 sites). The ratio of urban to rural sites for sites with non-infinity estimates was unchanged with respect to the full mammal subset (0.44). Additionally, the distribution of sites across values of human population density and the Human Footprint Index did not suggest any bias after removing sites with infinite effective population size estimates (Fig. S4).

Non-migratory Birds. Out of 129 sites for non-migratory birds we had 87 non-infinite values. Again, the ratio of urban to rural sites sampled remained consistent with the overall subset of non-migratory birds (0.74), and we saw no indication of bias with regard to human population density or the Human Footprint Index (Fig. S4).

Spatial autocorrelation

We found spatial patterns underlying the distribution of genetic diversity in both mammals and birds. dbMEMs capture spatial patterns at all scales in the data, starting broadly (dbMEM 1) and progressing towards increasingly finer scales. In general, we noted more spatial patterns, and more patterns at finer scales in mammals for all response variables. In mammals, following stepwise regression, 5 dbMEMs were significantly related to effective population size (dbMEMs 2, 27, 80, 93, 101). Significant patterns were also found for genetic diversity: 13 dbMEMs were significantly related to gene diversity (dbMEMs 2, 4, 5, 11, 22, 30, 31, 32, 47, 49, 102, 143, 193), and 21 to allelic richness (dbMEMs 2, 4, 5, 7, 8, 11, 12, 13, 21, 22, 29, 30, 31, 32, 47, 49, 102, 108, 143, 185, 190). Finally, we found 10 dbMEMs related to site -specific F_{ST} (dbMEMs 2, 10, 14, 27, 48, 70, 125, 127, 170, 197).

Patterns of spatial variation were less apparent in our subset of non-migratory birds. There were no significant dbMEMs for effective population size nor allelic richness. Gene diversity showed the most spatial variation, with 3 significant dbMEMs selected (dbMEMs 3, 6, 18). For site-specific FST, 2 dbMEMs were significant (dbMEMs 3, 6).

Among all birds, only 1 dbMEMs was significant for effective population size (dbMEM 2), 2 were retained for gene diversity (dbMEMs 2, 6), 1 for allelic richness (dbMEM 2) and1 (dbMEM 6) for site-specific F_{ST}.

All significant dbMEMs were incorporated into later models to account for spatial patterns of genetic diversity measures across North America.

References

Bivand, R., Keitt, T., and Rowlingson, B. 2017. rgdal: Bindings for the Geospatial Data Abstraction Library. Available from https://cran.r-project.org/package=rgdal.

Borcard, D., and Legendre, P. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecol. Modell. **153**(1–2): 51–68. doi:10.1016/S0304-3800(01)00501-4.

Borcard, D., Legendre, P., Avois-Jacquet, C., and Tuomisto, H. 2004. Dissecting the spatial structure of ecological data at multiple scales. Ecology **85**(7): 1826–1832.

Bürkner, P.-C. 2019. brms: Bayesian Regression Models using "Stan." Available from https://cran.r-project.org/package=brms.

Charlesworth, B., and Charlesworth, D. 2010. Elements of evolutionary genetics. Roberts & Company Publishers, Greenwood Village, Colorado, USA.

Clergeau, P., Jokimäki, J., and Savard, J.P.L. 2001. Are urban bird communities influenced by the bird diversity of adjacent landscapes? J. Appl. Ecol. **38**(5): 1122–1134. doi:10.1046/j.1365-2664.2001.00666.x.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J., and Ovenden, J.R. 2014. NeEstimator

v2: Re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Mol. Ecol. Resour. **14**(1): 209–214. doi:10.1111/1755-0998.12157.

Dray, S., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., and Wagner, H.H. 2017. adespatial: Multivariate Multiscale Spatial Analysis. Available from https://cran.r-project.org/package=adespatial.

Dray, S., Legendre, P., and Peres-Neto, P.R. 2006. Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol. Modell. **196**(3–4): 483–493. doi:10.1016/j.ecolmodel.2006.02.015.

Goudet, J., and Jombart, T. 2015. hierfstat: Estimation and Tests of Hierarchical F-Statistics. Available from https://cran.r-project.org/package=hierfstat.

Harrison, X.A., Donaldson, L., Correa-Cano, M.E., Evans, J., Fisher, D.N., Goodwin, C.E.D., Robinson, B.S., Hodgson, D.J., and Inger, R. 2018. A brief introduction to mixed effects modelling and multi-model inference in ecology. PeerJ **6**: e4794. doi:10.7717/peerj.4794.

Hijmans, R.J. 2017. raster: Geographic Data Analysis and Modeling. Available from https://cran.r-project.org/package=raster.

Jombart, T., Kamvar, Z.N., Collins, C., Lustrik, R., Beugin, M.-P., Knaus, B.J., Solymos, P., Mikryukov, V., Schliep, K., Maié, T., Morkovsky, L., Ahmed, I., Cori, A., Calboli, F., and Ewing, R.J. 2017. adegenet: Exploratory Analysis of Genetic and Genomic Data. Available from https://cran.r-project.org/package=adegenet.

Jones, M.B., Slaughter, P., Nahf, R., Boettiger, C., Jones, C., Read, J., Walker, L., Hart, E., and Chamberlain, S. 2017. dataone: R Interface to the DataONE REST API. Available from https://cran.r-project.org/package=dataone.

Kim, J., and Zambom, A.Z. 2017. SignifReg: Significant Variable Selection in Linear Regression. Available from https://cran.r-project.org/package=SignifReg.

Leberg, P.L. 2002. Estimating allelic richness: Effects of sample size and bottlenecks. Mol. Ecol. **11**: 2445–2449. doi:10.1046/j.1365-294X.2002.01612.x.

Lüdecke, D., Makowski, D., and Waggoner, P. 2019. performance: Assessment of Regression Models Performance. Available from https://cran.r-project.org/package=performance.

Manel, S., Poncet, B.N., Legendre, P., Gugerli, F., and Holderegger, R. 2010. Common factors drive adaptive genetic variation at different spatial scales in Arabis alpina. Mol. Ecol. **19**(17): 3824–3835. doi:10.1111/j.1365-294X.2010.04716.x.

Moran, P.A.P. 1948. The Interpretation of Statistical Maps. J. R. Stat. Soc. Ser. B **10**(2): 243–251. Available from https://www.jstor.org/stable/2983777.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U. S. A. **70**(12): 3321–3323. doi:10.1073/pnas.70.12.3321.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. Vienna, Austria. Available from http://www.r-project.org/.

Rodewald, P. (*Editor*). 2015. The Birds of North America. *In* August. Cornell Laboratory of Ornithology, Ithaca, NY. Available from https://birdsna-org.uml.idm.oclc.org.

Sanderson, E.W., Jaiteh, M., Levy, M.A., Redford, K.H., Wannebo, A. V., and Woolmer, G. 2002. The Human Footprint and the Last of the Wild. Bioscience **52**(10): 891. doi:10.1641/0006-3568(2002)052[0891:THFATL]2.0.CO;2.

Shafer, A.B.A., Cullingham, C.I., Côté, S.D., and Coltman, D.W. 2010. Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North America. Mol. Ecol. **19**(21): 4589–4621. doi:10.1111/j.1365-294X.2010.04828.x.

Statistics Canada. 2016. Population Centres Census Division Cartographic Boundary File. 2016 Census. Statistics Canada Catalogue no. 92-160-X. Available from https://www12.statcan.gc.ca/census-recensement/2011/geo/bound-limit/bound-limit-2016-eng.cfm.

U.S. Census Bureau. 2016. TIGER/Line Shapefiles: Urban Areas (machinereadable data

files). Available from https://www.census.gov/geo/maps-data/data/tiger-line.html.

Waples, R.S., and Do, C. 2008. LDNE: A program for estimating effective population size from data on linkage disequilibrium. Mol. Ecol. Resour. **8**(4): 753–756. doi:10.1111/j.1755-0998.2007.02061.x.

Waples, R.S., and Do, C. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evol. Appl. **3**(3): 244–262. Wiley/Blackwell (10.1111). doi:10.1111/j.1752-4571.2009.00104.x.

WCS, and CIESIN. 2005. Last of the Wild Project, Version 2, 2005 (LWP-2): Global Human Footprint Dataset (Geographic). NASA Socioeconomic Data and Applications Center (SEDAC), Palisades, NY. doi:10.7927/H4M61H5F.

Weir, B.S., and Cockerham, C.C. 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution. **38**(6): 1358–1370. doi:10.2307/2408641.

Weir, B.S., and Goudet, J. 2017. A unified characterization of population structure. Genetics **206**: 2085–2103.

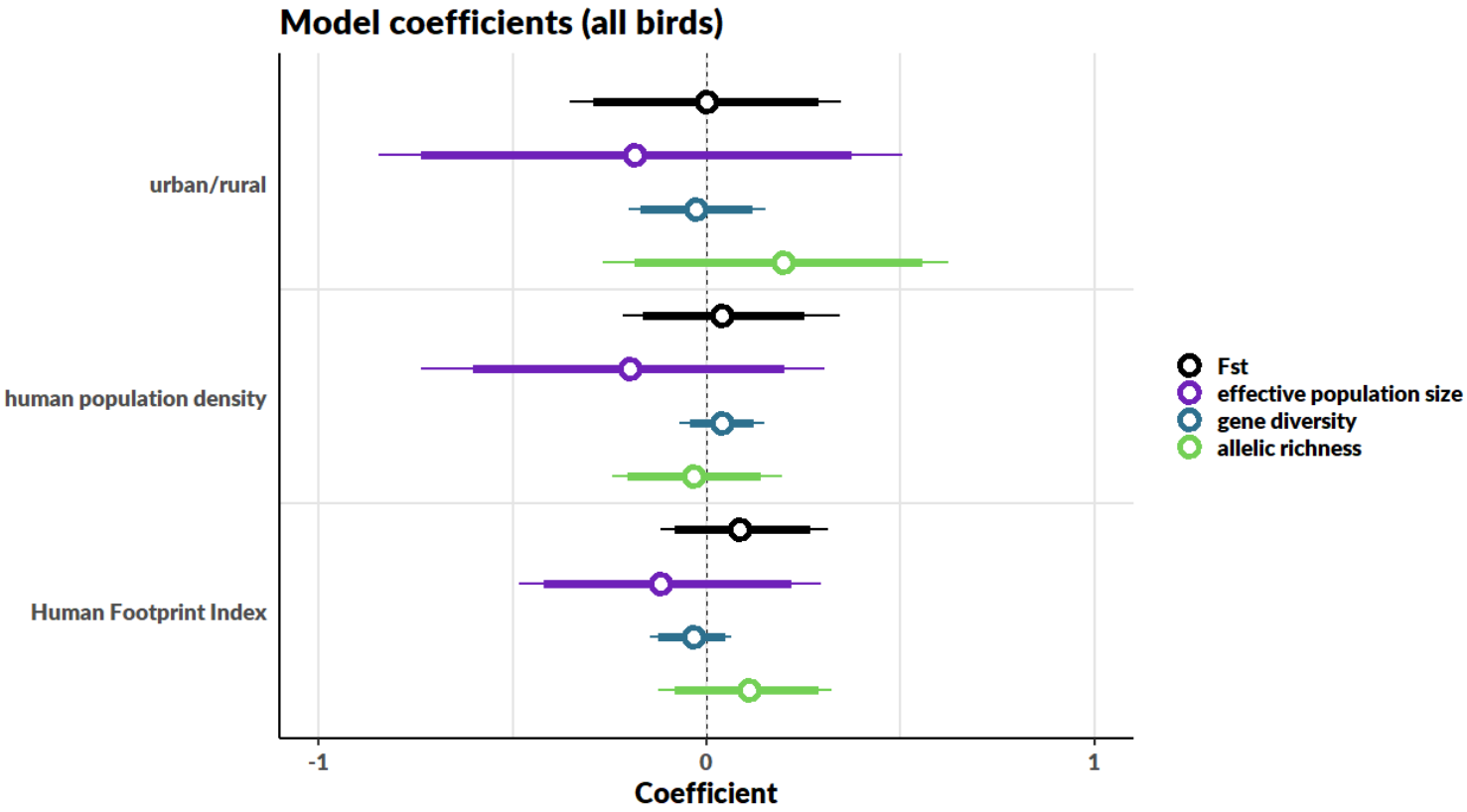


Figure S2.1.

Plotted model coefficients all birds (both migratory and non-migratory species). Open circles represent coefficient estimates, bold lines are 90% credible intervals, and narrow lines are 95% credible intervals. Intervals that overlap zero (dashed vertical line) indicate the disturbance variable has no effect on the response variable. Sample size differed between variables due to limitations estimating effective population size population-specific F_{ST}. Sample sizes for each variable are given in Table S2.1.

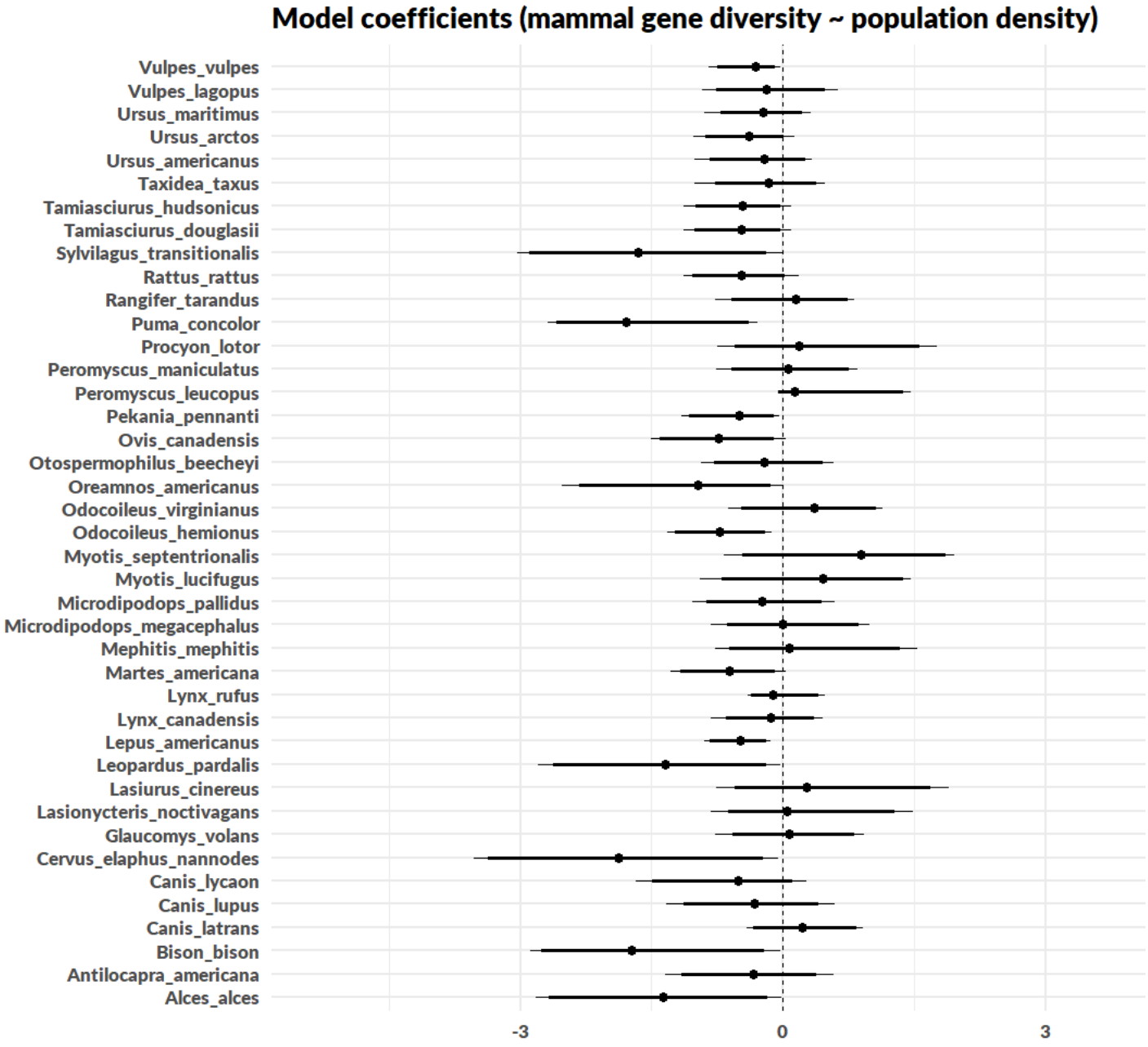


Figure S2.2.

Species-specific parameter estimates for the effect of human population density (which had the strongest effects for mammals) on mammal gene diversity. Ranges are 90% (thick line) and 95% (thin line) credible intervals.

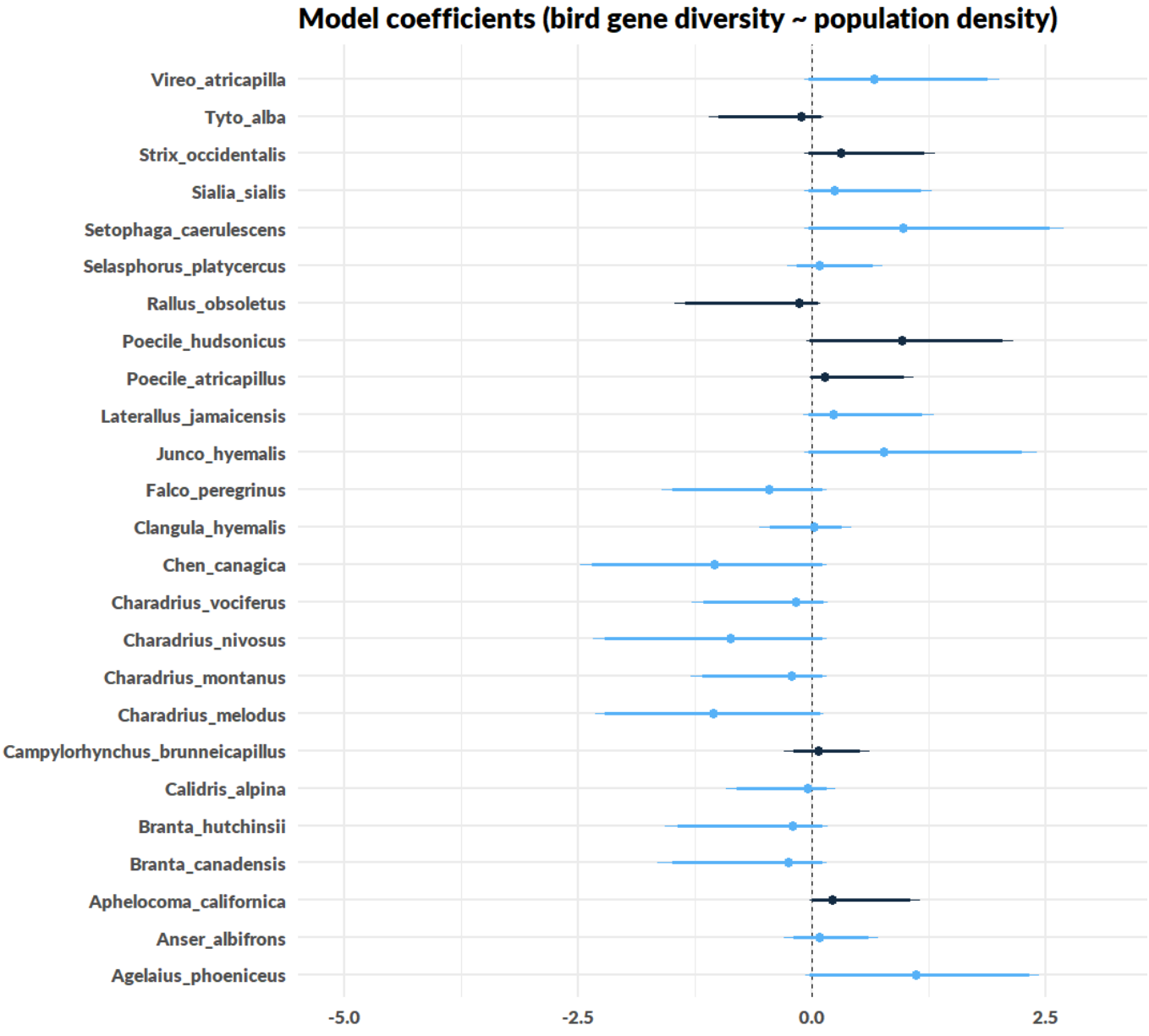


Figure S2.3.

Species-specific parameter estimates for the effect of human population density on gene diversity in migratory (blue) and non-migratory birds (black). Ranges are 90% (thick line) and 95% (thin line) credible intervals.

Figure S2.4.

Histograms showing the number of sites included (non-infinity) or excluded (infinity) from effective population size analyses in mammals (top graphs), non-migratory birds (center graphs), and all birds (bottom graphs). (A, C, E) Distribution of sites across human population density. The x axis represents log-transformed human population density plus a

constant (0.1). (B, D, F) Distribution of sites across the Human Footprint Index. Human Footprint is measured on a scale from 0 (most wild) to 100 (most disturbed). Overlapping, similar distributions indicate that excluding sites for which we were unable to estimate effective population size likely did not bias our analyses.

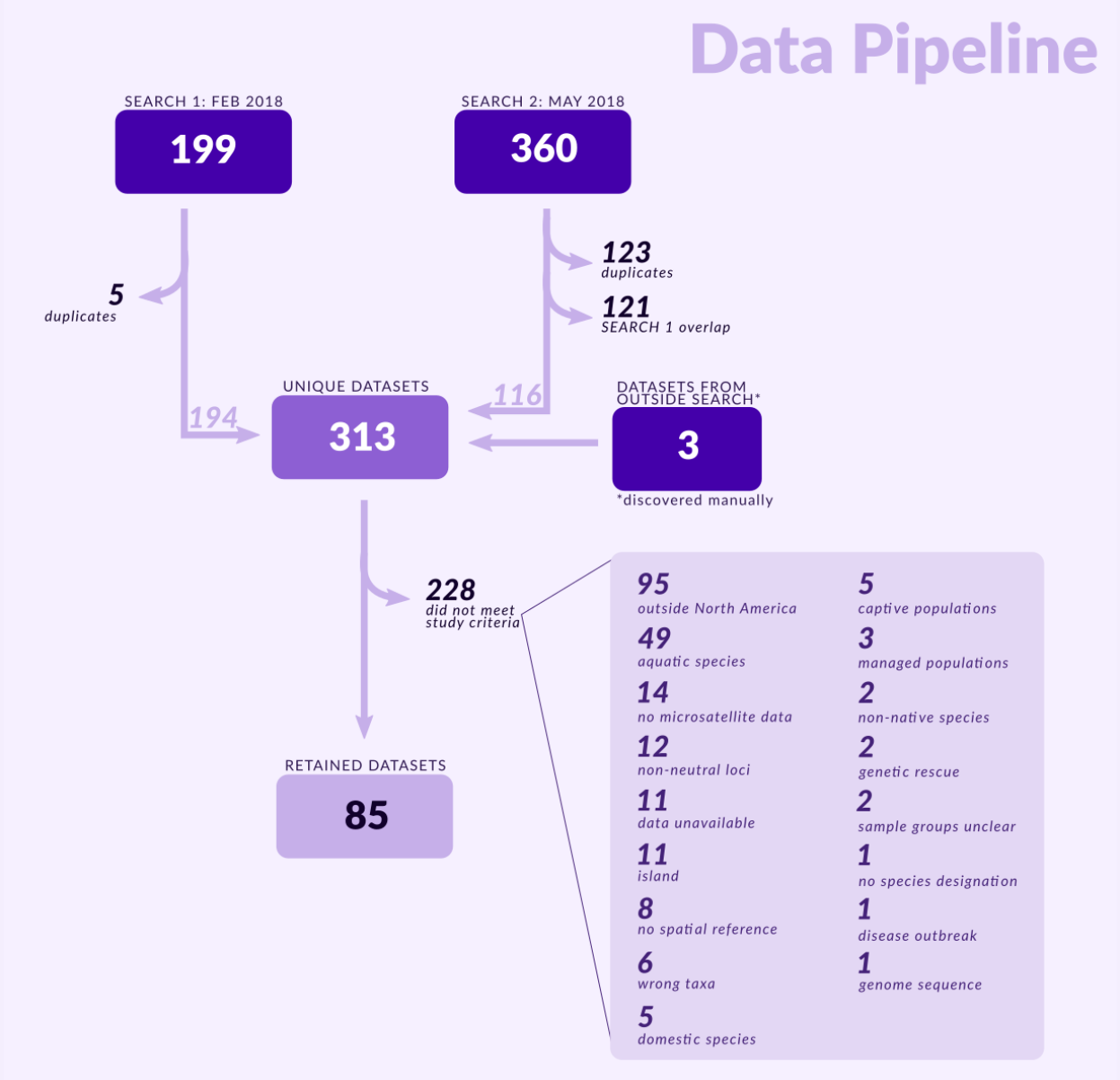


Figure S2.5. Data pipeline. Schematic shows the number of results from two systematic searches in the DataONE network, and filtering steps taken to arrive at our final dataset. Three microsatellite datasets did not appear in our search results, but were discovered manually and included in our analysis. See Table 2 for a complete list of included work, and Data S1 for raw search results.

Table S2.1.

Summary of number of species, sites, individuals and loci per taxonomic class (mammals and birds). For dataset-specific information, refer to Table S3.

class	species	number of sites			number of individuals		number of loci	
		total sites	urban sites	rural sites	median	range	median	range
mammals	41	812	250	562	26	5 – 2444	13	5 – 210
birds (all)	25	196	71	125	19	5 – 602	11	6 – 30
birds (non-migratory)	7	129	55	74	19	5 – 141	11	6 – 20

Table S2.2.

Summary and reference information for data used in this study. **Class**: mammals (M) or birds (B). **Repository**: location of data (USGS = U.S. Geological Survey). **Loci**: number of loci sampled, **populations**: number of populations, **individuals**: total number of individuals summed over all populations. **Coordinates**: method of assigning coordinate locations to each site. C = site coordinates provided in study; AC = coordinates given per sample in study, averaged to obtain site coordinates; GM = site name searched in Google Maps; GNIS = site name searchable in the Geographic Names Information System; M = map provided in study, georeferenced in ArcMap; P = site polygons (shapefile) provided, centroid coordinates taken.

Species	Class	Repository	Loci	Populations	Individuals	Coordinates	Search Date	Reference
Alces alces	M	Dryad	10	2	89	AC	February	(1, 2)
Canis latrans	M	Dryad	10	41	482	GM	February	(3, 4)
Cervus elaphus nannodes	M	Dryad	20	2	54	GM	February	(5, 6)
Lasionycteris noctivagans	M	Dryad	18	1	87	C	February	(7, 8)
Lasiurus cinereus	M	Dryad	19	1	132	C		
Lynx rufus	M	Dryad	10	5	95	GM	February	(9, 10)
Leopardus pardalis	M	Dryad	10	2	70	GM		
Lynx rufus	M	Dryad	9	4	365	AC	February	(11, 12)
Lynx rufus	M	Dryad	15	52	1646	AC	February	(13, 14)
Mephitis mephitis	M	Dryad	9	1	345	AC	February	(15, 16)
Procyon lotor	M	Dryad	10	1	330	AC		

Microdipodops megacephalus	M	Dryad	11	3	184	GM	February	(17, 18)
Microdipodops pallidus	M	Dryad	10	2	105	GM		
Myotis lucifugus	M	Dryad	9	15	735	C	February	(19, 20)
Myotis lucifugus	M	Dryad	11	21	1142	M	February	(21, 22)
Myotis lucifugus	M	Dryad	8	29	1310	C	February	(23, 24)
Myotis septentrionalis	M	Dryad	5	15	896	C		
Odocoileus hemionus	M	Dryad	10	60	1831	C	February	(25, 26)
Oreamnos americanus	M	Dryad	22	1	102	AC	February	(27, 28)
Ovis canadensis	M	Dryad	208	1	276	C	February	(29, 30)
Ovis canadensis	M	Dryad	210	1	216	GM		
Peromyscus leucopus	M	Dryad	10	12	134	C	February	(31, 32)
Peromyscus leucopus	M	Dryad	11	11	367	C	February	(33, 34)
Peromyscus leucopus	M	Dryad	18	13	312	GM	February	(35, 36)
Peromyscus maniculatus	M	Dryad	10	1	31	GM	February	(37, 38)
Puma concolor	M	Dryad	13	1	739	AC	February	(39, 40)
Puma concolor	M	Dryad	18	2	667	AC	February	(41, 42)
Puma concolor	M	Dryad	46	8	354	M	February	(43, 44)
Puma concolor	M	Dryad	10	2	196	M	February	(45, 46)
Rangifer tarandus	M	Dryad	19	18	655	M	February	(47, 48)

Rangifer tarandus	M	Dryad	18	27	606	M	February	(49, 50)
Rangifer tarandus	M	Dryad	21	5	230	M	February	(51, 52)
Rangifer tarandus	M	Dryad	14	27	802	M	February	(53, 54)
Rattus rattus	M	Dryad	9	8	126	GM	February	(55, 56)
Taxidea taxus	M	Dryad	12	1	233	AC	February	(57, 58)
Taxidea taxus	M	Dryad	12	3	917	AC	February	(59, 60)
Taxidea taxus	M	Dryad	20	8	236	M	February	(61, 62)
Ursus americanus	M	Dryad	15	7	269	M	February	(63, 64)
Ursus americanus	M	Dryad	15	4	250	AC	February	(65, 66)
Ursus maritimus	M	Dryad	21	14	2232	AC	February	(67, 68)
Ursus arctos	M	Dryad	8	16	831	M	February	(69, 70)
Ursus maritimus	M	Dryad	8	11	319	M		
Ursus americanus	M	Dryad	8	1	32	M		
Ursus arctos	M	Dryad	20	1	729	GM	February	(71, 72)
Ursus maritimus	M	Dryad	9	2	610	AC	February	(73, 74)
Ursus maritimus	M	Dryad	8	4	402	GM	February	(75, 76)
Vulpes vulpes	M	Dryad	8	5	257	M	February	(77, 78)
Vulpes lagopus	M	Dryad	9	3	78	M		
Vulpes vulpes	M	Dryad	13	11	376	AC	February	(79, 80)

Canis lupus	M	Dryad	12	1	62	C	February	(81, 82)
Canis lycaon	M	Dryad	12	1	62	GM		
Odocoileus_hemionus	M	Dryad	10	2	410	AC	February	(83, 84)
Tamiasciurus hudsonicus	M	Dryad	9	12	199	AC	February	(85, 86)
Tamiasciurus douglasii	M	Dryad	9	14	198	AC		
Lepus americanus	M	Dryad	8	39	853	AC	February	(87, 88)
Martes americana	M	Dryad	12	34	653	C	February	(89, 90)
Agelaius phoeniceus	B	Dryad	10	29	240	C	February	(91, 92)
Aphelocoma californica	B	Dryad	13	7	493	AC	February	(93, 94)
Calidris alpina	B	Dryad	8	30	236	C	February	(95, 96)
Charadrius melodus	B	Dryad	8	3	220	C	February	(97, 98)
Charadrius montanus	B	Dryad	14	7	94	C		
Charadrius nivosus	B	Dryad	15	4	68	C		
Charadrius vociferus	B	Dryad	14	2	49	C		
Laterallus jamaicensis	B	Dryad	15	2	336	M	February	(99, 100)
Selasphorus platycercus	B	Dryad	8	2	58	C	February	(101, 102)
Poecile hudsonicus	B	Dryad	6	2	260	C	February	(103, 104)
Poecile atricapillus	B	Dryad	11	13	913	C	February	(105, 106)
Poecile atricapillus	B	Dryad	14	32	142	C	February	(107, 108)

Setophaga caerulescens	B	Dryad	6	8	401	C	February	(109, 110)
Sialis sialis	B	Dryad	12	1	73	C	February	(111, 112)
Antilocapra americana	M	Dryad	19	4	175	GM	May	(113, 114)
Bison bison	M	Dryad	29	1	188	GM	May	(115, 116)
Odocoileus virginianus	M	Dryad	14	8	2069	C	May	(117, 118)
Otospermophilus beecheyi	M	Dryad	11	64	205	AC	May	(119, 120)
Ovis canadensis nelsoni	M	Dryad	16	3	579	P	May	(121, 122)
Rangifer tarandus	M	Dryad	16	14	480	AC	May	(123, 124)
Sylvilagus transitionalis	M	Dryad	10	5	157	AC	May	(125, 126)
Anser albifrons	B	USGS	8	3	115	C	May	(127)
Campylorhynchus brunneicapillus	B	Dryad	22	4	363	AC	May	(128, 129)
Clangula hyemalis	B	USGS	12	12	109	AC	May	(130)
Falco peregrinus	B	USGS	12	7	112	GNIS	May	(131)
Junco hyemalis	B	Dryad	7	8	602	GM	May	(132, 133)
Strix occidentalis	B	USGS	10	1	423	AC	May	(134)
Rallus obsoletus	B	USGS	9	17	107	C	May	(135)
Vireo atricapilla	B	Dryad	12	7	160	AC	May	(136, 137)
Ursus americanus	M	Dryad	12	1	2444	AC	May	(138, 139)
Lynx canadensis	M	Dryad	15	2	556	M	May	(140, 141)

Lynx rufus	M	Dryad	17	1	106	AC	May	(142, 143)
Odocoileus hemionus	M	Dryad	18	5	135	M	May	(144, 145)
Ursus americanus	M	Dryad	20	4	101	M, AC	May	(146, 147)
Ursus arctos	M	Dryad	20	4	113	M, AC		
Lynx canadensis	M	Dryad	14	2	702	C	February	(148, 149)
Glaucomys volans	M	Dryad	7	2	278	C		
Odocoileus hemionus	M	USGS	15	28	73	AC	May	(150)
Anser albifrons	B	USGS	19	8	10	GM	May	(151)
Branta canadensis	B	USGS	19	1	10	GM		
Branta hutchinsii	B	USGS	18	1	9	GM		
Chen canagica	B	USGS	30	1	27	GM		
Ursus americanus	M	Dryad	15	1	506	AC	May	(152, 153)
Ursus maritimus	M	Dryad	24	3	78	M	May	(154, 155)
Peromyscus maniculatus	M	Dryad	11	10	109	C	May	(156, 157)
Pekania pennanti	M	Dryad	16	28	722	C	NA	(158, 159)
Tyto alba	B	NA	20	4	292	M	NA	(160)
Vireo atricapilla	B	Dryad	9	9	67	M	NA	(161, 162)

References

1. Wilson RE, Farley SD, McDonough TJ, Talbot SL, Barboza PS (2015) A genetic discontinuity in moose (*Alces alces*) in Alaska corresponds with fenced transportation infrastructure. *Conserv Genet* 16(4):791–800.

2. Wilson RE, Farley SD, McDonough TJ, Talbot SL, Barboza PS (2015) Data from: A genetic discontinuity in moose (*Alces alces*) in Alaska corresponds with fenced transportation infrastructure. *Conserv Genet*. doi:doi:10.5061/dryad.bp907.

3. Heppenheimer E, et al. (2017) Demographic history influences spatial patterns of genetic diversityin recently expanded coyote (*Canis latrans*) populations. *Heredity*:1–13.

4. Heppenheimer E, et al. (2017) Data from: Demographic history influences spatial patterns of genetic diversity in recently expanded coyote (*Canis latrans*) populations. *Heredity*. doi:doi:10.5061/dryad.2t965.

5. Sacks BN, Lounsberry ZT, Kalani T, Meredith EP, Langner C (2016) Development and characterization of 15 polymorphic dinucleotide microsatellite markers for tule elk using hiseq3000. *J Hered* 107(7):666–669.

6. Sacks BN, Lounsberry ZT, Kalani T, Meredith EP, Langner C (2016) Data from: Development and characterization of 15 polymorphic di-nucleotide microsatellite markers for tule elk using HiSeq3000. *J Hered*. doi:doi:10.5061/dryad.j2654.

7. Baerwald EF, Barclay RMR (2016) Are migratory behaviours of bats socially transmitted? *R Soc Open Sci* 3(150658). doi:10.1093/jmammal/gyv135.

8. Baerwald EF, Barclay RMR (2016) Data from: Are migratory behaviours of bats socially transmitted? *R Soc Open Sci*. doi:doi:10.5061/dryad.4d3n1.

9. Janecka JE, et al. (2016) Genetic differences in the response to landscape fragmentation by a habitat generalist, the bobcat, and a habitat specialist, the ocelot. *Conserv Genet* 17(5):1093–1108.

10. Janecka JE, et al. (2016) Data from: Genetic differences in the response to landscape fragmentation by a habitat generalist, the bobcat, and a habitat specialist, the ocelot. *Conserv Genet*. doi:doi:10.5061/dryad.5b2k6.

11. Serieys LEK, Lea A, Pollinger JP, Riley SPD, Wayne RK (2015) Disease and freeways drive genetic change in urban bobcat populations. *Evol Appl* 8(1):75–92.

12. Serieys LE, Lea A, Pollinger JP, Riley SPD, Wayne RK (2014) Data from: Disease and freeways drive genetic change in urban bobcat populations. *Evol Appl*. doi:doi:10.5061/dryad.7j8q0.

13. Reding DM, Bronikowski AM, Johnson WE, Clark WR (2012) Pleistocene and ecological effects on continental-scale genetic differentiation in the bobcat (*Lynx rufus*). *Mol Ecol* 21(12):3078–3093.

14. Reding DM, Bronikowski AM, Johnson WE, Clark WR (2012) Data from: Pleistocene and ecological effects on continental-scale genetic differentiation in the bobcat (*Lynx rufus*). *Mol Ecol*. doi:doi:10.5061/dryad.d3t16pd2.

15. Rioux Paquette S, Talbot B, Garant D, Mainguy J, Pelletier F (2014) Modelling the dispersal of the two main hosts of the raccoon rabies variant in heterogeneous environments with landscape genetics. *Evol Appl* 7(7):734–749.

16. Rioux Paquette S, Talbot B, Garant D, Mainguy J, Pelletier F (2014) Data from: Modelling the dispersal of the two main hosts of the raccoon rabies variant in heterogeneous environments with landscape genetics. *Evol Appl*. doi:doi:10.5061/dryad.jr66k.

17. Andersen JJ, Portnoy DS, Hafner JC, Light JE (2013) Populations at risk: Conservation genetics of kangaroo mice (*Microdipodops*) of the great basin desert. *Ecol Evol* 3(8):2497–2513.

18. Andersen JJ, Portnoy DS, Hafner JC, Light JE (2013) Data from: Populations at risk: conservation genetics of kangaroo mice (*Microdipodops*) of the Great Basin Desert. *Ecol Evol*. doi:doi:10.5061/dryad.r531f.

19. Burns LE, Frasier TR, Broders HG (2014) Genetic connectivity among swarming sites in the wide ranging and recently declining little brown bat (*Myotis lucifugus*). *Ecol Evol* 4(21):4130–4149.

20. Burns LE, Frasier TR, Broders HG (2014) Data from: Genetic connectivity among swarming sites in the wide ranging and recently declining little brown bat (*Myotis lucifugus*). *Ecol Evol*. doi:doi:10.5061/dryad.hs37s.

21. Davy CM, et al. (2017) Prelude to a panzootic: gene flow and immunogenetic variation in northern little brown *Myotis* vulnerable to bat white-nose syndrome. *FACETS* 2:690–714.

22. Davy CM, et al. (2017) Data from: Prelude to a panzootic: gene flow and immunogenetic variation in northern little brown *Myotis* vulnerable to bat white-nose syndrome. *FACETS*. doi:doi:10.5061/dryad.h7n25.

23. Johnson LNL, et al. (2015) Population Genetic Structure Within and among Seasonal Site Types in the Little Brown Bat (*Myotis lucifugus*) and the Northern Long-Eared Bat (M. septentrionalis). *PLoS One* 10(5):1–18.

24. Johnson LNL, et al. (2015) Data from: Population genetic structure within and among seasonal site types in the little brown bat (*Myotis lucifugus*) and the northern long-eared bat (M. septentrionalis). *PLoS One*. doi:doi:10.5061/dryad.47nm0.

25. Latch EK, Reding DM, Heffelfinger JR, Alcalá-Galván CH, Rhodes OE (2014) Range-wide analysis of genetic structure in a widespread, highly mobile species (*Odocoileus hemionus*) reveals the importance of historical biogeography. *Mol Ecol* 23(13):3171–3190.

26. Latch EK, Reding DM, Heffelfinger JR, Alcalá-Galván CH, Rhodes OE (2014) Data from: Range-wide analysis of genetic structure in a widespread, highly mobile species (*Odocoileus hemionus*) reveals the importance of historical biogeography. *Mol Ecol*. doi:doi:10.5061/dryad.ns2jn.

27. Shafer ABA, et al. (2012) Data from: Habitat selection predicts genetic relatedness in

an alpine ungulate. *Ecology*. doi:doi:10.5061/dryad.6b6k8b83.

28. Shafer ABA, et al. (2012) Habitat selection predicts genetic relatedness in an alpine ungulate. *Ecology* 93(6):1317–1329.

29. Miller JM, Poissant J, Malenfant RM, Hogg JT, Coltman DW (2015) Temporal dynamics of linkage disequilibrium in two populations of bighorn sheep. *Ecol Evol* 5(16):3401–3412.

30. Miller JM, Poissant J, Malenfant RM, Hogg JT, Coltman DW (2015) Data from: Temporal dynamics of linkage disequilibrium in two populations of bighorn sheep. *Ecol Evol*. doi:doi:10.5061/dryad.n2cj3.

31. Taylor ZS, Hoffman SMG (2014) Landscape models for nuclear genetic diversity and genetic structure in white-footed mice (*Peromyscus leucopus*). *Heredity* 112(6):588–595.

32. Taylor ZS, Hoffman SMG (2014) Data from: Landscape models for nuclear genetic diversity and genetic structure in white-footed mice (*Peromyscus leucopus*). *Heredity*. doi:doi:10.5061/dryad.j272c.

33. Rogic A, Tessier N, Legendre P, Lapointe F-J, Millien V (2013) Genetic structure of the white-footed mouse in the context of the emergence of Lyme disease in southern Québec. *Ecol Evol* 3(7):2075–2088.

34. Rogic A, Tessier N, Legendre P, Lapointe F-J, Millien V (2013) Data from: Genetic structure of the white-footed mouse in the context of the emergence of Lyme disease in southern Québec. *Ecol Evol*. doi:doi:10.5061/dryad.43vg5.

35. Munshi-South J, Kharchenko K (2010) Rapid, pervasive genetic differentiation of urban white-footed mouse (*Peromyscus leucopus*) populations in New York City. *Mol Ecol* 19(19):4242–4254.

36. Munshi-South J, Kharchenko K (2010) Data from: Rapid, pervasive genetic differentiation of urban white-footed mouse (*Peromyscus leucopus*) populations in

New York City. *Mol Ecol*. doi:doi:10.5061/dryad.1893.

37. Ozer F, Gellerman H, Ashley M V. (2011) Genetic impacts of Anacapa deer mice reintroductions following rat eradication. *Mol Ecol* 20(17):3525–3539.

38. Ozer F, Gellerman H, MV A (2011) Data from: Genetic impacts of Anacapa deer mice reintroductions following rat eradication. *Mol Ecol*. doi:doi:10.5061/dryad.778c9.

39. Andreasen AM, Stewart KM, Longland WS, Beckmann JP, Forister ML (2012) Identification of source-sink dynamics in mountain lions of the Great Basin. *Mol Ecol* 21(23):5689–5701.

40. Andreasen AM, Stewart KM, Longland WS, Beckmann JP, Forister ML (2012) Data from: Identification of source-sink dynamics in mountain lions of the Great Basin. *Mol Ecol*. doi:doi:10.5061/dryad.mj74q.

41. Warren MJ, Wallin DO, Beausoleil RA, Warheit KI (2016) Forest cover mediates genetic connectivity of northwestern cougars. *Conserv Genet* 17(5):1011–1024.

42. Warren MJ, Wallin DO, Beausoleil RA, Warheit KI (2016) Data from: Forest cover mediates genetic connectivity of northwestern cougars. *Conserv Genet*. doi:doi:10.5061/dryad.3bn62.

43. Ernest HB, Vickers TW, Morrison SA, Buchalski MR, Boyce WM (2014) Fractured genetic connectivity threatens a Southern California puma (*Puma concolor*) population. *PLoS One* 9(10). doi:10.1371/journal.pone.0107985.

44. Ernest HB, Vickers TW, Morrison SA, Buchalski MR, Boyce WM (2014) Data from: Fractured genetic connectivity threatens a southern California puma (*Puma concolor*) population. *PLoS One*. doi:10.5061/dryad.dp0qj.

45. Holbrook JD, Deyoung RW, Tewes ME, Young JH (2012) Demographic history of an elusive carnivore: Using museums to inform management. *Evol Appl* 5(6):619–628.

46. Holbrook JD, Deyoung RW, Tewes ME, Young JH (2012) Data from: Demographic history of an elusive carnivore: using museums to inform management. *Evol Appl*.

doi:doi:10.5061/dryad.k5d9470m.

47. Mager KH, Colson KE, Groves P, Hundertmark KJ (2014) Population structure over a broad spatial scale driven by nonanthropogenic factors in a wide-ranging migratory mammal, Alaskan caribou. *Mol Ecol* 23(24):6045–6057.

48. Mager KH, Colson KE, Groves P, Hundertmark KJ (2014) Data from: Population structure over a broad spatial scale driven by non-anthropogenic factors in a wide-ranging migratory mammal, Alaskan caribou. *Mol Ecol*. doi:doi:10.5061/dryad.3hp5v.

49. Serrouya R, et al. (2012) Population size and major valleys explain microsatellite variation better than taxonomic units for caribou in western Canada. *Mol Ecol* 21(11):2588–2601.

50. Serrouya R, et al. (2012) Data from: Population size and major valleys explain microsatellite variation better than taxonomic units for caribou in western Canada. *Mol Ecol*. doi:doi:10.5061/dryad.250c3s47.

51. Colson KE, Mager KH, Hundertmark KJ (2014) Reindeer introgression and the population genetics of caribou in southwestern Alaska. *J Hered* 105(5):585–596.

52. Colson KE, Mager KH, Hundertmark KJ (2014) Data from: Reindeer introgression and the population genetics of caribou in Southwestern Alaska. *J Hered*. doi:doi:10.5061/dryad.9qh56.

53. Weckworth B V., Musiani M, McDevitt AD, Hebblewhite M, Mariani S (2012) Reconstruction of caribou evolutionary history in Western North America and its implications for conservation. *Mol Ecol* 21(14):3610–3624.

54. Weckworth B V., Musiani M, McDevitt AD, Hebblewhite M, Mariani S (2012) Data from: Reconstruction of caribou evolutionary history in Western North America and its implications for conservation. *Mol Ecol*. doi:doi:10.5061/dryad.gn22271h.

55. Lack JB, et al. (2012) Invasion facilitates hybridization with introgression in the

Rattus rattus species complex. *Mol Ecol* 21(14):3545–3561.

56. Lack JB, et al. (2012) Data from: Invasion facilitates hybridization with introgression in the Rattus rattus species complex. *Mol Ecol*. doi:doi:10.5061/dryad.sj168561.

57. Kierepka EM, Latch EK (2016) Fine-scale landscape genetics of the American badger (*Taxidea taxus*): disentangling landscape effects and sampling artifacts in a poorly understood species. *Heredity* 116(1):33–43.

58. Kierepka EM, Latch EK (2015) Data from: Fine-scale landscape genetics of the American badger (*Taxidea taxus*): disentangling landscape effects and sampling artifacts in a poorly understood species. *Heredity*. doi:doi:10.5061/dryad.mr909.

59. Kierepka EM, Latch EK (2016) High gene flow in the American badger overrides habitat preferences and limits broadscale genetic structure. *Mol Ecol* 25(24):6055–6076.

60. Kierepka EM, Latch EK (2016) Data from: High gene flow in the American badger overrides habitat preferences and limits broadscale genetic structure. *Mol Ecol*. doi:doi:10.5061/dryad.3qt42.

61. Rico Y, et al. (2016) Spatial patterns of immunogenetic and neutral variation underscore the conservation value of small, isolated American badger populations. *Evol Appl* 9(10):1271–1284.

62. Rico Y, et al. (2016) Data from: Spatial patterns of immunogenetic and neutral variation underscore the conservation value of small, isolated American badger populations. *Evol Appl*. doi:doi:10.5061/dryad.qb87r.

63. Draper JP, Waits LP, Adams JR, Seals CL, Steury TD (2017) Genetic health and population monitoring of two small black bear (*Ursus americanus*) populations in Alabama, with a regional perspective of genetic diversity and exchange. *PLoS One* 12(11). doi:10.1371/journal.pone.0186701.

64. Draper JP, Waits LP, Adams JR, Seals CL, Steury TD (2017) Data from: Genetic and

population monitoring of two small black bear (*Ursus americanus*) populations in Alabama, within a regional context. *PLoS One*. doi:doi:10.5061/dryad.bj7r3.

65. Puckett EE, et al. (2014) Influence of drift and admixture on population structure of American black bears (*Ursus americanus*) in the Central Interior Highlands, USA, 50 years after translocation. *Mol Ecol* 23(10):2414–2427.

66. Puckett EE, et al. (2014) Data from: Influence of drift and admixture on population structure of American black bears (*Ursus americanus*) in the Central Interior Highlands, U.S.A. 50 years after translocation. *Mol Ecol*. doi:doi:10.5061/dryad.q405j.

67. Peacock E, et al. (2015) Implications of the circumpolar genetic structure of polar bears for their conservation in a rapidly warming Arctic. *PLoS One* 10(1):1–30.

68. Peacock E, et al. (2015) Data from: Implications of the circumpolar genetic structure of polar bears for their conservation in a rapidly warming Arctic. *PLoS One*. doi:doi:10.5061/dryad.v2j1r.

69. Cronin MA, MacNeil MD (2012) Genetic relationships of extant brown bears (*Ursus arctos*) and polar bears (*Ursus maritimus*). *J Hered* 103(6):873–881.

70. Cronin MA, MacNeil MD (2012) Data from: Genetic relationships of extant brown bears (*Ursus arctos*) and polar bears (*Ursus maritimus*). *J Hered*. doi:doi:10.5061/dryad.q30rt.

71. Kamath PL, et al. (2015) Multiple estimates of effective population size for monitoring a long-lived vertebrate: An application to Yellowstone grizzly bears. *Mol Ecol* 24(22):5507–5521.

72. Kamath PL, et al. (2015) Data from: Multiple estimates of effective population size for monitoring a long-lived vertebrate: an application to Yellowstone grizzly bears. *Mol Ecol*. doi:10.5061/dryad.s6764.

73. Campagna L, et al. (2013) Extensive sampling of polar bears (*Ursus maritimus*) in the Northwest Passage (Canadian Arctic Archipelago) reveals population differentiation

across multiple spatial and temporal scales. *Ecol Evol* 3(9):3152–3165.

74. Campagna L, et al. (2013) Data from: Extensive sampling of polar bears (*Ursus maritimus*) in the Northwest Passage (Canadian Arctic Archipelago) reveals population differentiation across multiple spatial and temporal scales. *Ecol Evol*. doi:doi:10.5061/dryad.h1v63.

75. Laidre KL, et al. (2018) Range contraction and increasing isolation of a polar bear subpopulation in an era of sea-ice loss. *Ecol Evol* 8:2062–2075.

76. Laidre KL, et al. (2018) Data from: Range contraction and increasing isolation of a polar bear subpopulation in an era of sea-ice loss. *Ecol Evol*. doi:doi:10.5061/dryad.74v52.

77. Goldsmith EW, et al. (2016) Distribution of Rabies Virus Variants in Alaska. *Mol Ecol* 25(3):675–688.

78. Goldsmith EW, et al. (2015) Data from: Population structure of two rabies hosts relative to the known distribution of rabies virus variants in Alaska. *Mol Ecol*. doi:doi:10.5061/dryad.dc1q8.2.

79. Sacks BN, Brazeal JL, Lewis JC (2016) Landscape genetics of the nonnative red fox of California. *Ecol Evol* 6(14):4775–4791.

80. Sacks BN, Brazeal JL, Lewis JC (2016) Data from: Landscape genetics of the nonnative red fox of California. *Ecol Evol*. doi:doi:10.5061/dryad.hj722.

81. Wheeldon TJ, Rutledge LY, Patterson BR, White BN, Wilson PJ (2013) Y-chromosome evidence supports asymmetric dog introgression into eastern coyotes. *Ecol Evol* 3(9):3005–3020.

82. Wheeldon TJ, Rutledge LY, Patterson BR, White BN, Wilson PJ (2013) Data from: Y-chromosome evidence supports asymmetric dog introgression into eastern coyotes. *Ecol Evol*. doi:doi:10.5061/dryad.72s5k.

83. Latch EK, Kierepka EM, Heffelfinger JR, Rhodes OE (2011) Hybrid swarm between

divergent lineages of mule deer (*Odocoileus hemionus*). *Mol Ecol* 20(24):5265–5279.

84. Latch EK, Kierepka EM, Heffelfinger JR, Rhodes OE (2011) Data from: Hybrid swarm between divergent lineages of mule deer (*Odocoileus hemionus*). *Mol Ecol*. doi:doi:10.5061/dryad.hm2047nh.

85. Chavez AS, Saltzberg CJ, Kenagy GJ (2011) Genetic and phenotypic variation across a hybrid zone between ecologically divergent tree squirrels (*Tamiasciurus*). *Mol Ecol* 20(16):3350–3366.

86. Chavez AS, Saltzberg CJ, Kenagy GJ (2011) Data from: Genetic and phenotypic variation across a hybrid zone between ecologically divergent tree squirrels (*Tamiasciurus*). *Mol Ecol*. doi:doi:10.5061/dryad.195qg.

87. Cheng E, Hodges KE, Melo-Ferreira J, Alves PC, Mills LS (2014) Conservation implications of the evolutionary history and genetic diversity hotspots of the snowshoe hare. *Mol Ecol* 23(12):2929–2942.

88. Cheng E, Hodges KE, Melo-Ferreira J, Alves PC, Mills LS (2014) Data from: Conservation implications of the evolutionary history and genetic diversity hotspots of the snowshoe hare. *Mol Ecol*. doi:10.5061/dryad.dh63p.

89. Koen EL, Bowman J, Wilson PJ (2016) Node-based measures of connectivity in genetic networks. *Mol Ecol Resour* 16(1):69–79.

90. Koen EL, Bowman J, Wilson PJ (2015) Data from: Node-based measures of connectivity in genetic networks. *Mol Ecol Resour*. doi:doi:10.5061/dryad.4tg23.

91. Liu IA, et al. (2015) Genetic diversity does not explain variation in extra-pair paternity in multiple populations of a songbird. *J Evol Biol* 28(5):1156–1169.

92. Liu IA, et al. (2015) Data from: Genetic diversity does not explain variation in extra-pair paternity in multiple populations of a songbird. *J Evol Biol*. doi:doi:10.5061/dryad.4nj00.

93. Gowen FC, et al. (2014) Speciation in Western Scrub-Jays, Haldane's rule, and genetic

clines in secondary contact. *BMC Evol Biol* 14(1):1–15.

94. Gowen FC, et al. (2014) Data from: Speciation in Western Scrub-Jays, Haldane's rule, and genetic clines in secondary contact. *BMC Evol Biol*. doi:doi:10.5061/dryad.57f48.

95. Miller MP, et al. (2015) Intercontinental genetic structure and gene flow in Dunlin (*Calidris alpina*), a potential vector of avian influenza. *Evol Appl* 8(2):149–171.

96. Miller MP, et al. (2014) Data from: Intercontinental genetic structure and gene flow in Dunlin (*Calidris alpina*), a potential vector of avian influenza. *Evol Appl*. doi:doi:10.5061/dryad.4t806.

97. D'Urban Jackson J, et al. (2017) Polygamy slows down population divergence in shorebirds. *Evolution* 71(5):1313–1326.

98. D'Urban Jackson J, et al. (2017) Data from: Polygamy slows down population divergence in shorebirds. *Evolution*. doi:doi:10.5061/dryad.vn77k.

99. Hall LA, Beissinger SR (2017) Inferring the timing of long-distance dispersal between Rail metapopulations using genetic and isotopic assignments: *Ecol Appl* 27(1):208–218.

100. Hall LA, Beissinger SR (2016) Data from: Inferring the timing of long-distance dispersal between Rail metapopulations using genetic and isotopic assignments. *Ecol Appl*. doi:10.5061/dryad.27mk2.

101. Malpica A, Ornelas JF (2014) Postglacial northward expansion and genetic differentiation between migratory and sedentary populations of the broad-tailed hummingbird (*Selasphorus platycercus*). *Mol Ecol* 23(2):435–452.

102. Malpica A, Ornelas JF (2014) Data from: Post-glacial northward expansion and genetic differentiation between migratory and sedentary populations of the broad-tailed hummingbird (*Selasphorus platycercus*). *Mol Ecol*. doi:doi:10.5061/dryad.14n6g.

103. Lait LA, Burg TM (2013) When east meets west: Population structure of a high-

latitude resident species, the boreal chickadee (*Poecile hudsonicus*). *Heredity* 111(4):321–329.

104. Lait LA, Burg TM (2013) Data from: When east meets west: population structure of a high- latitude resident species, the boreal chickadee (*Poecile hudsonicus*). *Heredity*. doi:doi:10.5061/dryad.82hs7.

105. Adams R V., Burg TM (2015) Influence of ecological and geological features on rangewide patterns of genetic structure in a widespread passerine. *Heredity* 114(2):143–154.

106. Adams R V., Burg TM (2014) Data from: Influence of ecological and geological features on rangewide patterns of genetic structure in a widespread passerine. *Heredity*. doi:doi:10.5061/dryad.k086v.

107. Adams R V., Lazerte SE, Otter KA, Burg TM (2016) Influence of landscape features on the microgeographic genetic structure of a resident songbird. *Heredity* 117(2):63–72.

108. Adams R V., Lazerte SE, Otter KA, Burg TM (2016) Data from: Influence of landscape features on the microgeographic genetic structure of a resident songbird. *Heredity*. doi:doi:10.5061/dryad.gs228.

109. Kaiser SA, et al. (2017) A comparative assessment of SNP and microsatellite markers for assigning parentage in a socially monogamous bird. *Mol Ecol Resour* 17(2):183–193.

110. Kaiser SA, et al. (2016) Data from: A comparative assessment of SNP and microsatellite markers for assigning parentage in a socially monogamous bird. *Mol Ecol Resour*. doi:doi:10.5061/dryad.1vn46.

111. Avery JD, Fonseca DM, Campagne P, Lockwood JL (2013) Cryptic introductions and the interpretation of island biodiversity. *Mol Ecol* 22(8):2313–2324.

112. Avery JD, Fonseca DM, Campagne P, Lockwood JL (2013) Data from: Cryptic introductions and the interpretation of island biodiversity. *Mol Ecol*.

doi:doi:10.5061/dryad.1tv93.

113. Dunn SJ, Clancey E, Waits LP, Byers JA (2011) Inbreeding depression in pronghorn (*Antilocapra americana*) fawns. *Mol Ecol* 20(23):4889–4898.

114. Dunn SJ, Clancey E, Waits LP, Byers JA (2011) Data from: Inbreeding depression in pronghorn (*Antilocapra americana*) fawns. *Mol Ecol*. doi:10.5061/dryad.ht46qk45.

115. Cronin MA, et al. (2013) Genetic variation and differentiation of bison (*Bison bison*) subspecies and cattle (*Bos taurus*) breeds and subspecies. *J Hered* 104(4):500–509.

116. Cronin MA, et al. (2013) Data from: Genetic variation and differentiation of extant bison (*Bison bison*) subspecies and cattle (*Bos taurus*) breeds and subspecies. *J Hered*. doi:doi:10.5061/dryad.7k66b.

117. Robinson SJ, Samuel MD, Lopez DL, Shelton P (2012) The walk is never random: Subtle landscape effects shape gene flow in a continuous white-tailed deer population in the Midwestern United States. *Mol Ecol* 21(17):4190–4205.

118. Robinson SJ, Samuel MD, Lopez DL, Shelton P (2012) Data from: The walk is never random: subtle landscape effects shape gene flow in a continuous white-tailed deer population in the Midwestern United States. *Mol Ecol*. doi:doi:10.5061/dryad.p7639.

119. Phuong MA, Lim MCW, Wait DR, Rowe KC, Moritz C (2014) Delimiting species in the genus Otospermophilus (Rodentia: *Sciuridae*), using genetics, ecology, and morphology. *Biol J Linn Soc* 113(4):1136–1151.

120. Phuong MA, Lim MCW, Wait DR, Rowe KC, Moritz C (2014) Data from: Delimiting species in the genus Otospermophilus (Rodentia: *Sciuridae*) using genetics, ecology, and morphology. *Biol J Linn Soc*. doi:doi:10.5061/dryad.s4h2j.

121. Epps CW, Crowhurst RS, Nickerson BS (2018) Assessing changes in functional connectivity in a desert bighorn sheep metapopulation after two generations. *Mol Ecol* 27:2334–2346.

122. Epps CW, Crowhurst RS, Nickerson BS (2018) Data from: Assessing changes in

functional connectivity in a desert bighorn sheep metapopulation after two generations. *Mol Ecol*. doi:doi:10.5061/dryad.mp71t50.

123. Yannic G, et al. (2014) Temporally dynamic habitat suitability predicts genetic relatedness among caribou. *Proceedings Biol Sci* 281(2014052). doi:10.1098/rspb.2014.0502.

124. Yannic G, et al. (2014) Data from: Temporally dynamic habitat suitability predicts genetic relatedness among caribou. *Proc R Soc B*. doi:doi:10.5061/dryad.qn1cj.

125. Fenderson LE, et al. (2014) A multiscale analysis of gene flow for the New England cottontail, an imperiled habitat specialist in a fragmented landscape. *Ecol Evol* 4(10):1853–1875.

126. Fenderson LE, et al. (2014) Data from: A multi-scale analysis of gene flow for the New England cottontail, an imperiled habitat specialist in a fragmented landscape. *Ecol Evol*. doi:10.5061/dryad.1s834.

127. Wilson RE (2006) Greater White-Fronted Goose (*Anser albifrons*) Microsatellite DNA Data, Alaska, 1989-2006. doi:10.5066/F71G0JGN.

128. Barr KR, et al. (2015) Habitat fragmentation in coastal southern California disrupts genetic connectivity in the cactus wren (*Campylorhynchus brunneicapillus*). *Mol Ecol* 24(10):2349–2363.

129. Barr KR, et al. (2015) Data from: Habitat fragmentation in coastal southern California disrupts genetic connectivity in the Cactus Wren (*Campylorhynchus brunneicapillus*). *Mol Ecol*. doi:doi:10.5061/dryad.j5h92.

130. Wilson RE, Talbot SL (2016) Long-tailed Duck (*Clangula hyemalis*) Microsatellite DNA Data; Alaska, Canada, Russia, 1994-2002. doi:10.5066/F7HX19RJ.

131. Pierson BJ (2017) Peregrine Falcon (*Falco peregrinus*) mtDNA and Microsatellite Genetic Data, Alaska, Canada and Russia, 1880-2012. doi:10.5066/F7F18WV0.

132. Liebgold EB, Gerlach NM, Ketterson ED (2013) Similarity in temporal variation in

sex-biased dispersal over short and long distances in the dark-eyed junco, *Junco hyemalis*. *Mol Ecol* 22(22):5548–5560.

133. Liebgold EB, Gerlach NM, Ketterson ED (2013) Data from: Similarity in temporal variation in sex-biased dispersal over short and long distances in the dark-eyed junco, *Junco hyemalis*. *Mol Ecol*. doi:doi:10.5061/dryad.1jd40.2.

134. Miller MP, Davis RJ, Mullins TD, Haig SM, Forsman ED (2017) Microsatellite markers, habitat quality, and sample location data for Northern Spotted Owls (*Strix occidentalis caurina*). doi:10.5066/F7J67FVW.

135. Wood DA, et al. (2016) A Century of Landscape Disturbance and Urbanization of the San Francisco Bay Region affects the Present-day Genetic Diversity of the California Ridgway's Rail (*Rallus obsoletus obsoletus*). doi:10.5066/F7HD7SQ0.

136. Athrey G, Lance RF, Leberg PL (2012) How far is too close? restricted, sex-biased dispersal in black-capped vireos. *Mol Ecol* 21(17):4359–4370.

137. Athrey G, Lance RF, Leberg PL (2012) Data from: How far is too close? Restricted, sex-biased dispersal in black-capped vireos. *Mol Ecol*. doi:doi:10.5061/dryad.d2fm8.

138. Moore JA, Xu R, Frank K, Draheim H, Scribner KT (2015) Social network analysis of mating patterns in American black bears (*Ursus americanus*). *Mol Ecol* 24(15):4010–4022.

139. Moore JA, Xu R, Frank K, Draheim H, Scribner KT (2015) Data from: Social network analysis of mating patterns in American black bears (*Ursus americanus*). *Mol Ecol*. doi:doi:10.5061/dryad.c61q0.

140. Prentice MB, et al. (2017) Selection and drift influence genetic differentiation of insular Canada lynx (*Lynx canadensis*) on Newfoundland and Cape Breton Island. *Ecol Evol* 7(9):3281–3294.

141. Prentice MB, et al. (2017) Data from: Selection and drift influence genetic differentiation of insular Canada lynx (*Lynx canadensis*) on Newfoundland and Cape

Breton Island. *Ecol Evol*. doi:doi:10.5061/dryad.8ff46.

142. Lee JS, et al. (2012) Gene flow and pathogen transmission among bobcats (*Lynx rufus*) in a fragmented urban landscape. *Mol Ecol* 21(7):1617–1631.

143. Lee JS, et al. (2012) Data from: Gene flow and pathogen transmission among bobcats (*Lynx rufus*) in a fragmented urban landscape. *Mol Ecol*. doi:doi:10.5061/dryad.mm317th7.

144. Northrup JM, Shafer ABA, Anderson CR, Coltman DW, Wittemyer G (2014) Fine-scale genetic correlates to condition and migration in a wild cervid. *Evol Appl* 7(8):937–948.

145. Northrup JM, Shafer ABA, Anderson CR, Coltman DW, Wittemyer G (2014) Data from: Fine scale genetic correlates to condition and migration in a wild Cervid. *Evol Appl*. doi:doi:10.5061/dryad.3vc1b.

146. Sawaya MA, Kalinowski ST, Clevenger AP (2014) Genetic connectivity for two bear species at wildlife crossing structures in Banff National Park. *Proc R Soc B* 281:1–10.

147. Sawaya MA, Kalinowski ST, Clevenger AP (2014) Data from: Genetic connectivity for two bear species at wildlife crossing structures in Banff National Park. *Proc R Soc B*. doi:doi:10.5061/dryad.5q3b3.

148. Marrotte RR, et al. (2017) Multi-species genetic connectivity in a terrestrial habitat network. *Mov Ecol* 5(1):1–11.

149. Marrotte RR, et al. (2017) Data from: Multi-species genetic connectivity in a terrestrial habitat network. *Mov Ecol*. doi:doi:10.5061/dryad.qn4kq.

150. Vandergast AG, Mitelberg A (2016) DNA fingerprinting of Southern Mule Deer (*Odocoileus hemionus fuliginatus*) to determine movement across California State Route 67. doi:10.5066/F7KW5D32.

151. Talbot SL (2017) DNA Microsatellite and Sex Identification Markers for Emperor Goose (*Chen canagica*) and Cross-Species Amplification of Microsatellites in Select

Goose Species, Alaska 2016. doi:10.5066/F71V5C4S.

152. Puckett EE, Etter PD, Johnson EA, Eggert LS (2015) Phylogeographic Analyses of American Black Bears (*Ursus americanus*) Suggest Four Glacial Refugia and Complex Patterns of Postglacial Admixture. *Mol Biol Evol* 32(9):2338–2350.

153. Puckett EE, Etter PD, Johnson EA, Eggert LS (2015) Data from: Phylogeographic analyses of American black bears (*Ursus americanus*) suggest four glacial refugia and complex patterns of post-glacial admixture. *Mol Biol Evol*. doi:doi:10.5061/dryad.dc02b.

154. Malenfant RM, Coltman DW, Davis CS (2015) Design of a 9K illumina BeadChip for polar bears (*Ursus maritimus*) from RAD and transcriptome sequencing. *Mol Ecol Resour* 15(3):587–600.

155. Malenfant RM, Coltman DW, Davis CS (2014) Data from: Design of a 9K SNP chip for polar bears (*Ursus maritimus*) from RAD and transcriptome sequencing. *Mol Ecol Resour*. doi:doi:10.5061/dryad.b35td.

156. Taylor ZS, Hoffman SMG (2012) Microsatellite genetic structure and cytonuclear discordance in naturally fragmented populations of deer mice (*Peromyscus maniculatus*). *J Hered* 103(1):71–79.

157. Taylor ZS, Hoffman SMG (2011) Data from: Microsatellite genetic structure and cytonuclear discordance in naturally fragmented populations of deer mice (*Peromyscus maniculatus*). *J Hered*. doi:doi:10.5061/dryad.gs048.

158. Bertrand P, Bowman J, Dyer R, Manseau M, PJ W (2017) Sex-specific graphs: Relating group-specific topology to demographic and landscape data. *Mol Ecol* 26:3898–3912.

159. Bertrand P, Bowman J, Dyer R, Manseau M, PJ W (2017) Data from: Sex-specific graphs: Relating group-specific topology to demographic and landscape data. *Mol Ecol*. doi:doi:10.5061/dryad.167d5.

160. Machado AP, Clément L, Uva V, Goudet J, Roulin A (2018) The Rocky Mountains as a

dispersal barrier between barn owl (*Tyto alba*) populations in North America. *J Biogeogr*:1–13.

161. Athrey G, Barr KR, Lance RF, Leberg PL (2012) Birds in space and time: Genetic changes accompanying anthropogenic habitat fragmentation in the endangered black-capped vireo (*Vireo atricapilla*). *Evol Appl* 5(6):540–552.

162. Athrey G, KR B, RF L, PL L (2012) Data from: Birds in space & time: genetic changes accompanying anthropogenic habitat fragmentation in the endangered black-capped vireo (*Vireo atricapilla*). *Evol Appl*. doi:doi:10.5061/dryad.2r030k5p.

Supplementary Information 3

Inconsistent effects of urbanization on amphibian genetic diversity

Contents:

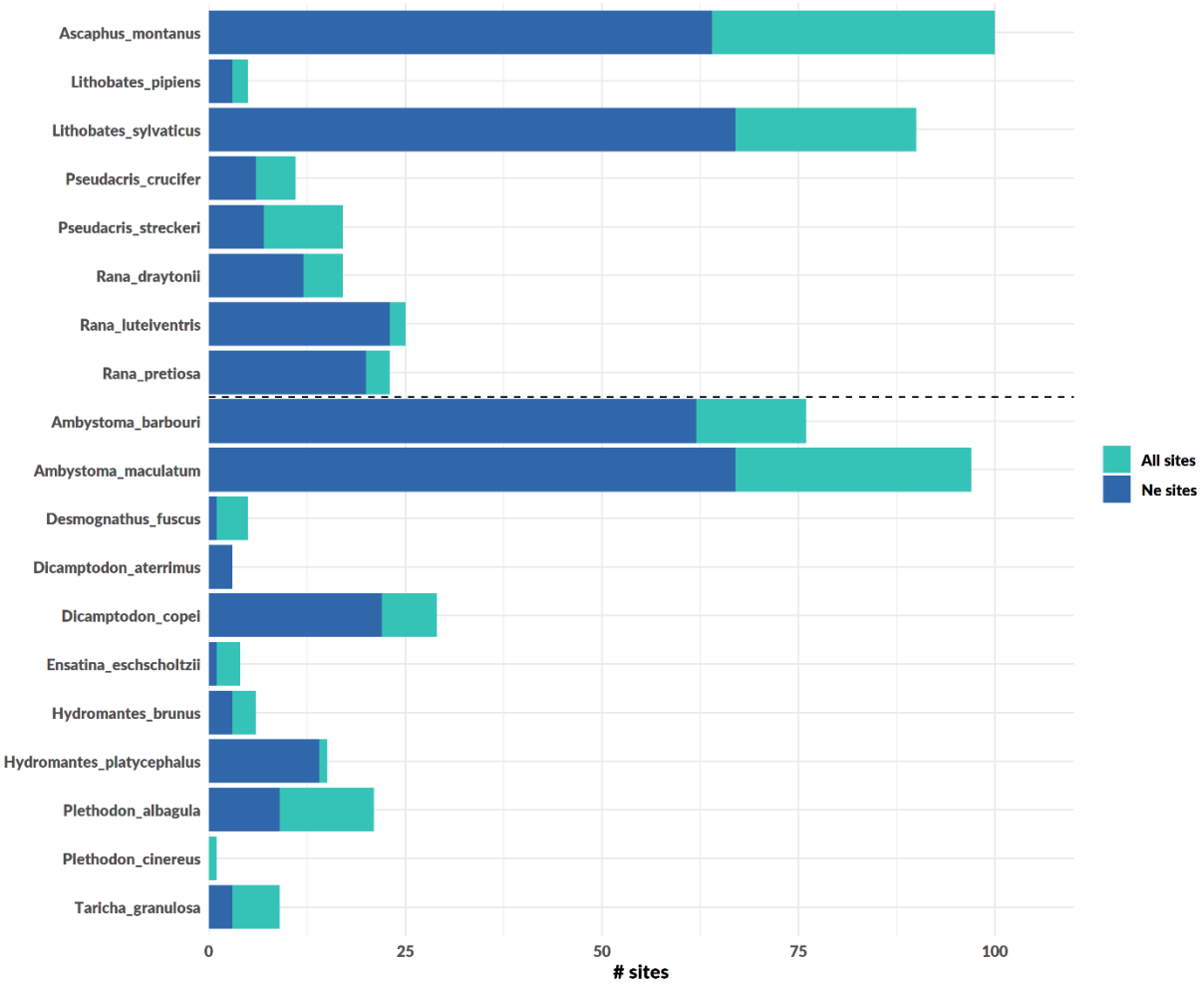


Figure S3.1. Comparison of the number of sites per species between the full dataset (*n* = 554 sites; turquoise bars) and the subset of data for which we were able to estimate effective population size (*n* = 387; dark blue bars). Frog species are above the dashed horizontal line, salamanders below. No species were disproportionately represented in the effective population size data subset.

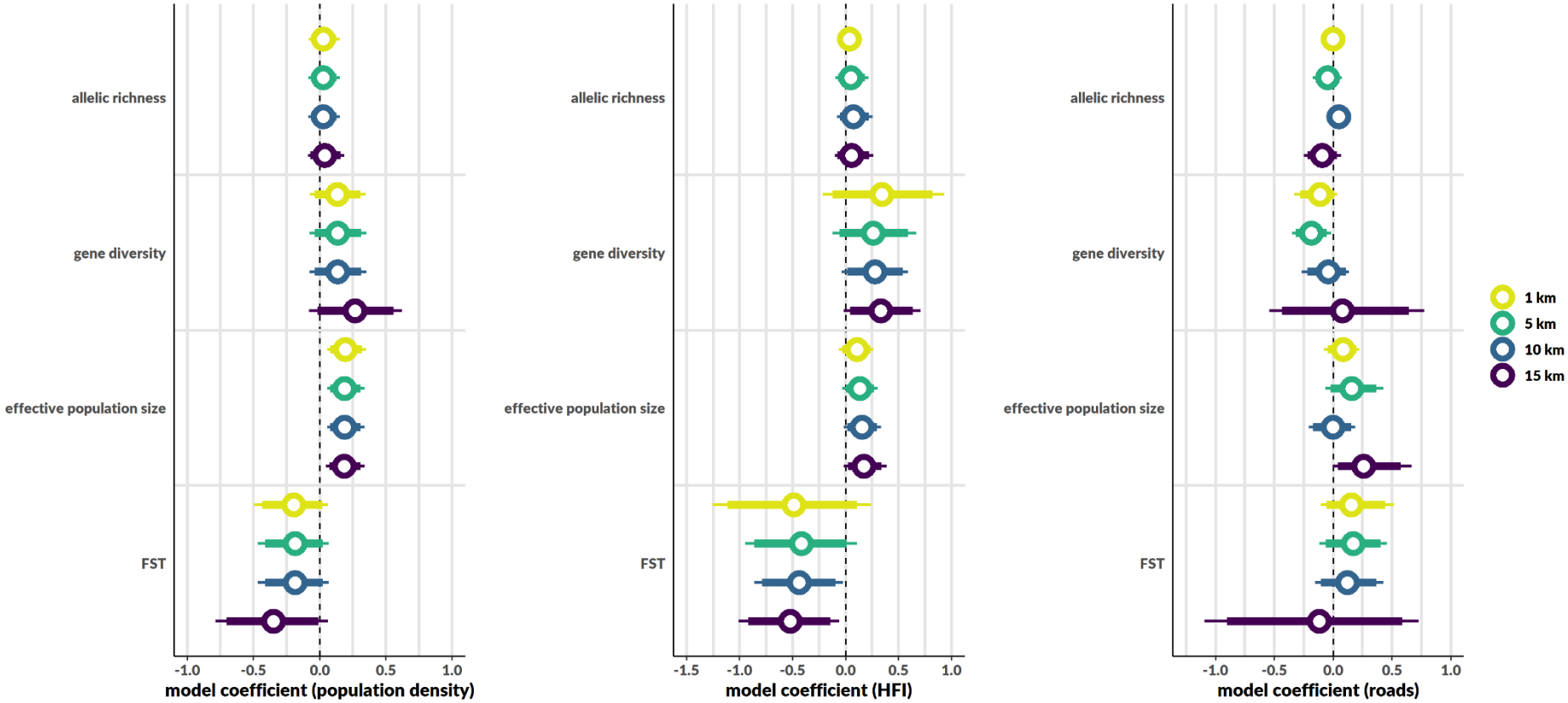


Figure S3.2. Effects of spatial scale of analysis. We extracted continuous measures of human presence within 1, 5, 10, and 15 km buffers around each site to test the effects of spatial scale on the relationships between genetic metrics and human presence. Responses to population density and Human Footprint Index (HFI) vary consistently in strength and direction regardless of scale, and effects of total road length at each site are more variable but generally weak at all scales.

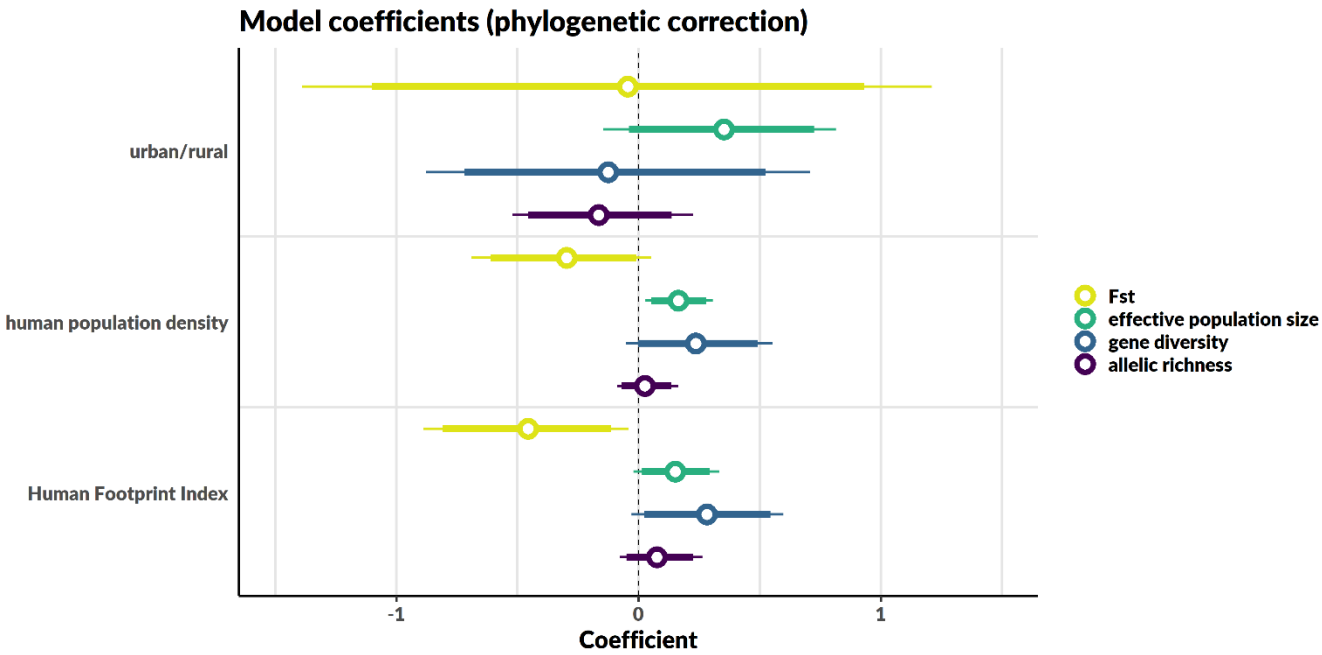


Figure S3.3. Coefficients for models presented in the main text reproduced including phylogeny as a random effect. Open circles are effect sizes, bold lines are 90% credible intervals, and narrow lines are 95% credible intervals. Similar effect sizes across all metrics suggest population genetic composition as measured here is not strongly affected by phylogeny.

Table S3.1. Model summaries for the effects of urbanization and human presence on population genetic composition corrected for phylogeny (shown in Figure S3.3). Four models were run for each response variable, three of which included a measure of human presence. The fourth was a null model including only spatial predictors as fixed effects if spatial autocorrelation was detected, otherwise was an intercept-only model. Marginal R^2 refers to the proportion of variation explained by fixed effects, and conditional R^2 is the variation explained by both fixed and random effects.

Variable	**Covariates**	**Coefficient (95% CI)**	**Marginal; Conditional R^2**
effective population size			
n = 387	urban/rural	0.35 (-0.14 – 0.82)	0.01; 0.32
	human population density	0.16 (0.03 – 0.31)	0.03; 0.33
	Human Footprint Index	0.15 (-0.02 – 0.33)	0.02; 0.36
	none	–	0.00; 0.31
gene diversity			
n = 554	urban/rural	-0.13 (-0.88 – 0.71)	0.11; 0.74
	human population density	0.24 (-0.05 – 0.55)	0.21; 0.76
	Human Footprint Index	0.28 (-0.03 – 0.60)	0.18; 0.77
	none	–	0.11; 0.74
allelic richness			
n = 554	urban/rural	-0.16 (-0.52 – 0.23)	0.03; 0.59
	human population density	0.03 (-0.09 – 0.16)	0.03; 0.59
	Human Footprint Index	0.08 (-0.08 – 0.26)	0.04; 0.60
	none	–	0.02; 0.59
F_{ST}			
n = 552	urban/rural	-0.04 (-1.39 – 1.21)	0.15; 0.58
	human population density	-0.30 (-0.69 – 0.05)	0.19; 0.60
	Human Footprint Index	-0.46 (-0.89 – -0.04)	0.21; 0.61
	none	–	0.18; 0.56

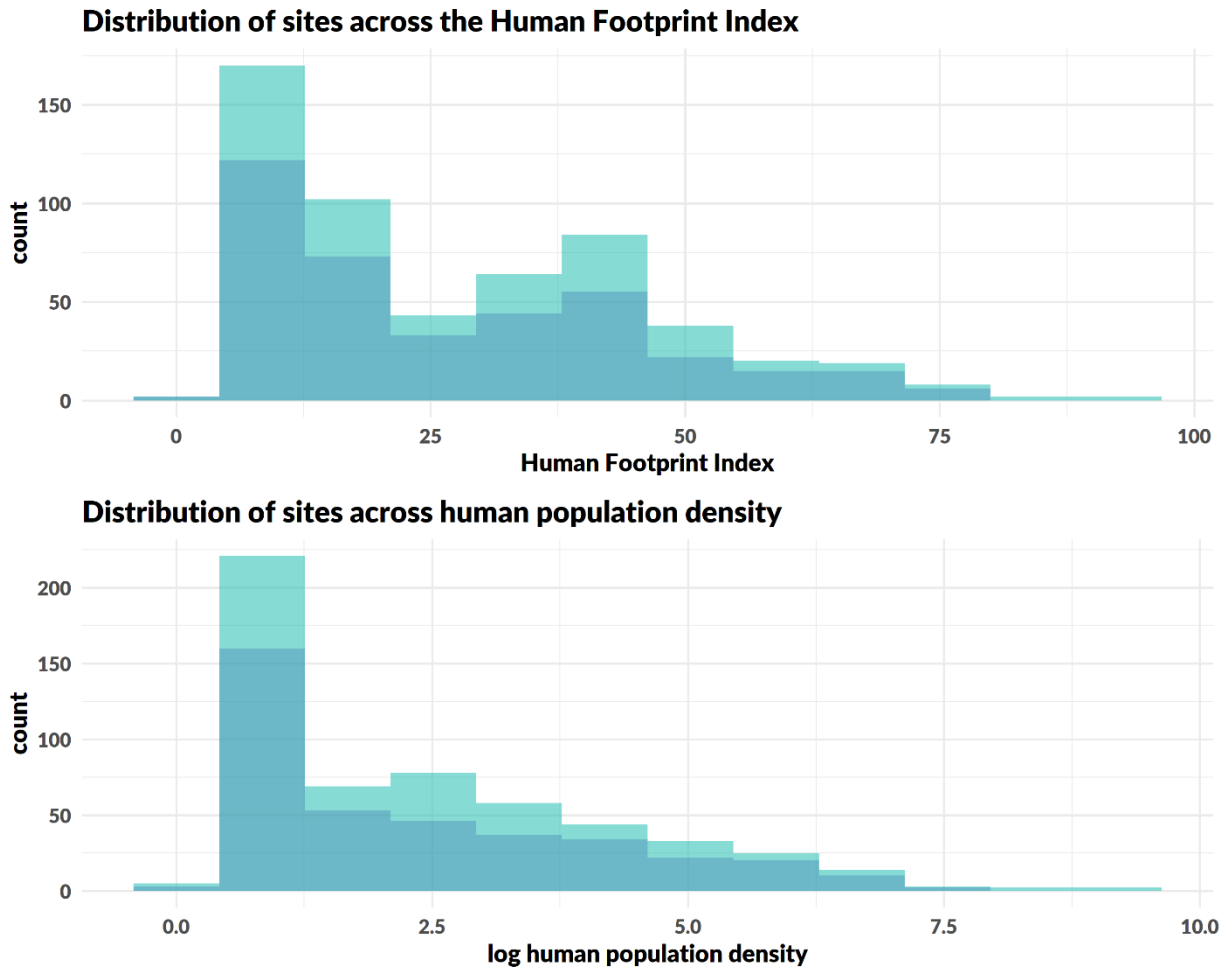


Figure S3.4. Distribution of sites across continuous measures of urbanization and human influence. A majority of sites are located in low to moderately transformed habitats. Lighter bars represent the full dataset (n = 554), and dark bars are the subset of data for which we were able to estimate effective population sizes (n = 387). Similar distributions indicate that excluding sites for which we were unable to estimate effective population size likely did not bias our analyses.

Table S3.2. Data synthesis summary. Raw microsatellite data was obtained from 19 studies. **Populations:** the number of populations with >5 individuals included in analyses. **Individuals**: the number of individuals summed across all populations. **Loci:** average number of loci sampled across populations. Mean and standard deviations are presented for gene diversity, allelic richness, population-specific F_{ST}, and effective population size (N_e).

Order	Species	Populations	Individuals	Loci	Gene diversity	Allelic richness	F_{ST}	N_e	Reference
Caudata	*Ambystoma barbouri*	76	1601	11.00	0.78 ± 0.07	6.50 ± 1.81	0.11 ± 0.08	151.53 ± 709.71	[1,2]
	Ambystoma maculatum	97	2407	9.16	0.69 ± 0.06	4.17 ± 0.44	0.05 ± 0.09	131.55 ± 262.17	[3–8]
	Desmognathus fuscus	5	140	5.00	0.39 ± 0.19	2.62 ± 0.85	0.40 ± 0.29	142.70 ± 0.00	[9,10]
	Dicamptodon aterrimus	3	361	9.00	0.48 ± 0.04	2.67 ± 0.27	0.22 ± 0.08	3.67 ± 2.84	[11,12]
	Dicamptodon copei	29	737	11.00	0.69 ± 0.13	4.98 ± 0.13	0.16 ± 0.15	583.46 ± 1752.00	[13,14]
	Ensatina eschscholtzii	4	47	10.00	0.80 ± 0.04	7.72 ± 1.35	0.09 ± 0.01	54.40 ± 0.00	[15,16]
	Hydromantes brunus	6	64	10.00	0.52 ± 0.08	3.52 ± 0.66	0.22 ± 0.12	81.57 ± 65.59	[17,18]
	Hydromantes platycephalus	15	195	10.00	0.44 ± 0.11	2.79 ± 0.16	0.48 ± 0.14	19.29 ± 19.20	[17,18]
	Plethodon albagula	21	343	20.00	0.48 ± 0.02	3.04 ± 0.09	0.02 ± 0.05	107.78 ± 105.99	[19,20]
	Plethodon cinereus	1	122	7.00	0.60 ± 0.00	3.51 ± 0.00	NA	NA	[21,22]
	Taricha granulosa	9	156	6.00	0.40 ± 0.15	2.64 ± 0.78	0.16 ± 0.24	20.07 ± 18.17	[23,24]
Anura	*Ascaphus montanus*	100	1968	13.00	0.74 ± 0.10	7.17 ± 2.26	0.07 ± 0.08	334.32 ± 701.47	[25,26]
	Lithobates pipiens	5	185	9.00	0.86 ± 0.02	6.58 ± 0.02	0.06 ± 0.02	81.00 ± 51.14	[27,28]
	Lithobates sylvaticus	90	2123	10.57	0.80 ± 0.04	6.18 ± 1.67	0.03 ± 0.04	169.63 ± 459.80	[3,4,7,8,29,30]
	Pseudacris crucifer	11	418	11.00	0.75 ± 0.05	4.96 ± 0.43	0.08 ± 0.04	878.17 ± 1197.22	[31,32]
	Pseudacris streckeri	17	181	14.00	0.59 ± 0.09	3.60 ± 0.80	0.15 ± 0.12	19.90 ± 24.10	[33,34]
	Rana draytonii	17	298	15.00	0.51 ± 0.11	2.95 ± 0.58	0.26 ± 0.16	55.06 ± 60.00	[35,36]
	Rana luteiventris	25	924	8.00	0.55 ± 0.08	3.43 ± 0.61	0.19 ± 0.15	22.78 ± 59.14	[37–40]
	Rana pretiosa	23	1410	10.39	0.32 ± 0.12	2.12 ± 0.47	0.38 ± 0.18	23.18 ± 20.60	[27,28,37,38]

References

1. Micheletti SJ, Storfer A. An approach for identifying cryptic barriers to gene flow that limit species' geographic ranges. Mol Ecol. 2017;26: 490–504. doi:10.1111/mec.13939

2. Micheletti SJ, Storfer A. Data from: An approach for identifying cryptic barriers to gene flow that limit species' geographic ranges. Dryad. 2016. doi:10.5061/dryad.c6kj2

3. Coster SS, Babbitt KJ, Cooper A, Kovach AI. Limited influence of local and landscape factors on finescale gene flow in two pond-breeding amphibians. Mol Ecol. 2015;24: 742–758. doi:10.1111/mec.13062

4. Coster SS, Babbitt KJ, Cooper A, Fraser University Kovach S, Kovach AI. Data from: Limited influence of local and landscape factors on finescale gene flow in two pond-breeding amphibians. Dryad. 2015. doi:10.5061/dryad.4903q

5. Johnson BB, White TA, Phillips CA, Zamudio KR, Shaffer HB. Asymmetric Introgression in a Spotted Salamander Hybrid Zone Corresponding editor. J Hered. 2015; 608–617. doi:10.1093/jhered/esv042

6. Johnson BB, White TA, Phillips CA, Zamudio KR. Data from: Asymmetric introgression in a spotted salamander hybrid zone. Dryad. 2015. doi:10.5061/dryad.v23r2

7. Richardson JL. Divergent landscape effects on population connectivity in two co-occurring amphibian species. Mol Ecol. 2012;21: 4437–4451. doi:10.1111/j.1365-294X.2012.05708.x

8. Richardson J. Data from: Divergent landscape effects on population connectivity in two co-occurring amphibian species. Dryad. 2012. doi:10.5061/dryad.51b94

9. Munshi-South J, Zak Y, Pehek E. Conservation genetics of extremely isolated urban populations of the northern dusky salamander (Desmognathus fuscus) in New York City. PeerJ. 2013;1: 1–16. doi:10.7717/peerj.64

10. Munshi-South J, Zak Y, Pehek E. Data from: Conservation genetics of extremely isolated urban populations of the Northern Dusky Salamander (Desmognathus fuscus) in New York City. Dryad. 2013. doi:10.5061/dryad.q1nc0

11. Mullen LB, Woods HA, Schwartz MK, Sepulveda AJ, Lowe WH. Scale-dependent genetic structure of the Idaho giant salamander (Dicamptodon aterrimus) in stream networks. Mol Ecol. 2010;19: 898–909. doi:10.1111/j.1365-294X.2010.04541.x

12. Mullen L, Woods H, Schwartz M, Mountain Research Station Sepulveda R, Sepulveda A, Lowe W. Data from: Scale-dependent genetic structure of the Idaho giant salamander (Dicamptodon aterrimus) in stream networks. Dryad. 2010. doi:10.5061/dryad.1187

13. Trumbo DR, Spear SF, Baumsteiger J, Storfer A. Rangewide landscape genetics of an endemic Pacific northwestern salamander. Mol Ecol. 2013;22: 1250–1266. doi:10.1111/mec.12168

14. Trumbo DR, Spear SF, Baumsteiger J, Storfer A. Data from: Rangewide landscape genetics of an endemic Pacific northwestern salamander. Dryad. 2012. doi:10.5061/dryad.s4sh6

15. Devitt TJ, Devitt SEC, Hollingsworth BD, McGuire JA, Moritz C. Montane refugia predict population genetic structure in the Large-blotched Ensatina salamander. Mol Ecol. 2013;22: 1650–1665. doi:10.1111/mec.12196

16. Devitt TJ, Cameron Devitt SE, Hollingsworth BD, McGuire JA, Moritz C. Data from: Montane refugia predict population genetic structure in the Large-blotched Ensatina salamander. Dryad. 2012. doi:10.5061/dryad.k9g50

17. Rovito SM, Schoville SD. Testing models of refugial isolation, colonization and population connectivity in two species of montane salamanders. Heredity (Edinb). 2017;119: 265–274. doi:10.1038/hdy.2017.31

18. Rovito SM;, Schoville SD. Data from: Testing models of refugial isolation, colonization and population connectivity in two species of montane salamanders. Dryad. 2017.

doi:10.5061/dryad.c197n

19. Peterman WE, Connette GM, Semlitsch RD, Eggert LS. Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander. Mol Ecol. 2014;23: 2402–2413. doi:10.1111/mec.12747

20. Peterman WE, Connette GM, Semlitsch RD, Eggert LS. Data from: Ecological resistance surfaces predict fine scale genetic differentiation in a terrestrial woodland salamander Dryad. Dryad. 2014. doi:10.5061/dryad.m4f17

21. Grant AH, Liebgold EB. Color-Biased Dispersal Inferred by Fine-Scale Genetic Spatial Autocorrelation in a Color Polymorphic Salamander. J Hered. 2017; 588–593. doi:10.1093/jhered/esx040

22. Grant AH, Liebgold EB. Data from: Color-biased dispersal inferred by fine-scale genetic spatial autocorrelation in a color polymorphic salamander. Dryad. 2017. doi:10.5061/dryad.732d5

23. Hague MTJ, Avila LA, Hanifin CT, Snedden WA, Stokes AN, Brodie ED. Toxicity and population structure of the Rough-Skinned Newt (Taricha granulosa) outside the range of an arms race with resistant predators. Ecol Evol. 2016;6: 2714–2724. doi:10.1002/ece3.2068

24. Hague MTJ, Avila LA, Hanifin CT, Snedden WA, Stokes AN, Brodie Jr ED, et al. Data from: Toxicity and population structure of the Rough-Skinned Newt (Taricha granulosa) outside the range of an arms race with resistant predators Data from: Toxicity and. Dryad. 2017. doi:10.5061/dryad.3c212

25. Metzger G, Espindola A, Waits LP, Sullivan J. Genetic Structure across Broad Spatial and Temporal Scales: Rocky Mountain Tailed Frogs (Ascaphus montanus; Anura: Ascaphidae) in the Inland Temperate Rainforest. J Hered. 2015; 700–710. doi:10.1093/jhered/esv061

26. Metzger G, Espindola A, Waits LP, Sullivan J. Data from: Genetic structure across broad spatial and temporal scales: Rocky Mountain tailed frogs (Ascaphus montanus;

Anura: Ascaphidae) in the inland temperate rainforest. Dryad. 2015. doi:10.5061/dryad.2pb57

27. Phillipsen IC, Funk WC, Hoffman EA, Monsen KJ, Blouin MS. Comparative analyses of effective population size within and among species: Ranid frogs as a case study. Evolution (N Y). 2011;65: 2927–2945. doi:10.1111/j.1558-5646.2011.01356.x

28. Phillipsen IC, Funk WC, Hoffman EA, Monsen KJ, Blouin MS. Data from: Comparative analyses of effective population size within and among species: ranid frogs as a case study. Dryad. 2011. doi:10.5061/dryad.q757q

29. Duncan SI, Crespi EJ, Mattheus NM, Rissler LJ. History matters more when explaining genetic diversity within the context of the core-periphery hypothesis. Mol Ecol. 2015;24: 4323–4336. doi:10.1111/mec.13315

30. Duncan SI, Crespi EJ, Mattheus NM, Rissler LJ. Data from: History matters more when explaining genetic diversity within the context of the core-periphery hypothesis. Dryad. 2015. doi:10.5061/dryad.fp1k7

31. Stewart KA, Austin JD, Zamudio KR, Lougheed SC. Contact zone dynamics during early stages of speciation in a chorus frog (Pseudacris crucifer). Heredity (Edinb). 2016;116: 239–247. doi:10.1038/hdy.2015.96

32. Stewart KA, Austin JD, Zamudio KR, Lougheed SC. Data from: Contact zone dynamics during early stages of speciation in a chorus frog (Pseudacris crucifer). Dryad. 2015.

33. Barrow LN, Bigelow AT, Phillips CA, Lemmon EM. Phylogeographic inference using Bayesian model comparison across a fragmented chorus frog species complex. Mol Ecol. 2015;24: 4739–4758. doi:10.1111/mec.13343

34. Barrow LN, Bigelow AT, Phillips CA, Lemmon EM. Data from: Phylogeographic inference using Bayesian model comparison across a fragmented chorus frog species complex Dryad. Dryad. 2015. doi:10.5061/dryad.h2082

35. Richmond JQ, Barr KR, Backlin AR, Vandergast AG, Fisher RN. Evolutionary dynamics of a rapidly receding southern range boundary in the threatened California Red-

Legged Frog (Rana draytonii). Evol Appl. 2013;6: 808–822. doi:10.1111/eva.12067

36. Richmond JQ, Barr KR, Backlin AR, Vandergast AG, Fisher RN. Data from: Evolutionary dynamics of a rapidly receding southern range boundary in the threatened California Red-Legged Frog (Rana draytonii). Dryad. 2013. Available: https://datadryad.org/stash/data_paper/doi:10.5061/dryad.g2b4h?

37. Robertson JM, Murphy MA, Pearl CA, Adams MJ, Páez-Vacas MI, Haig SM, et al. Regional variation in drivers of connectivity for two frog species (Rana pretiosa and R. luteiventris) from the U.S. Pacific Northwest. Mol Ecol. 2018;27: 3242–3256. doi:10.1111/mec.14798

38. Robertson JM, Murphy MA, Pearl CA, Adams MJ, Páez-Vacas MI, Haig SM, et al. Data from: Regional variation in drivers of connectivity for two frog species (Rana pretiosa and R. luteiventris) from the U.S. Pacific Northwest. Dryad. 2018. doi:10.5061/dryad.5n18790

39. Seaborn T, Hauser SS, Konrade L, Waits LP, Goldberg CS. Landscape genetic inferences vary with sampling scenario for a pond-breeding amphibian. Ecol Evol. 2019;9: 5063–5078. doi:10.1002/ece3.5023

40. Seaborn T, Hauser SS, Konrade L, Waits LP, Goldberg CS. Data from: Landscape genetic inferences vary with sampling scenario for a pond-breeding amphibian. Dryad. 2018. doi:10.5061/dryad.1nq73

Supplementary Information 4

Systemic racism alters genetic diversity in wildlife

Contents:

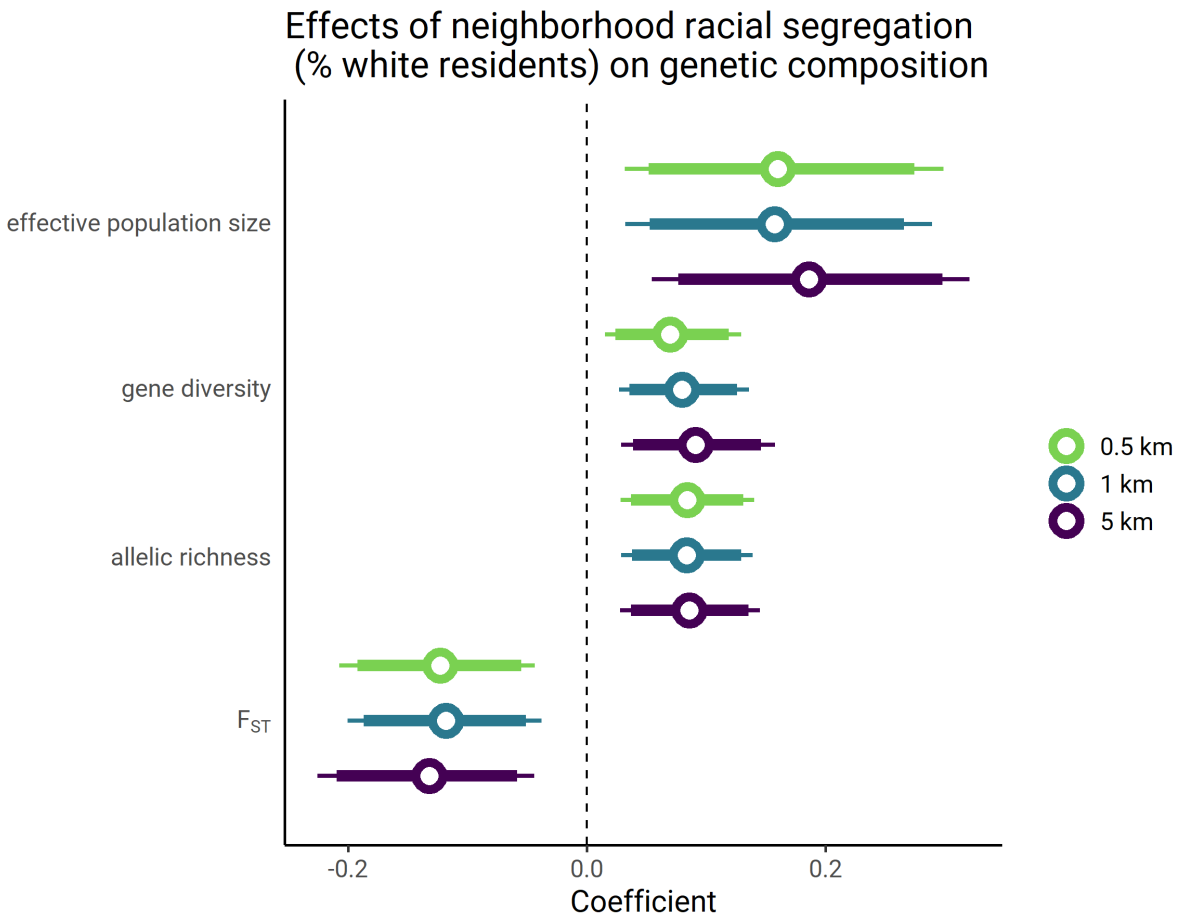


Figure S4.1.

Model results for the percent of residents identifying as white within 1 and 5 km of a sample site. Results within 0.5 km of a site (main text) are shown for comparison. Coefficient estimates (open circles) are given with 90% (narrow lines) and 95% (thick lines) credible intervals. Effects of neighborhood racial composition hold across all tested scales.

Table S4.1.

Amount of variation in genetic composition explained by fixed and random effects, shown for models including either racial segregation (neighborhood racial composition), the Human Footprint Index, or both, as covariates. Marginal R^2 (R^2_m) is the proportion of variation explained by fixed effects, and conditional R^2 (R^2_c) is that explained by fixed and random effects.

Variable	**Covariate**	**R^2_m; R^2_c**
allelic richness	Racial segregation (% white residents, 0.5 km)	0.01; 0.82
n = 202 sites	Human Footprint Index	0.01; 0.82
	both	0.00; 0.82
gene diversity	Racial segregation (% white residents, 0.5 km)	0.00; 0.90
n = 202 sites	Human Footprint Index	0.01; 0.91
	both	0.02; 0.90
effective population size	Racial segregation (% white residents, 0.5 km)	0.03; 0.34
n = 202 sites	Human Footprint Index	0.03; 0.36
	both	0.05; 0.37
F_{ST}	Racial segregation (% white residents, 0.5 km)	0.01; 0.66
n = 198 sites	Human Footprint Index	0.02; 0.69
	both	0.04; 0.69

Table S4.2. Data summary. List of classes, species, and the number of populations sampled within each species. Number of individuals are summed across all populations; loci is the mean number of loci used across studies. Species means and standard deviations (mean ± SD) are given for gene diversity, allelic richness, effective population size (N_e), and population-specific F_{ST}.

Class	Species	Populations	Individuals	Loci	Gene diversity	Allelic richness	N_e	F_{ST}
amphibian	*Ambystoma barbouri*	3	63	11.00	0.69 ± 0.03	5.91 ± 1.88	59.87 ± 31.10	0.21 ± 0.03
amphibian	*Ambystoma maculatum*	9	257	13.22	0.67 ± 0.02	4.2 ± 0.24	326.07 ± 623.47	0.05 ± 0.03
amphibian	*Desmognathus fuscus*	1	22	5.00	0.43	2.80	142.70	0.35
amphibian	*Lithobates sylvaticus*	10	223	14.10	0.81 ± 0.03	6.06 ± 0.22	116.72 ± 94.42	0.03 ± 0.02
amphibian	*Rana draytonii*	1	10	15.00	0.41	2.53	13.90	0.39
amphibian	*Rana luteiventris*	3	46	8.00	0.49 ± 0.12	3.07 ± 0.75	8.2 ± 3.75	0.27 ± 0.17
amphibian	*Taricha granulosa*	1	20	6.00	0.77	4.65	38.70	-0.39
bird	*Agelaius phoeniceus*	2	53	10.00	0.85 ± 0.02	6.44 ± 0.32	55.35 ± 17.75	0.02 ± 0.02
bird	*Aphelocoma californica*	6	111	13.00	0.7 ± 0.02	4.79 ± 0.11	74.8 ± 72.85	0.03 ± 0.01
bird	*Campylorhynchus brunneicapillus*	11	347	15.00	0.61 ± 0.04	5.14 ± 0.26	30.79 ± 23.00	0.05 ± 0.02
bird	*Charadrius melodus*	2	93	8.00	0.33 ± 0.10	1.92 ± 0.2	23.95 ± 16.19	0.12 ± 0.28
bird	*Laterallus jamaicensis*	1	123	15.00	0.71	4.35	1027.20	0.00
bird	*Poecile atricapillus*	5	89	11.00	0.68 ± 0.04	6.08 ± 1.63	208.6 ± 324.27	0.04 ± 0.05

bird	*Rallus obsoletus*	5	80	9.00	0.46 ± 0.02	2.77 ± 0.15	24.56 ± 32.18	0.05 ± 0.04
bird	*Selasphorus platycercus*	1	8	8.00	0.59	4.22	17.80	0.03
bird	*Sialia sialis*	3	49	12.00	0.69 ± 0.02	4.48 ± 0.06	68.33 ± 4.50	0.01 ± 0.00
bird	*Strix occidentalis*	1	39	10.00	0.73	4.23	139.00	0.02
bird	*Tyto alba*	6	154	20.00	0.5 ± 0.01	3.21 ± 0.07	133.28 ± 108.12	0.02 ± 0.02
bird	*Vireo atricapilla*	1	34	9.00	0.77	5.03	35.90	0.01
mammal	*Canis latrans*	20	180	9.95	0.77 ± 0.03	5.76 ± 0.91	51.19 ± 83.06	0.03 ± 0.03
mammal	*Cervus elaphus nannodes*	1	21	20.00	0.39	2.03	40.00	0.08
mammal	*Leopardus pardalis*	1	28	10.00	0.58	2.98	8.40	-0.02
mammal	*Lepus americanus*	4	154	8.00	0.65 ± 0.11	4.67 ± 0.70	67.2 ± 30.34	0.17 ± 0.13
mammal	*Lynx rufus*	20	957	13.95	0.71 ± 0.05	4.23 ± 0.42	213.78 ± 399.37	0.06 ± 0.05
mammal	*Odocoileus hemionus*	10	506	10.50	0.63 ± 0.05	3.7 ± 0.35	144.98 ± 185.49	0.08 ± 0.04
mammal	*Odocoileus virginianus*	33	1126	14.00	0.81 ± 0.02	5.55 ± 0.13	6309.87 ± 34696.13	0.01 ± 0.01
mammal	*Otospermophilus beecheyi*	1	40	11.00	0.72	4.81	37.80	0.15
mammal	*Pekania pennanti*	1	22	16.00	0.61	3.37	25.10	0.09
mammal	*Peromyscus leucopus*	20	362	15.20	0.81 ± 0.03	5.64 ± 0.46	35.33 ± 46.07	0.08 ± 0.03
mammal	*Peromyscus maniculatus*	1	31	10.00	0.80	5.38	15.10	NA
mammal	*Puma concolor*	8	448	37.00	0.45 ± 0.07	2.51 ± 0.38	30.74 ± 31.34	0.21 ± 0.14
mammal	*Sylvilagus transitionalis*	2	151	10.00	0.47 ± 0.00	2.58 ± 0.08	17.7 ± 19.94	0.07 ± 0.00
mammal	*Tamiasciurus douglasii*	1	18	9.00	0.66	3.91	106.60	0.03

mammal	*Taxidea taxus*	3	369	14.67	0.74 ± 0.07	4.42 ± 0.73	158.77 ± 191.75	0.13 ± 0.01
mammal	*Ursus americanus*	7	142	15.00	0.64 ± 0.13	3.78 ± 0.92	95.61 ± 105.79	0.22 ± 0.13
mammal	*Vulpes vulpes*	11	364	13.00	0.63 ± 0.04	3.67 ± 0.33	15.81 ± 11.04	0.1 ± 0.07
reptile	*Chrysemys picta*	2	56	11.00	0.75 ± 0.02	5.81 ± 0.03	107.7 ± 88.67	0.04 ± 0.00
reptile	*Gopherus polyphemus*	6	224	20.00	0.61 ± 0.03	3.75 ± 0.10	78.87 ± 67.29	0.17 ± 0.04
reptile	*Uma inornata*	2	52	11.00	0.58 ± 0.07	3.64 ± 0.01	95 ± 117.80	0.01 ± 0.05

Table S4.3. References for newly acquired raw microsatellite datasets. References for other mammal and bird datasets can be found in Appendix S2, and for amphibians in Appendix S3.

Class	Species	Search date	References
reptile	*Chrysemys picta*	Feb-19	(B. N. Reid et al., 2019; Brendan N. Reid et al., 2018)
reptile	*Gopherus polyphemus*	Feb-19	(D. Gaillard et al., 2017; Daniel Gaillard et al., 2017; White, Rothermel, Zamudio, & Tuberville, 2018b, 2018a; Yuan, Dean, Longo Berrios, et al., 2015; Yuan, Dean, Longo, et al., 2015)
reptile	*Uma inornata*	Feb-19	(Vandergast et al., 2016a, 2016b)
mammal	*Cynomys leucurus*	Nov-20	(Hoogland, Trott, & Keller, 2019b, 2019a)
mammal	*Dipodomys ingens*	Nov-20	(Statham, Bean, Alexander, Westphal, & Sacks, 2019b, 2019a)
mammal	*Myotis lucifugus*	Nov-20	(C. L. Lausen et al., 2019; C. Lausen et al., 2018)
mammal	*Myotis septentrionalis*	Nov-20	(C. L. Lausen et al., 2019; C. Lausen et al., 2018)
mammal	*Myotis thysanodes*	Nov-20	(C. L. Lausen et al., 2019; C. Lausen et al., 2018)
mammal	*Canis latrans*	Nov-20	DeCandia, Murphy(DeCandia, Henger, Krause, Gormezano, Weckel, Nagy, Munshi-South, & Vonholdt, 2019; DeCandia, Henger, Krause, Gormezano, Weckel, Nagy, Munshi-South, & VonHoldt, 2019; Murphy, Adams, Cox, & Waits, 2018, 2019)
mammal	*Martes americana*	Nov-20	(Manlick, Romanksi, & Pauli, 2019; Manlick, Romanski, & Pauli, 2018)
mammal	*Taxidea taxus*	Nov-20	(Ford, Weir, Lewis, Larsen, & Russello, 2019, 2020)
mammal	*Vulpes vulpes*	Nov-20	(Quinn, Alden, & Sacks, 2019b, 2019a)

Table S4.3 References

DeCandia, A.L., Henger, C.S., Krause, A., Gormezano, L.J., Weckel, M., Nagy, C., Munshi-South, J., and Vonholdt, B.M. 2019a. Genetics of urban colonization: Neutral and adaptive variation in coyotes (*Canis latrans*) inhabiting the New York metropolitan area. J. Urban Ecol. **5**(1). Oxford University Press. doi:10.1093/jue/juz002.

DeCandia, A.L., Henger, C.S., Krause, A., Gormezano, L.J., Weckel, M., Nagy, C., Munshi-South, J., and VonHoldt, B.M. 2019b. Dryad Data -- Genetics of urban colonization: neutral and adaptive variation in coyotes (*Canis latrans*) inhabiting the New York metropolitan area. Dryad. doi:10.5061/dryad.c0282c8.

Ford, B.M., Weir, R.D., Lewis, J.C., Larsen, K.W., and Russello, M.A. 2019. Fine-scale genetic structure and conservation status of American badgers at their northwestern range periphery. Conserv. Genet. **20**(5): 1023–1034. Springer Netherlands. doi:10.1007/s10592-019-01192-2.

Ford, B.M., Weir, R.D., Lewis, J.C., Larsen, K.W., and Russello, M.A. 2020. Dryad Data -- Fine-scale genetic structure and conservation status of American badgers at their northwestern range periphery. Dryad. doi:10.5061/dryad.cj3v894.

Gaillard, D., Ennen, J.R., Kreiser, B.R., Qualls, C.P., Sweat, S.C., Birkhead, R., Tuberville, T.D., Aresco, M., McCoy, E.D., Mushinsky, H.R., and Hentges, T.W. 2017a. Range-wide and regional patterns of population structure and genetic diversity in the gopher tortoise. J. Fish Wildl. Manag. **8**(2): 497–512. Allen Press. doi:10.3996/022017-JFWM-010.

Gaillard, D., Ennen, J.R., Kreiser, B.R., Qualls, C.P., Sweat, S.C., Birkhead, R., Tuberville, T.D., Aresco, M., McCoy, E.D., Mushinsky, H.R., Hentges, T.W., Kreiser, B.R., Qualls, C.P., Tuberville, T.D., McCoy, E.D., Mushinsky, H.R., and Hentges, T.W. 2017b. Dryad Data -- Range-wide and regional patterns of population structure and genetic diversity in the gopher tortoise. Dryad. doi:10.5061/dryad.nk064.

Hoogland, J.L., Trott, R., and Keller, S.R. 2019a. Polyandry and polygyny in a social rodent: An integrative perspective based on social organization, copulations, and genetics. Front. Ecol. Evol. **7**(FEB). Frontiers Media S.A. doi:10.3389/fevo.2019.00003.

Hoogland, J.L., Trott, R., and Keller, S.R. 2019b. Dryad Data -- Polyandry and polygyny in a social rodent: an integrative perspective based on social organization, copulations, and

genetics. Dryad. doi:10.5061/dryad.jn365c2.

Lausen, C., Proctor, M.F., Nagorsen, D., Burles, D., Paetkau, D., Harmston, E., Blejwas, K., Govindarajulu, P., and Friis, L. 2018. Dryad Data -- Population genetics reveal Myotis keenii (Keen's myotis) and Myotis evotis (long-eared myotis) to be a single species. Dryad. doi:10.5061/dryad.h9b3d30.

Lausen, C.L., Proctor, M., Nagorsen, D.W., Burles, D., Paetkau, D., Harmston, E., Blejwas, K., Govindarajulu, P., and Friis, L. 2019. Population genetics reveal myotis keenii (Keen's myotis) and myotis evotis (long-eared myotis) to be a single species. Can. J. Zool. **97**(3): 267–279. Canadian Science Publishing. doi:10.1139/cjz-2018-0113.

Manlick, P.J., Romanksi, M.C., and Pauli, J.N. 2019. Dryad Data -- Dynamic colonization history in a rediscovered Isle Royale carnivore. Dryad. doi:10.5061/dryad.m58q16m.

Manlick, P.J., Romanski, M.C., and Pauli, J.N. 2018. Dynamic colonization history in a rediscovered Isle Royale carnivore. Sci. Rep. **8**(1). Nature Publishing Group. doi:10.1038/s41598-018-31130-0.

Murphy, S.M., Adams, J.R., Cox, J.J., and Waits, L.P. 2018. Dryad Data -- Substantial red wolf genetic ancestry persists in wild canids of southwestern Louisiana. Dryad. doi:10.5061/dryad.46c39p1.

Murphy, S.M., Adams, J.R., Cox, J.J., and Waits, L.P. 2019, March 1. Substantial red wolf genetic ancestry persists in wild canids of southwestern Louisiana. Wiley-Blackwell. doi:10.1111/conl.12621.

Quinn, C.B., Alden, P.B., and Sacks, B.N. 2019a. Dryad Data -- Noninvasive sampling reveals short-term genetic rescue in an insular red fox population. Dryad. doi:10.5061/dryad.5k8q374.

Quinn, C.B., Alden, P.B., and Sacks, B.N. 2019b. Noninvasive Sampling Reveals Short-Term Genetic Rescue in an Insular Red Fox Population. J. Hered. **110**(5): 559–576. Oxford University Press. doi:10.1093/jhered/esz024.

Reid, B.N., Kass, J.M., Wollney, S., Jensen, E.L., Russello, M.A., Viola, E.M., Pantophlet, J., Iverson, J.B., Peery, M.Z., Raxworthy, C.J., and Naro-Maciel, E. 2018. Dryad Data -- Disentangling the genetic effects of refugial isolation and range expansion in a trans-continentally distributed species. Dryad. doi:10.5061/dryad.8rb35rj.

Reid, B.N., Kass, J.M., Wollney, S., Jensen, E.L., Russello, M.A., Viola, E.M., Pantophlet, J., Iverson, J.B., Peery, M.Z., Raxworthy, C.J., and Naro-Maciel, E. 2019. Disentangling the genetic effects of refugial isolation and range expansion in a trans-continentally distributed species. Heredity. **122**(4): 441–457. doi:10.1038/s41437-018-0135-5.

Schmidt, C., Domaratzki, M., Kinnunen, R.P., Bowman, J., and Garroway, C.J. 2020. Continent-wide effects of urbanization on bird and mammal genetic diversity. Proc. R. Soc. B Biol. Sci. **287**(1920): 20192497. doi:10.1098/rspb.2019.2497.

Schmidt, C., and Garroway, C.J. 2020. Inconsistent effects of urbanization on amphibian genetic diversity. bioRxiv: 1–30. doi:10.1101/2020.08.16.253104.

Statham, M.J., Bean, W.T., Alexander, N., Westphal, M.F., and Sacks, B.N. 2019a. Dryad Data -- Historical population size change and differentiation of relict populations of the endangered giant kangaroo rat. Dryad. doi:10.5061/dryad.5jh21k3.

Statham, M.J., Bean, W.T., Alexander, N., Westphal, M.F., and Sacks, B.N. 2019b. Historical Population Size Change and Differentiation of Relict Populations of the Endangered Giant Kangaroo Rat. J. Hered. **110**(5): 548–558. Oxford University Press. doi:10.1093/jhered/esz006.

Vandergast, A.G., Wood, D.A., Thompson, A.R., Fisher, M., Barrows, C.W., and Grant, T.J. 2016a. Dryad Data -- Drifting to oblivion? Rapid genetic differentiation in an endangered lizard following habitat fragmentation and drought. Dryad. doi:10.5061/dryad.30t5b.

Vandergast, A.G., Wood, D.A., Thompson, A.R., Fisher, M., Barrows, C.W., and Grant, T.J. 2016b. Drifting to oblivion? Rapid genetic differentiation in an endangered lizard following habitat fragmentation and drought. Divers. Distrib. **22**(3): 344–357. Blackwell Publishing Ltd. doi:10.1111/ddi.12398.

White, K.N., Rothermel, B.B., Zamudio, K.R., and Tuberville, T.D. 2018a. Dryad Data -- Male body size predicts reproductive success but not within-clutch paternity patterns in gopher tortoises (Gopherus polyphemus). Dryad. doi:10.5061/dryad.31bc37q.

White, K.N., Rothermel, B.B., Zamudio, K.R., and Tuberville, T.D. 2018b. Male body size predicts reproductive success but not within-clutch paternity patterns in gopher tortoises (Gopherus polyphemus). J. Hered. **109**(7): 791–801. Oxford University Press.

doi:10.1093/jhered/esy036.

Yuan, M.L., Dean, S.H., Longo, A. V., Rothermel, B.B., Tuberville, T.D., and Zamudio, K.R. 2015a. Kinship, inbreeding and fine-scale spatial structure influence gut microbiota in a hindgut-fermenting tortoise. Mol. Ecol. **24**(10): 2521–2536. Blackwell Publishing Ltd. doi:10.1111/mec.13169.

Yuan, M.L., Dean, S.H., Longo Berrios, A. V., Rothermel, B.B., Tuberville, T.D., Zamudio, K.R., and Longo, A. V. 2015b. Dryad Data -- Kinship, inbreeding, and fine-scale spatial structure influence gut microbiota in a hindgut-fermenting tortoise. Dryad. doi:10.5061/dryad.54bm8.

Table S4.4. Raw search results for reptile and additional mammal datasets performed in February 2019 and November 2020, respectively. Reason for exclusion is NA if study met inclusion criteria.

Class	Search Date	Reason for exclusion	URL
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.6r7qn?ver=2017-07-19T10:01:44.901-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.6r7qn?ver=2017-08-16T11:46:06.333-04:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.pp6bm/9?ver=2015-07-24T12:53:55.745-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.pp6bm/13?ver=2015-06-03T17:09:06.080-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.pp6bm/10?ver=2015-06-03T17:05:53.305-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.pp6bm/11?ver=2015-06-03T17:06:56.070-04:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.4gb62?ver=2016-04-05T20:17:58.764-04:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.1tv72?ver=2017-02-13T11:11:53.277-05:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.1tv72?ver=2017-03-10T11:14:04.837-05:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.32h8t/1?ver=2015-10-01T17:00:02.499-04:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.3780c/1?ver=2016-05-06T14:07:01.196-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.1tv72/1?ver=2017-03-10T11:14:09.117-05:00
reptile	February 2019	NA	https://doi.org/10.5061/dryad.cc6r3?ver=2017-11-08T08:18:53.221-05:00
reptile	February 2019	duplicate	https://doi.org/10.5061/dryad.cc6r3/1?ver=2017-11-08T08:18:55.630-05:00
reptile	February 2019	NA	http://dx.doi.org/10.5061/dryad.30t5b/1?ver=2015-11-19T12:07:35.851-05:00
reptile	February 2019	SNP	http://dx.doi.org/10.5061/dryad.8br5c?ver=2016-11-29T11:42:36.904-05:00

reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.30t5b?ver=2016-03-03T10:39:21.500-05:00
reptile	February 2019	island	http://dx.doi.org/10.5061/dryad.6c7p5/1?ver=2016-11-23T11:51:06.881-05:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.6c7p5?ver=2016-11-23T11:50:57.890-05:00
reptile	February 2019	marine species	http://dx.doi.org/10.5061/dryad.q1kf0/2?ver=2014-10-14T14:13:13.498-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.q1kf0/1?ver=2014-10-14T14:13:12.459-04:00
reptile	February 2019	marine species	http://dx.doi.org/10.5061/dryad.7dk0m36r/1?ver=2013-05-16T02:30:08.080-04:00
reptile	February 2019	outside North America	https://doi.org/10.5061/dryad.7db01?ver=2018-05-22T14:04:14.702+00:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.q1kf0?ver=2014-10-14T14:13:12.507-04:00
reptile	February 2019	duplicate	https://doi.org/10.5061/dryad.7db01/2?ver=2018-04-23T16:35:58.186+00:00
reptile	February 2019	duplicate	https://doi.org/10.5061/dryad.7db01/3?ver=2018-04-23T16:35:58.616+00:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.mb2sf?ver=2014-04-09T16:58:42.922-04:00
reptile	February 2019	outside North America	https://doi.org/10.5061/dryad.d3kk74r?ver=2018-08-13T13:06:33.980+00:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.t5952?ver=2012-10-02T11:02:41.091-04:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.6k2qm/1?ver=2015-04-16T12:43:02.465-04:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.048kf?ver=2016-08-31T17:30:53.860-04:00
reptile	February 2019	NA	http://dx.doi.org/10.5061/dryad.77rf2/2?ver=2014-06-06T17:18:52.451-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.77rf2/1?ver=2014-06-06T17:18:45.299-04:00
reptile	February 2019	NA	http://dx.doi.org/10.5061/dryad.t0j7s/1?ver=2013-05-21T10:58:46.161-04:00
reptile	February 2019	wrong taxa	http://dx.doi.org/10.5061/dryad.rb7h0/3?ver=2016-06-16T11:55:54.677-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.rb7h0/2?ver=2016-06-16T11:55:51.774-04:00

reptile	February 2019	genetic restoration	http://dx.doi.org/10.5061/dryad.ps736/1?ver=2013-07-11T12:11:02.666-04:00
reptile	February 2019	SNP	http://dx.doi.org/10.5061/dryad.p6m94/4?ver=2016-06-08T09:53:41.409-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.p6m94/5?ver=2016-06-08T09:53:47.011-04:00
reptile	February 2019	SNP	https://doi.org/10.5061/dryad.k7k4m/3?ver=2018-05-03T12:50:08.216+00:00
reptile	February 2019	NA	http://dx.doi.org/10.5061/dryad.50070?ver=2014-02-11T10:57:33.277-05:00
reptile	February 2019	SNP	http://dx.doi.org/10.5061/dryad.19gp1?ver=2017-01-26T10:46:46.236-05:00
reptile	February 2019	wrong taxa	http://dx.doi.org/10.5061/dryad.j7260?ver=2014-02-14T15:12:14.172-05:00
reptile	February 2019	wrong taxa	http://dx.doi.org/10.5061/dryad.c62gg/1?ver=2013-12-18T15:34:27.101-05:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.c62gg/2?ver=2013-12-18T15:35:14.414-05:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.c62gg/3?ver=2013-12-18T15:35:58.876-05:00
reptile	February 2019	hybrid zone	http://dx.doi.org/10.5061/dryad.14811?ver=2016-11-17T10:31:59.918-05:00
reptile	February 2019	wrong taxa	http://dx.doi.org/10.5061/dryad.6mt23?ver=2015-06-03T09:35:09.158-04:00
reptile	February 2019	NA	http://dx.doi.org/10.5061/dryad.v22n5/1?ver=2014-07-29T02:30:04.181-04:00
reptile	February 2019	no coordinates	http://dx.doi.org/10.5061/dryad.s6f76?ver=2016-05-18T16:29:09.831-04:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.ct849?ver=2016-01-04T11:46:03.357-05:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.rq430/1?ver=2014-02-04T02:30:10.986-05:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.rq430?ver=2014-02-04T02:30:11.019-05:00
reptile	February 2019	outside North America	http://dx.doi.org/10.5061/dryad.6697t?ver=2017-08-10T14:53:05.430-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.ct849/1?ver=2015-11-25T09:18:46.004-05:00
reptile	February 2019	non-neutral	http://dx.doi.org/10.5061/dryad.7ck13/5?ver=2017-04-13T10:58:03.807-04:00

reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.7ck13/6?ver=2017-04-13T10:58:09.420-04:00
reptile	February 2019	no microsatellite data	http://dx.doi.org/10.5061/dryad.40c7c/2?ver=2016-08-02T16:54:46.211-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.40c7c/1?ver=2016-08-02T16:54:44.683-04:00
reptile	February 2019	NA	http://dx.doi.org/10.5061/dryad.nk064/1?ver=2017-07-28T10:33:28.805-04:00
reptile	February 2019	NA	https://doi.org/10.5061/dryad.31bc37q?ver=2018-08-06T12:14:39.063+00:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.7ck13?ver=2017-06-27T23:15:03.209-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.nk064/2?ver=2017-07-28T10:33:31.619-04:00
reptile	February 2019	duplicate	https://doi.org/10.5061/dryad.31bc37q/1?ver=2018-07-30T21:50:40.332+00:00
reptile	February 2019	NA	http://dx.doi.org/10.5061/dryad.54bm8/2?ver=2015-03-31T21:34:30.861-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.40c7c?ver=2017-05-15T20:31:23.849-04:00
reptile	February 2019	mtDNA	http://dx.doi.org/10.5061/dryad.4hs71t6t?ver=2012-06-26T10:59:57.747-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.nk064?ver=2017-08-24T08:15:25.367-04:00
reptile	February 2019	wrong taxa	http://dx.doi.org/10.5061/dryad.3c212?ver=2016-03-18T15:59:30.598-04:00
reptile	February 2019	NA	https://doi.org/10.5061/dryad.8rb35rj?ver=2018-07-24T18:11:49.412+00:00
reptile	February 2019	parentage analysis	https://doi.org/10.5061/dryad.121sk?ver=2017-10-26T13:32:16.379-04:00
reptile	February 2019	duplicate	https://doi.org/10.5061/dryad.121sk/1?ver=2017-10-26T13:32:18.977-04:00
reptile	February 2019	marine species	https://doi.org/10.5061/dryad.q2kf0?ver=2018-01-29T09:54:13.769-05:00
reptile	February 2019	NA	http://dx.doi.org/10.5061/dryad.p5c04?ver=2016-08-31T17:27:54.752-04:00
reptile	February 2019	duplicate	http://dx.doi.org/10.5061/dryad.p5c04/1?ver=2017-01-06T15:45:11.178-05:00
mammal	November 2020	NA	https://datadryad.org/stash/dataset/doi:10.5061/dryad.jn365c2

mammal	November 2020	already included	https://datadryad.org/stash/dataset/doi:10.5061/dryad.qn4kq
mammal	November 2020	NA	https://datadryad.org/stash/dataset/doi:10.5061/dryad.5jh21k3
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.dk73qp7
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.nvx0k6dqm
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.th71ss0
mammal	November 2020	already included	https://datadryad.org/stash/dataset/doi:10.5061/dryad.s04h8
mammal	November 2020	NA	https://datadryad.org/stash/dataset/doi:10.5061/dryad.h9b3d30
mammal	November 2020	already included	https://datadryad.org/stash/dataset/doi:10.5061/dryad.h7n25
mammal	November 2020	duplicate	https://datadryad.org/stash/dataset/doi:10.5061/dryad.h9b3d30
mammal	November 2020	samples not from populations	https://datadryad.org/stash/dataset/doi:10.5061/dryad.9nb07pr
mammal	November 2020	duplicate	https://datadryad.org/stash/dataset/doi:10.5061/dryad.5b2k6
mammal	November 2020	data too sparse	https://datadryad.org/stash/dataset/doi:10.5061/dryad.t77f1p4
mammal	November 2020	NA	https://datadryad.org/stash/dataset/doi:10.5061/dryad.j76c4k4
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.xwdbrv195
mammal	November 2020	NA	https://datadryad.org/stash/dataset/doi:10.5061/dryad.c0282c8
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.17r39p2
mammal	November 2020	already included	https://datadryad.org/stash/dataset/doi:10.5061/dryad.p2ngf1vp0
mammal	November 2020	duplicate	https://datadryad.org/stash/dataset/doi:10.5061/dryad.qn4kq
mammal	November 2020	NA	https://datadryad.org/stash/dataset/doi:10.5061/dryad.5k8q374
mammal	November 2020	already included	https://datadryad.org/stash/dataset/doi:10.5061/dryad.8ff46

mammal	November 2020	already included	https://datadryad.org/stash/dataset/doi:10.5061/dryad.bj7r3
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.vr61ks2
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.dv41ns1ts
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.54p37
mammal	November 2020	NA	https://datadryad.org/stash/dataset/doi:10.5061/dryad.46c39p1
mammal	November 2020	already included	https://datadryad.org/stash/dataset/doi:10.5061/dryad.f81c5
mammal	November 2020	SNP	https://datadryad.org/stash/dataset/doi:10.5061/dryad.gb5mkkwkw
mammal	November 2020	duplicate	https://datadryad.org/stash/dataset/doi:10.5061/dryad.qn4kq
mammal	November 2020	data too sparse	https://datadryad.org/stash/dataset/doi:10.5061/dryad.m58q16m
mammal	November 2020	non-neutral	https://datadryad.org/stash/dataset/doi:10.5061/dryad.rr4xgxd55
mammal	November 2020	NA	https://datadryad.org/stash/dataset/doi:10.5061/dryad.cj3v894
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.c6t0470
mammal	November 2020	non-urban populations	https://datadryad.org/stash/dataset/doi:10.5061/dryad.8931zcrmb
mammal	November 2020	outside North America	https://datadryad.org/stash/dataset/doi:10.5061/dryad.43j74d0
mammal	November 2020	non-urban populations	https://datadryad.org/stash/dataset/doi:10.5061/dryad.7k2g187
mammal	November 2020	already included	https://datadryad.org/stash/dataset/doi:10.5061/dryad.n8v973b

Supplementary Information 5

Genetic and species-level biodiversity patterns are linked by demography and ecological opportunity

Contents

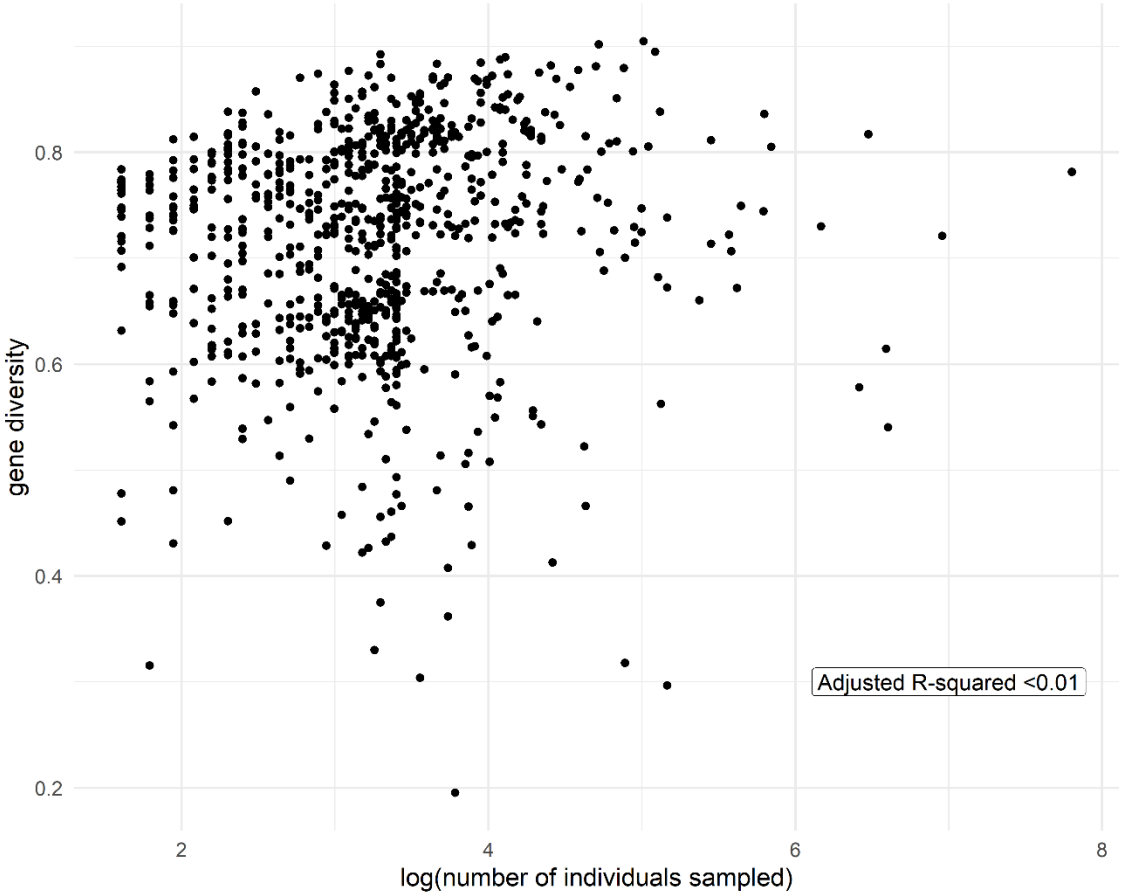


Figure S5.1. Plot of gene diversity vs. sample size. Gene diversity as a metric of genetic diversity depends on allele frequencies and is minimally affected by sample size. Larger populations have more rare alleles, which contribute little to gene diversity.

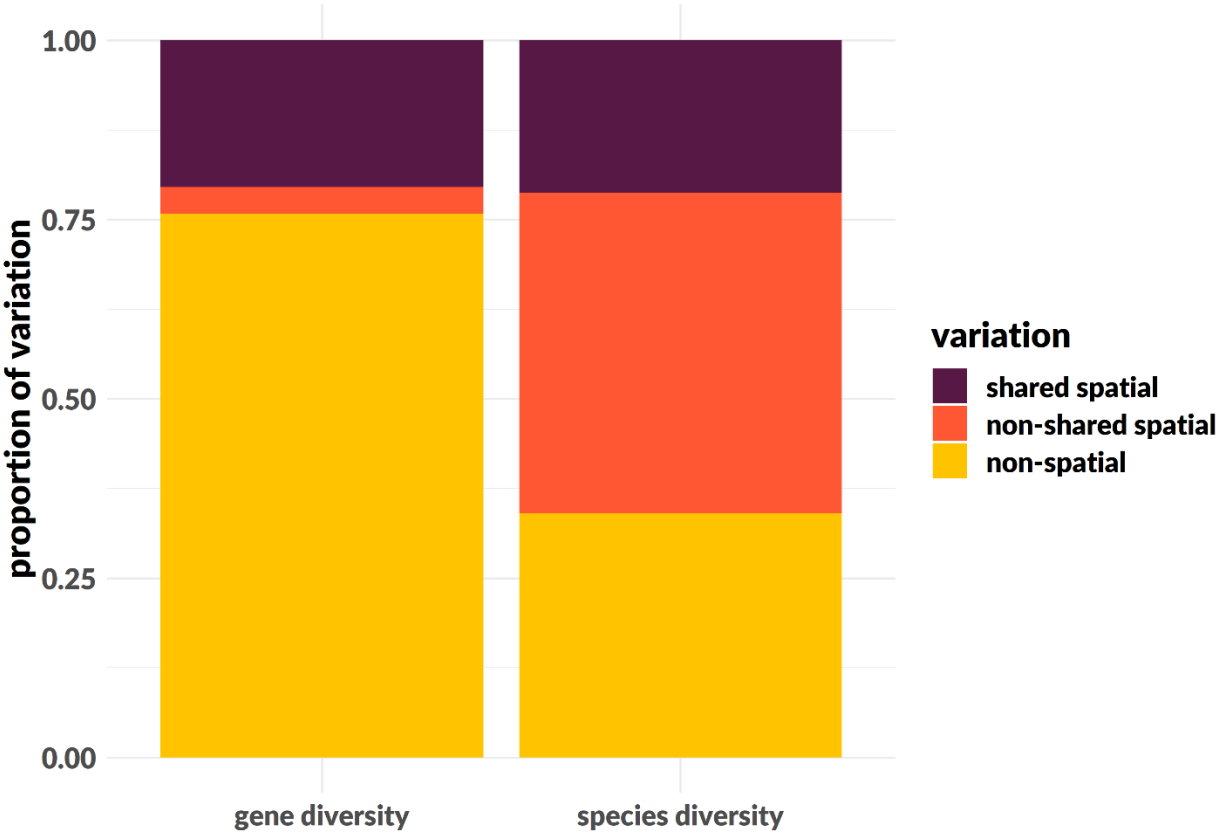


Figure S5.2. Variation partitioning results. This graph shows the proportion of variation in genetic diversity and species richness which can be explained by spatial factors, determined using Moran's eigenvector maps (MEMs). Spatial variation is further broken down into shared and non-shared spatial variation. Shared spatial variation is variation in genetic diversity and species richness explained by shared MEMs; non-shared variation is the remaining fraction of spatial variation not accounted for by shared MEMs.

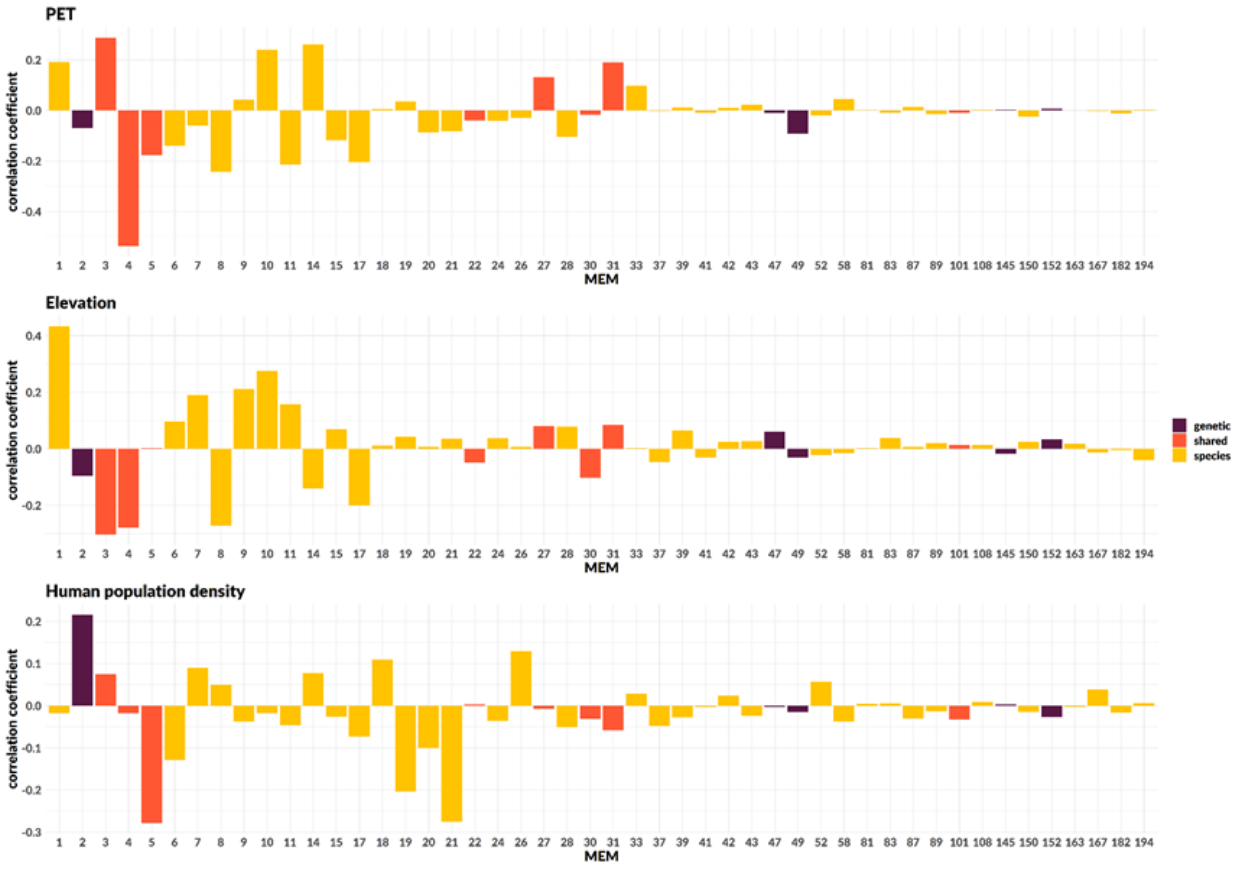


Figure S5.3. Correlation coefficients for spatial patterns (MEMs) and environmental variables measured at the site level: potential evapotranspiration (PET), elevation, and human population density. MEMs describe spatial patterns in genetic diversity, species richness, or both (shared spatial patterns). MEMs are ordered from broad (MEM1) to fine scale (MEM194) patterns. Strong correlations indicate that environmental variables included in structural equation models account for broad scale spatial patterns present in genetic diversity and species richness.

Table S5.1. Path coefficients and standard errors for SEM model (Fisher's C = 2.92, p = 0.23, 2 degrees of freedom).

Response	Predictor	Estimate ± SE
genetic diversity	human population density	-0.07 ± 0.02
genetic diversity	mass	-0.55 ± 0.13
genetic diversity	heterogeneity	-0.30 ± 0.07
mass	PET	-0.23 ± 0.03
mass	heterogeneity	0.14 ± 0.03
mass	human population density	-0.15 ± 0.03
species richness	mass	-0.09 ± 0.01
species richness	heterogeneity	0.70 ± 0.01
species richness	human population density	-0.05 ± 0.01
species richness	PET	0.44 ± 0.01
species richness	genetic diversity	-0.12 ± 0.01

Table S5.2. Summary of genetic data included in analyses. Number of populations, mean number of loci, mean number of individuals per population, and the total number of individuals summed across populations are given per species.

Species	Populations	Mean loci	Mean individuals	Total individuals
Alces alces	2	10	44.5	89
Antilocapra americana	1	19	175	175
Bison bison	8	29	23	184
Canis latrans	41	10	7.39	303
Canis lupus	1	12	62	62
Glaucomys volans	8	7	34.75	278
Lasionycteris noctivagans	1	18	87	87
Lasiurus cinereus	1	19	132	132
Leopardus pardalis	2	10	35	70
Lepus americanus	39	8	21.87	853
Lynx canadensis	33	14.15	38.12	1258
Lynx rufus	65	14.37	33.97	2208
Martes americana	29	12	22.52	653
Mephitis mephitis	1	9	345	345
Microdipodops megacephalus	3	11	60	180
Microdipodops pallidus	2	10	52.5	105
Myotis lucifugus	65	9.2	47.68	3099
Myotis septentrionalis	15	5	59.73	896
Odocoileus hemionus	67	10.55	34.81	2332
Odocoileus virginianus	64	14	32.33	2069
Oreamnos americanus	1	22	102	102
Otospermophilus beecheyi	3	11	68.33	205
Ovis canadensis	16	40.12	66.94	1071
Pekania pennanti	34	16	21.24	722
Peromyscus leucopus	36	13.19	21.53	775
Peromyscus maniculatus	10	10.9	13.6	136
Procyon lotor	1	10	330	330
Puma concolor	13	33.62	150.46	1956
Rangifer tarandus	82	16.96	32.16	2637
Sylvilagus transitionalis	3	10	52.33	157
Tamiasciurus douglasii	14	9	13.29	186
Tamiasciurus hudsonicus	12	9	15.67	188
Taxidea taxus	12	17.33	115.5	1386
Ursus americanus	43	15	83.72	3600
Ursus arctos	19	9.89	88.05	1673
Ursus maritimus	35	15.09	104	3640
Vulpes lagopus	3	9	26	78
Vulpes vulpes	16	11.44	38.81	621

Supplementary Information 6

Determinants of genetic diversity and species richness of North American amphibians

Contents:

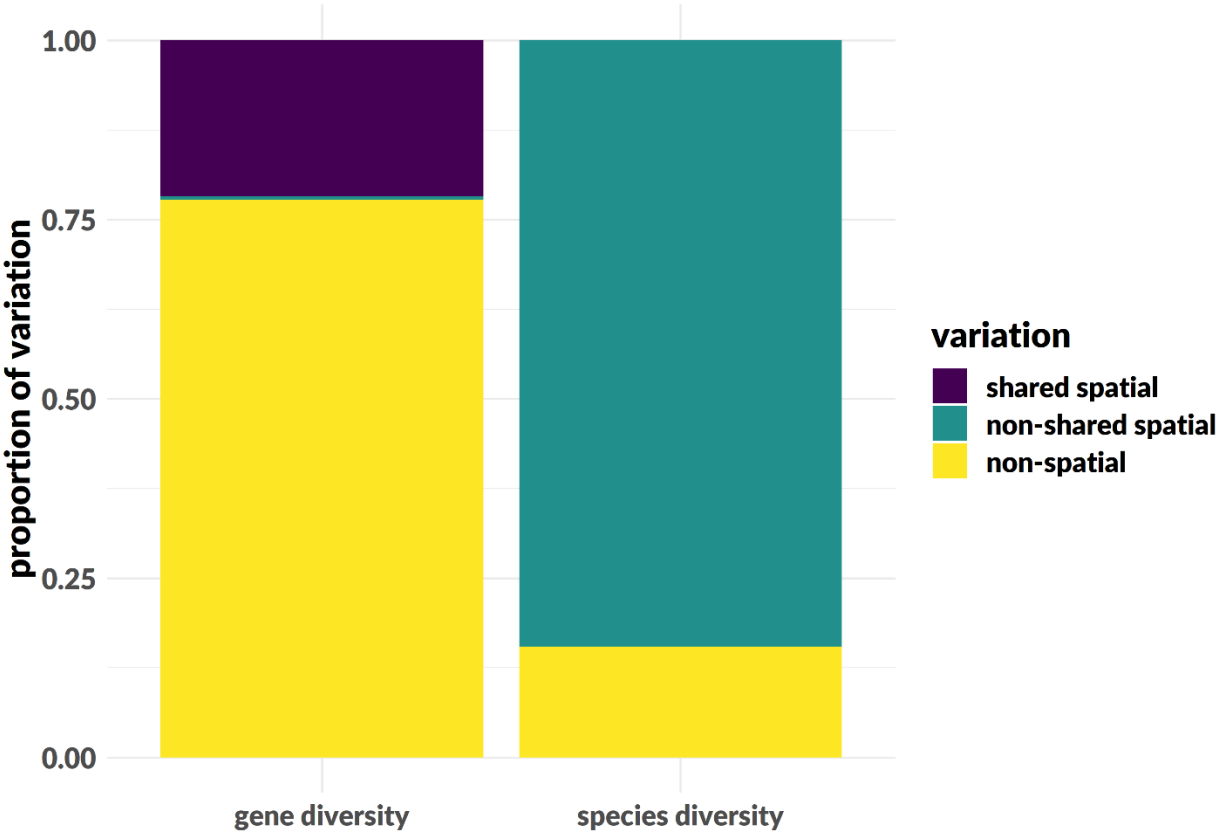


Figure S6.1. Variation partitioning results. Shared spatial patterns (MEMs) explain a substantial proportion of variation in genetic diversity and none in species richness.

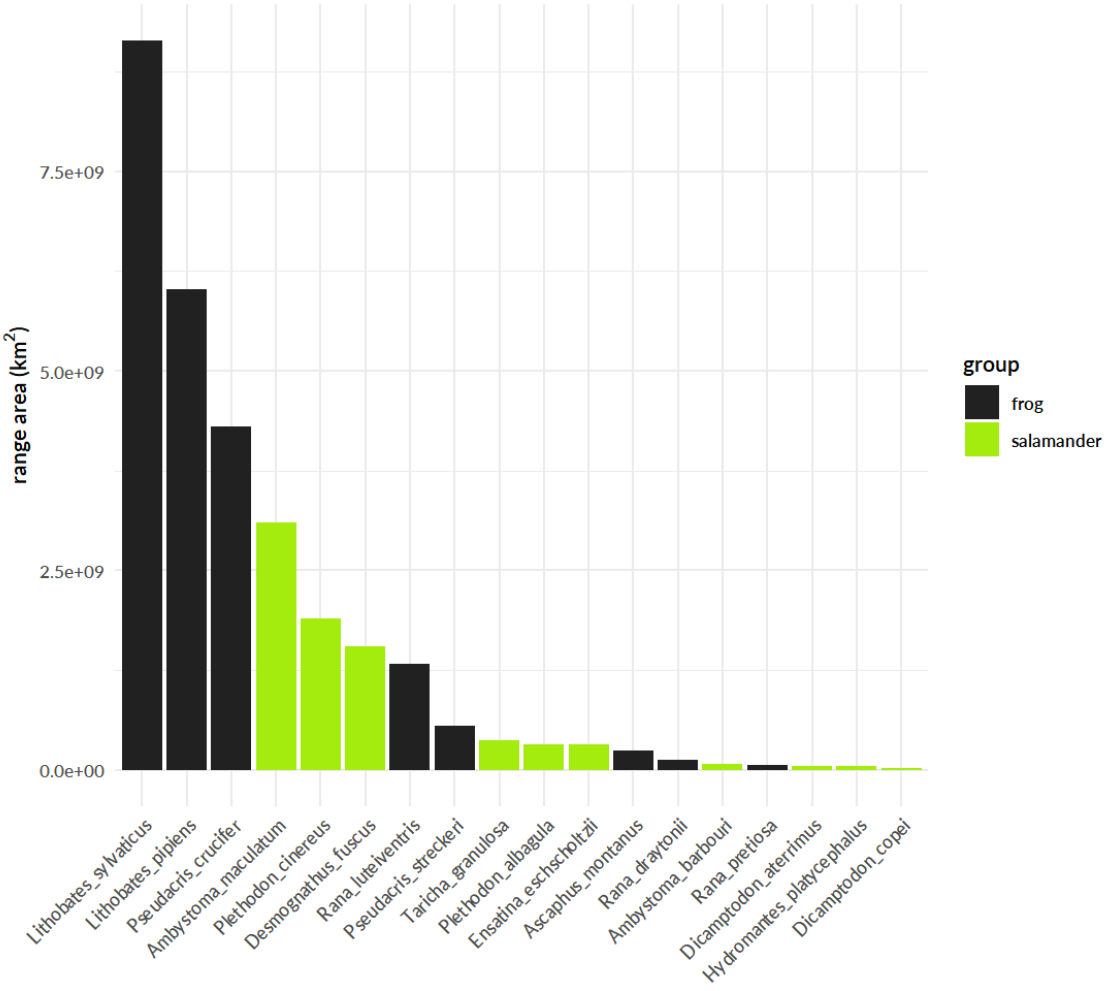


Figure S6.2. Range areas (km^2) for sampled species. The largest ranges belong to generalist frogs: wood frogs (*Lithobates sylvaticus*), northern leopard frogs (*L. pipiens*), and spring peepers (*Pseudacris crucifer*).

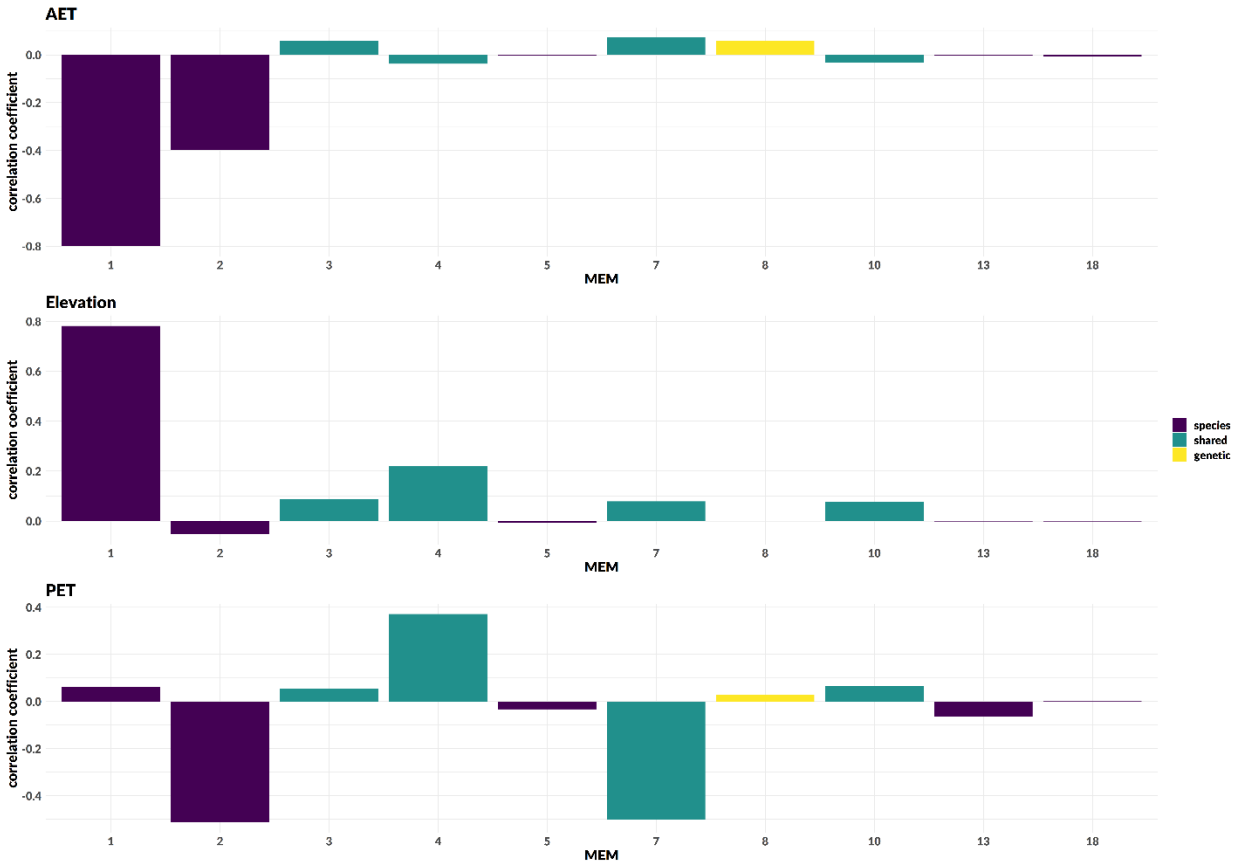


Figure S6.3. Spatial patterns (MEMs) are correlated with environments: actual evapotranspiration (AET), site elevation, and potential evapotranspiration (PET). MEMs that uniquely describe spatial patterns in species richness are more strongly correlated with environmental variables than shared patterns or patterns in genetic diversity. MEMs on the x-axis are ordered from broad to increasingly fine spatial scales.

Table S6.1. Path coefficients and standard errors for SEM model with orders combined (Fisher's C = 3.56, p = 0.47, 4 degrees of freedom).

Response	Predictor	Estimate ± SE
gene diversity	body size	-0.04 ± 0.21
gene diversity	elevation range	-0.40 ± 0.24
body size	elevation range	0.01 ± 0.04
body size	AET mean	0.72 ± 0.03
body size	PET mean	-0.23 ± 0.04
species richness	body size	-0.04 ± 0.03
species richness	elevation range	0.82 ± 0.03
species richness	AET mean	0.71 ± 0.03
species richness	PET mean	-0.15 ± 0.03
species richness	gene diversity	0.13 ± 0.03

Table S6.2. Overall model statistics, path coefficients, and standard errors for SEM model in frogs and salamanders.

	Response	**Predictor**	**Estimate ± SE**
Frogs	gene diversity	body size	0.07 ± 0.17
Fisher's C = 2.46	gene diversity	elevation range	-0.77 ± 0.28
p = 0.65	body size	AET mean	-0.44 ± 0.07
4 degrees of freedom	body size	PET mean	0.14 ± 0.09
n = 288	body size	elevation range	-0.24 ± 0.08
	species richness	body size	0.43 ± 0.03
	species richness	elevation range	0.59 ± 0.04
	species richness	AET mean	0.45 ± 0.04
	species richness	PET mean	0.03 ± 0.04
	species richness	gene diversity	-0.22 ± 0.04
Salamanders	gene diversity	body size	0.09 ± 0.23
Fisher's C = 2.05	gene diversity	elevation range	0.02 ± 0.41
p = 0.73	body size	AET mean	-0.44 ± 0.03
4 degrees of freedom	body size	PET mean	-0.60 ± 0.03
n = 260	body size	elevation range	-0.76 ± 0.03
	species richness	body size	0.07 ± 0.03
	species richness	elevation range	1.16 ± 0.03
	species richness	AET mean	0.62 ± 0.03
	species richness	PET mean	0.13 ± 0.02
	species richness	gene diversity	0.19 ± 0.02

Table S6.3. Data summary. The dataset included 8 frog species (Anura) and 10 salamander species (Caudata). Sites = the number of sites where genetic data was sampled; loci = the mean number of microsatellite loci sampled; individuals per site = mean number of individuals sampled; total individuals = the total number of sampled individuals summed across all sites.

Order	Species	Sites	loci	Individuals per site	Total individuals
Anura	*Ascaphus montanus*	100	13.00	19.68	1968
	Lithobates pipiens	5	9.00	37.00	185
	Lithobates sylvaticus	90	10.57	23.59	2123
	Pseudacris crucifer	11	11.00	38.00	418
	Pseudacris streckeri	17	14.00	10.65	181
	Rana draytonii	17	15.00	17.53	298
	Rana luteiventris	25	8.00	36.96	924
	Rana pretiosa	23	10.39	61.30	1410
Caudata	*Ambystoma barbouri*	76	11.00	21.07	1601
	Ambystoma maculatum	97	9.16	24.81	2407
	Desmognathus fuscus	5	5.00	28.00	140
	Dicamptodon aterrimus	3	9.00	120.33	361
	Dicamptodon copei	29	11.00	25.41	737
	Ensatina eschscholtzii	4	10.00	11.75	47
	Hydromantes platycephalus	15	10.00	13.00	195
	Plethodon albagula	21	20.00	16.33	343
	Plethodon cinereus	1	7.00	122.00	122
	Taricha granulosa	9	6.00	17.33	156