The ecology of urban wildlife:

How do species respond to rapid environmental change?

by

Riikka Kinnunen

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Department of Biological Sciences
University of Manitoba
Winnipeg

Abstract

Human activities alter ecosystems globally through land-use conversion. Human-caused environmental alterations tend to be rapid and wildlife increasingly find themselves in ecologically and evolutionarily new settings where their persistence is uncertain. Cities, as the planet's newest ecosystems, offer an opportunity to study the ecological responses of animals to rapid environmental change. Yet, how wildlife responds to urbanization is poorly understood. I explored this by characterizing the mechanisms that enable wildlife to colonize and persist in cities. Using continent-wide synthetic analyses, I first investigated which species colonize new environments after rapid environmental change by examining the phenotypic traits of Passerines and the species richness of migratory birds across structurally and socioeconomically varying cities. I then used focal species-specific studies to determine whether behavioral modifications facilitate population persistence in urban environments by investigating differences in the activity of Eastern grey squirrels (*Sciurus carolinensis)* and North American red squirrels (*Tamiasciurus hudsonicus*) between urban and non-urban areas. Finally, I used molecular and serological methods to explore the possible parasite-related ecological consequences of urban colonization by squirrels (Sciuridae) for domestic cats, and humans, in the newly assembled urban ecosystem. I specifically asked whether squirrels are important intermediate hosts of the parasite *Toxoplasma gondii* and whether *T. gondii* infection among some squirrel species is more common in a city than in a rural area. Overall, I show that cities that vary in their structural and socioeconomic features filter for species with different subsets of traits. I also show that behavioral adjustments likely allow squirrel populations to persist in cities and that competition might alter the temporal activity patterns of animals in cities. Lastly, I found no evidence of *T. gondii* infection in squirrels. This likely suggests that squirrels are not important intermediate hosts of *T. gondii* in cities and do not need to be considered as sources of infection to cats. This thesis advances our knowledge of how human activities can predict biodiversity following urbanization and how species and animal populations are responding to rapid environmental change.

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Dedication

To Anniina,

for inspiring me to challenge myself and simply for being who you are.

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Contributions of authors

Chapter 2: RPK, CS, and CJG conceived of the study. RPK and KCF collected data and RPK built the dataset. RPK performed statistical analyses with input from CS and CJG. All authors contributed to data interpretation. RPK and CJG wrote the first draft of the manuscript, and all authors contributed to subsequent revisions.

Chapter 3: RPK, KCF, and CJG conceived of the study with input from CS. RPK and KCF collected data and RPK built the dataset. RPK performed statistical analyses with input from CS and CJG. RPK wrote the first draft of the manuscript, and all authors contributed to editing subsequent revisions.

Chapter 4: RPK and CJG conceived of the study. RPK collected and manipulated data. RPK performed the analyses with input from CJG. RPK wrote the first draft of the manuscript, and CJG contributed to editing subsequent drafts.

Chapter 5: RPK and CJG conceived of the study. RPK and CS collected data. AHO processed data. RPK wrote the first draft of the manuscript, and all authors contributed to editing subsequent revisions.

Chapter 1. Introduction

"Only within the moment of time represented by the present century has one species -- man -- acquired significant power to alter the nature of the world."

— Rachel Carson, *Silent Spring*

My Ph.D. thesis aims to characterize some of the ways that species respond to rapid environmental change by studying population formation and persistence in cities. I will start by broadly explaining which species, in general, colonize new environments after rapid environmental change. I will then determine if behavioral modifications facilitate population persistence in urban environments. Finally, I explore the parasite-related ecological consequences of urban colonization for humans and their pets in the newly assembled urban ecosystem. A better understanding of the predictors of biodiversity following environmental change, the details of how animal populations cope with environmental change, and the potential consequences of urban host-parasite interactions for humans and their pets will significantly add to our knowledge of the earliest ecological steps that allow for subsequent evolutionary change.

Environmental filtering under rapid environmental change

Community ecology has a long history, reaching back to the 17th to early 20th century, when natural historians like Maria Sibylla Merian (1647-1717), Alexander von Humboldt (1769-1859), Charles Darwin (1809-1882), Arthur Tansley (1871-1955) and Charles Elton (1900-1991) observed differences in the distribution and behavior of plants and animals (Langenheim 1996; Vellend 2016). This history laid the ground for the more contemporary ideas in community ecology that branched into many different foundational directions, reflected in the hundreds of theoretical or conceptual models (e.g., island biogeography, neutral theory, metacommunity theory) developed over the past 150 years to explain community dynamics (Hanski & Gilpin 1991; Hubbell 2011; MacArthur & Wilson 1967). This apparent "mess" (Lawton 1999) led Mark Vellend to seek a way to simply organize these theoretical ideas in building his *Theory of Ecological Communities* (Vellend 2016). The theory states that at the most general level four processes underlie all models of community dynamics: selection between individuals of different species (e.g., ecological selection), ecological drift, speciation, and dispersal (Vellend 2010). These processes parallel selection, drift, migration, and mutation—familiar from evolutionary biology. Out of these processes, my thesis focus is on ecological selection.

One result of ecological selection is environmental filtering - this concept , in general, centers around a species ability to colonize or persist in new environments (Kraft et al. 2015). Not all species will tolerate the same abiotic and biotic conditions, and consequently, some species will be "filtered" by environments after an environmental change (i.e., are unable to colonize those new environments). This is the result of ecological selection (Vellend 2016), where the environment is acting as a selective force acting at the species level. Here only species with phenotypic traits that confer tolerance to the environmental conditions in that location will survive and successfully establish populations. This may lead to the clustering of similar species in a community—species that have generally similar phenotypic traits may co-occur in similar environments (e.g., Pontarp et al. 2019; Vellend 2016). The original environmental filtering concept (that first arose in the plant ecology field) considered only abiotic factors to be the filtering forces in the environment (e.g., Woodward & Diament 1991), however biotic factors, especially competition and predation may also lead to both the clustering or differentiation of species traits (Kraft et al. 2015).

Human activities have, often irreversibly, altered ecosystems globally through, for example, land-use conversion and climate change (Alberti et al. 2003). Urbanization is one of the most pervasive and human-governed forms of land use (Seto et al. 2011) and the global urban landcover has doubled between 1985 and 2015 increasing from 362,747 km^2 to 653,354 km^2 (Liu et al. 2020). This rate of global urban expansion outpaced the growth of the global human population during the same period (United Nations 2019) and is expected to have major impacts on biodiversity worldwide (Seto et al. 2013). Cities are fundamentally different from natural environments in the dominance of humans and their activities on the urban ecosystem. 55% of the world's population was urban in 2018, and this figure is expected to increase to 68% by 2050 (United Nations 2019). Although cities cover only ~ 3% of all habitable land globally (Liu et al. 2014), urbanization can have significant ecological impacts on the environment, as well as strong, multifaceted effects on ecosystems (Alberti et al. 2003). This rapid human-caused environmental change is likely to cause ecological selection and filter animal communities. The formation of cities thus offers a great platform to study environmental filtering and how wildlife responds to rapid environmental change.

Urban ecology

Over the past 30-years there has been an increasing interest in the effects of urbanization on the ecology and evolution of organisms (Alberti 2015; Johnson & Munshi-South 2017), biodiversity (e.g., Aronson et al. 2014; Lepczyk et al. 2017; Spotswood et al. 2021), species traits (e.g., Alberti et al. 2017; Aronson et al. 2016; Hensley et al. 2019; Santini et al. 2019; Silva et al. 2016), as well as patterns in global urban land-use change (e.g., Liu et al. 2020; Liu et al. 2014; Seto et al. 2011; Seto & Fragkias 2005). The early urban ecology, conducted in Asia, Europe, and North America (under the umbrella of environmental sciences), was highly applied in nature, focused on solving problems to improve the human condition, however basic and theoretical research was largely missing (McDonnell 2013). This was mainly due to a belief in the "balance of nature", popularised by George Perkins Marsh in his book Man and Nature (Marsh 1864). The book enforced the idea that to effectively study nature, research must be done far from people, in areas as untouched by humans as possible (McDonnell 2013). Although there was some research on the ecology of human settlements during the 1970s and 1980s, the field of urban ecology did not fully take off before the late 1990s, mainly due to the belief that human-dominated environments were not legitimate subjects of ecological study—a belief that was now deeply rooted in the ecological community (McDonnell 2013). Early urban ecology research thus followed a framework familiar from "natural" ecology studying the natural aspects of cities (e.g., parks, rivers, soil) rather than focusing on the features of cities (Forman 2016).

Urban ecology today is an evolving discipline that integrates ecological, geographical, planning, and social sciences. Because of this interdisciplinary nature of urban ecology, the focus of research is currently branching to different directions based on its origins. Namely, urban ecology generally describes the study of humans in cities, the study of nature in cities, or the coupled relationships between humans and nature (Marzluff et al. 2008). Some consider cities as socio-economic systems or consider the whole city as an ecosystem (e.g., Alberti et al. 2003; Grimm et al. 2000; Pickett et al. 2017), whilst others look at cities from a landscape ecology perspective (Luck & Wu 2002). Urban ecosystems are heterogeneous environments, consisting of remnants of natural habitat present in the area before urbanization, and of human constructed more or less natural habitats like parks, backyards and managed wetlands, as well as parking lots, roads, or sports fields. Despite the varying nature of built environments, urban ecology research to date has still largely followed a framework similar to that developed in natural areas (Forman 2016; Grimm et al. 2008; Wu 2014). The most recent urban ecology literature, however, has called for further integration of humans and their social and cultural patterns and processes into the urban eco-evolutionary framework to gain a more complete understanding of the ecological and evolutionary patterns happening in cities (Alberti et al. 2020; Des Roches et al. 2020; Schell et al. 2020).

Urbanization is a complex phenomenon (Alberti et al. 2020) where humans and their activities are writing a new eco-evolutionary play by changing both the actors and the stage of G. Evelyn Hutchison's ecological theatre (Hutchinson 1965). Humans are the defining feature of cities, and our actions and decisions are molding the city environment in numerous ways and driving ecological and evolutionary processes in urban areas (Johnson & Munshi-South 2017; Shochat et al. 2006). Human activities and preferences alter the overall structural composition of the city (Des Roches et al. 2020; Schell et al. 2020)—and with that also the vegetation patterns (Pearse et al. 2018), the level of traffic, noise, and artificial light (McKinney 2002), and the amount of human-provided food and nesting structures available for wildlife (Lepczyk et al. 2004; Tryjanowski et al. 2015), just to name a few. These are all likely to vary with the number, density, and wealth of human populations. Consequently, humans are ecosystem engineers that can change their environment, and that of the wildlife inhabiting cities. Although previous research has tried—by investigating for example the amount of vegetation or tree cover in cities—to capture some of the mechanisms that cities influence animal communities, the complex and diverse nature of cities is unlikely to be captured by these kinds of single natural environmental metrics. Consequently, research focused on human activities should more likely capture the highly variable sources of human-related disturbance or resources that human niche building may provide to wildlife in cities. However, research directly exploring the effects of cities' socioeconomic status on wildlife communities is still rare, particularly at a macroecological level, although the complex multidimensional nature of human activities in cities could have consistent effects on wildlife communities.

How does urbanization filter animal communities?

As urbanization can have considerable effects on wildlife, there has been a rise in studies aiming to discover how urbanization filters animal communities. These studies have generally either investigated differences in species richness and abundance in response to some degree of urbanization (i.e., urbanization gradient) or compared differences between urban and non-urban wildlife (Shanahan et al. 2014). However, evidence from both birds and mammals suggests that cities filter for subsets of local species that have traits suited to population persistence in urban environments (e.g., Alberti et al. 2017; Aronson et al. 2016; Chace & Walsh 2006; Croci et al. 2008; Hensley et al. 2019; Jokimäki et al. 2016; Kark et al. 2007; Leveau 2013; Leveau et al. 2017; Meffert & Dziock 2013; Meillère et al. 2015; Santini et al. 2019; Silva et al. 2016). Some of the general traits found in these studies that may enable population persistence in urban environments include large litter sizes, larger body masses, and bigger brains (Croci et al. 2008; Iglesias-Carrasco et al. 2019; Maklakova et al. 2011; Santini et al. 2019). However, behavioral flexibility under changing environmental conditions can also be an important phenotypic response for succeeding in urban environments (Lowry et al. 2013; Sol et al. 2013) (see discussion below). In addition, cities seem to filter bird species based on their urban tolerance, and studies to date have largely categorized birds found in cities either as urban avoiders, adapters, or exploiters (*sensu* McKinney 2006), or based on, for example, their diet or nesting guilds, with omnivores, granivores, and cavity-nesting birds often the most dominant (e.g., Allen & O'Connor 2000; Chace & Walsh 2006; Murgui & Hedblom 2017). However, although cities also seem to host many migratory bird species both during breeding and non-breeding seasons (Brawn &

Stotz 2001; Hostetler et al. 2005; Partridge & Clark 2018), and urban green areas may offer the only sites available for refueling for numerous migrants crossing large metropolitan areas (Brawn & Stotz 2001; Mehlman et al. 2005) studies to date have largely overlooked the potential importance of city habitat to migratory birds. There is some evidence that Neotropical and short-distance migrants are generally underrepresented in urban ecosystems as compared to resident species (Allen & O'Connor 2000; Kluza et al. 2000; Poague et al. 2000), but generally, the ways that different migratory species use the habitat are still largely unknown (but see Partridge & Clark 2018; Seewagen et al. 2011; Seewagen & Slayton 2008). As many migratory birds are currently facing declines and fragmentation of their preferred natural breeding habitats mainly due to land cover change (Drummond & Loveland 2010) further knowledge of their preferences and their ability to tolerate human-caused disturbance in different cities is of high importance.

Much of the previous work done in cities to date has focused on urban-rural species trait comparisons that treat different cities as homogeneous and comparable concerning the features of species that can colonize them. Furthermore, most have been local or regional investigations, conducted in one or a few cities only, and broad-scale, continent-wide investigations are still largely lacking. However, it is notable that in instances where species filtering has been explored across multiple cities, different cities seem to filter for slightly different subsets of traits (Hensley et al. 2019). This finding suggests that different cities are suited to hosting particular types of species and that heterogeneity between cities may select for phenotypes that confer tolerance to the particular, mostly human-modified, environmental conditions in each city.

Do behavioral modifications facilitate population persistence in urban environments?

Cities differ in many ways from non-urban, more natural areas. Cities are designed with humans in mind and compared to more natural areas cities have more traffic, pollution, noise, impervious surfaces, buildings, and people, as well as modified distribution of food and nesting resources for wildlife (Grimm et al. 2008; Shochat et al. 2006; Swaddle et al. 2015). Cities can also have elevated temperatures (the urban heat island effect) (Arnfield

2003; Leal Filho et al. 2018), as well as higher levels of artificial light at night compared to neighboring rural areas (Gaston et al. 2014). Temperature and light, in particular, can affect animals in a multitude of ways, yet are still often neglected in urban studies (Hölker et al. 2010). Cities can also have altered biotic interactions, as urbanization can influence the circadian cycles of animals (Łopucki & Kiersztyn 2020), create or decouple predator-prey associations (Haskell et al. 2001; Rodewald et al. 2011), and influence inter-and intraspecific competition patterns within communities (Lewis et al. 2015).

Regardless of the challenges that these new selection pressures in cities pose to colonizing wildlife, some species thrive in these evolutionarily new environments. Behavioral modifications that improve an individual's reproductive success, probability of survival, or fitness in cities can prevent population extirpation and give time for genetic adaptation (Lowry et al. 2013; Tuomainen & Candolin 2011). As a result, these are likely to be important for the success and persistence of animals in cities (Tuomainen & Candolin 2011). Previous studies have indeed identified behavioral differences between urban and non-urban animals (reviewed in Ritzel & Gallo 2020), for example in exploratory behavior (Atwell et al. 2012; Miranda et al. 2013; Thompson et al. 2018), aggression (Grunst et al. 2018), and fear response (Van Donselaar et al. 2018). Being a faster, bolder, more aggressive explorer in a new environment might enable animals to quickly obtain information of possible predators or competing individuals and gain access to better food and nesting resources, consequently facilitating persistence in urban environments (Smith & Blumstein 2008; Sol et al. 2013). Additionally, alterations to diet or foraging patterns, the timing and duration of breeding, or inter-, and intraspecies interactions may also facilitate species persistence in cities (reviewed in Ritzel & Gallo 2020), yet research investigating these kinds of behavioral modifications in cities is still relatively rare and research on mammals is particularly underrepresented (Ritzel & Gallo 2020).

Host-parasite interactions in cities

In a similar way to filtering mammals and birds, cities can also filter parasites. In some cases, the overall species richness and diversity of parasites can be reduced in cities as urbanization can lead to a decline in host species richness. With reduced access to hosts, parasites with one or a few host species may then also become extirpated (Bradley & Altizer 2007). However, wildlife in cities can also act as reservoirs for parasites that can infect domestic pets and have implications for human health (Mackenstedt et al. 2015). Indeed, previous work has found increased levels of wildlife parasitism in cities compared to rural areas (Deplazes et al. 2004; Giraudeau et al. 2014; Lehrer et al. 2010; Reperant et al. 2009). This increased level of parasitism could reflect both the increased population density of urban host species (Parker & Nilon 2008) and higher within and between species contact rates in response to resource provisioning in cities relative to rural areas (Bradley & Altizer 2007; Gliwicz et al. 1994). Parasites that spread through direct contact or oral-fecal routes are likely to be favored in urban areas, (Bradley & Altizer 2007), but general knowledge underlying the ways host-parasite interactions operate in cities is still limited (Mackenstedt et al. 2015).

Toxoplasma gondii are particularly interesting Coccidian parasites within the context of emerging urban ecosystems because they rely on our pets for reproduction and can infect humans. Domestic cats are the only known definitive host of *T. gondii*, but other mammals can act as intermediate hosts, acting as reservoirs for the parasite and, in some cases, as possible sources of infection for cats in cities. However, not much is known about *T. gondii* host-parasite interactions in cities, nor whether *T. gondii* prevalence in wildlife might be higher in urban areas than in more natural habitats. A significant proportion of the human population globally is infected with *T. gondii,* but most healthy people do not experience symptoms of infection (Tenter et al. 2000). However, immunocompromised people and pregnant women require medical intervention to avoid serious health issues (Dixon 1992; Hill et al. 2005; Tenter et al. 2000). Consequently, further knowledge of *T. gondii* infection dynamics in cities is needed.

Objectives and thesis organization

My thesis is organized in a sandwich thesis format. First, I asked whether there are relationships between characteristics of cities and the life-history traits of species that are able to persist in cities (Chapter 2). Next, I wanted to further focus on human activities and the urban environmental variation it creates and asked if socioeconomic and structural variation across cities can predict the species richness of migratory birds belonging to different breeding biome groups (e.g., forest generalists) and are found in cities during the breeding season (Chapter 3). Third, I investigated how animal populations within cities are responding to urbanization and asked if the activity of Eastern grey squirrels (*Sciurus carolinensis*) and North American red squirrels (*Tamiasciurus hudsonicus*) differs between urban and rural areas (Chapter 4). Finally, I explored the potential host-parasite interactions in cities and asked whether squirrels are important intermediate hosts of *Toxoplasma gondii* and whether *T. gondii* infection is more common in a city than in more natural habitats (Chapter 5). Overall, my thesis will provide an overview of the ecology of wildlife in cities and further our knowledge of how species and populations are responding to rapid environmental change.

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Chapter 2: The socioeconomic status of cities covaries with avian life-history strategies

RP Kinnunen[1], KC Fraser[1], C Schmidt[1], CJ Garroway[1]

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

https://esajournals-onlinelibrary-wiley-com.uml.idm.oclc.org/doi/10.1002/ecs2.3918

Abstract

Cities are the planet's newest ecosystem and thus provide the opportunity to study community formation directly following major permanent environmental change. The human social and built components of environments can vary widely in different cities, yet it is largely unknown how features of cities covary with the traits of colonizing species despite humans being the ultimate cause of environments and disturbances in cities. We constructed a dataset from open-source data comprised of 13,502 breeding season observations of 213 Passerine species observed in 551 Census-defined urban areas across the United States. We found that as a city became more compact with less sprawl it tended to support more migratory species and species with lower body mass, shorter lifespans, and larger clutches. We also found that species had lower body mass in cities with higher median income, and higher body mass in highly populated cities. Our results highlight the complexity of human-dominated urban ecosystems, where human socioeconomic actions and everyday activities intermix leading to structurally heterogeneous environments that support the colonization of some species over others.

Keywords

colonization, community formation, ecological selection, life history, socioeconomic variation, urbanization

Introduction

The recent growth of cities has created a natural experiment well suited for exploring how rapid land-use changes reshape animal communities. Urban landcover doubled between 1985 and 2015 increasing from 362,747 km^2 to 653,354 km^2 (Liu et al. 2020). This rate of expansion outpaced the growth of the global human population during the same period (United Nations 2019). It is now within cities that most people interact with and benefit from local biodiversity (e.g., through recreational activities and ecosystem services; Bolund and Hunhammar 1999). Although cities can be a substantial threat to wildlife they also provide the opportunity for the conservation and management of native species (Spotswood et al. 2021). It is thus increasingly important for us to understand how cities support nature.

In natural settings, species with generally similar phenotypic traits tend to co-occur in similar environments (e.g., Pontarp et al. 2019; Vellend 2016). This is a result of ecological selection (in the sense of Vellend 2016)—the phenotype-based differential survival and reproduction of members of different species due to environmental filtering (Kraft et al. 2015) and biotic interactions. The ubiquity of humans in cities is the defining feature of a city's environment and our social composition and decision-making processes create urban environmental heterogeneity (Des Roches et al. 2020; Hobbs et al. 2006; Pickett et al. 2017; Schell et al. 2020). This heterogeneity is likely to cause ecological selection. A complete understanding of the evolutionary and ecological dynamics of urbanization requires us to identify the pathways through which human societies influence urban organisms (Des Roches et al. 2020; Schell et al. 2020). Here we test for associations between aspects of a city's socioeconomic status and phenotypic traits of urban passerines at the species level.

Our focus on a city's socioeconomic status as a species filter emphasizes the importance of human activities and decision-making in urban ecosystems (Des Roches et al. 2020; Pickett et al. 2017; Schell et al. 2020). We chose this emphasis because humans, as the ultimate ecosystem engineers in cities, provide and remove resources, pollute the environment, and generally influence wildlife populations in both negative and positive ways (Gaston et al.

2014; Johnson & Munshi-South 2017; Kyba et al. 2017; Shochat et al. 2006). Noise, light, and chemical pollution, impermeable surfaces, green spaces, and human supplemented food sources are all likely to vary with the number, density, and wealth of human populations. Consequently, focusing on humans should capture these likely highly variable sources of disturbance in a cohesive measure. However, research directly exploring the effects of cities' socioeconomic status on wildlife communities is still rare although various human activities that cause disturbance (e.g., traffic, different types of pollution) could have consistent effects on wildlife communities.

The consequences of human social and economic decisions scale from individual households to whole cities (Rigolon et al. 2018). We hypothesized that a city's compactness or sprawl, human population size, and median income were likely to capture the overarching patterns related to how human decisions might shape environments and thus animal communities. For example, plant diversity, vegetation cover, and food provisioning—important predictors of urban biodiversity—are higher in wealthier areas (Hope et al. 2003; Iverson & Cook 2000; Kinzig et al. 2005; Leong et al. 2018; Talarchek 1990). Sprawl, characterized by scattered, low-density development extending across vast areas of land, has become the prevailing development pattern for nearly all metropolitan areas across the U.S. (Bullard et al. 2000; Ewing et al. 2002; Huang et al. 2007), with wide-reaching effects on habitat availability and quality, biodiversity, and the health and well-being of people living in those cities (Bullard et al. 2000; Matlack 1993; Robinson et al. 2005; Zuidema et al. 1996). Highly populated and sprawling cities will also be the most disturbed and polluted with high-traffic road networks connecting suburban areas to city centers. Migration is another important trait associated with resource availability and environmental variability that allows species to cope with seasonality and periods of low productivity, particularly among birds, and migratory species generally select resource-rich, stable habitats during the breeding season (Somveille et al. 2015). The use of cities by migratory species is relatively unexplored, but there is some evidence that migratory birds may be underrepresented in urban ecosystems (Allen & O'Connor 2000; Kluza et al. 2000; Poague et al. 2000). We therefore also tested for associations between a city's socioeconomic status and the migratory status of the species found in that city.

There is now considerable evidence from both birds and mammals suggesting that cities filter for subsets of local species that have traits suited to population persistence in urban environments (Alberti et al. 2017; Aronson et al. 2016; Chace & Walsh 2006; Croci et al. 2008; Hensley et al. 2019; Jokimäki et al. 2016; Kark et al. 2007; Leveau 2013; Leveau et al. 2017; Meffert & Dziock 2013; Meillère et al. 2015; Santini et al. 2019; Sepp et al. 2018; Silva et al. 2016; Sol et al. 2014). Much of this previous work has focused on urban-rural species trait comparisons that treat different cities as homogeneous and comparable with respect to the features of species that can colonize them. However, it is notable that in instances where species filtering has been explored across multiple cities, different cities seem to filter for slightly different subsets of traits (Hensley et al. 2019; Tryjanowski et al. 2013; however see Morelli et al. 2016). This finding suggests that different cities are suited to hosting particular types of species. Our work differs from the more typical comparisons of urban and rural communities in that we treat cities as a spatially variable habitat type and explore trait variation across different cities.

We tested for associations between city socioeconomics and the median body size, lifespan, reproductive output, and migratory status of the species it hosted using a dataset we constructed from open-source data. Our overall goal was to investigate the effects of human activities on birds per se and we thus considered city features as general metrics of human social and socioeconomic activity. The socioeconomic status of cities is correlated with resource availability and environmental stability, with increasing human population size and sprawl positively correlated with disturbance, and the compactness of a city and increasing median income correlated with resource stability and abundance (Bullard et al. 2000; Leong et al. 2018; Matlack 1993; Robinson et al. 2005; Zuidema et al. 1996). Natural environments characterized by disturbance tend to be positively correlated with species body size and lifespan and negatively related to reproductive output (Lack 1947, 1954; Martin 1987; Ricklefs 2000). This is thought to be because 1) large-bodied species are better able to withstand periods of low resource availability; 2) investment in fewer, better-quality offspring in poor environments increases their likelihood of survival; and 3) being long-lived allows for multiple reproductive attempts given higher offspring mortality rates (Sol et al. 2012, 2014). We thought these same patterns would manifest across cities.

Methods

Data compilation

We compiled our dataset from open data sources (see SI Fig 1 for detailed data compilation process). First, we downloaded the eBird Basic Dataset for the United States from eBird.org (Sullivan et al. 2009). eBird is an online bird abundance and distribution checklist program jointly coordinated by the Cornell Laboratory of Ornithology and the National Audubon Society. The eBird project relies on citizen science volunteer observers who submit georeferenced observations of species to a centralized database. Observations are submitted in a checklist format, listing species seen during one bird-watching occasion. Checklists are designated as either complete checklists, where all birds detected and identified were recorded, or incomplete checklists, where some species seen were not recorded. Regional reviewers identify outliers and verify each species observation based on sighting coordinates (Wood et al. 2011). We used eBird observations of Passerines from the United States so that we were working with related and trophically similar species groups across cities with relatively comparable histories. We focused on Passerines because they are a broadly comparable group with many species that have colonized cities. More precisely, we focused on native Passerines noted as being present in cities during the breeding season. We discarded observations of transient birds likely on route to their breeding grounds and observations of birds from outside of the breeding season. We chose observations from May 27th to July 7th as our breeding season, following the practices of the North American Breeding Bird Survey (available from https://www.pwrc.usgs.gov/bbs/index.cfm). We note that our species are found in cities during the breeding season but may not necessarily breed in cities. Our focus on the breeding season ensured that our observations were focused on a period of high resource demand. Our observations were filtered to match data available for city socioeconomic status metrics which were last measured in 2010. This period also allowed for the maximum accumulation of species detections in cities. The observation dates were filtered using the R package *auk* (version 0.3.3; Strimas-Mackey et al. 2018) in R version 3.5.0 (R Core Team 2018).

We extracted species-level data for body mass, clutch size, and longevity from the Amniote Life-History database (Myhrvold et al. 2015). This is a systematically compiled database of life-history traits for birds, mammals, and reptiles built for comparative life-history analyses (Myhrvold et al. 2016). For species with multiple raw data points, the median value is reported. There were no obvious mistakes in this database for our species. We compared species lists from eBird and the Amniote Life-History database and found 17 species of Passerines present in the eBird dataset that were not found in the Amniote database (SI Table 2.1). A few of these were introduced species, some were species with a limited range or a specific habitat type, and most were unlikely to breed in cities. So, overall, not many species were lost using the data from the Amniote Life-History database. Bird species that are typically present in a single U.S. state year-round were classified as residents, and bird species that migrate to breed were classified as migrants. Some populations of migratory birds can choose to stay in cities overwinter and therefore could be classed as residents, but our data did not allow the determination of these kinds of population-level differences.

We assigned georeferenced eBird species records to urban areas using U.S. Census-defined urban area maps provided by the U.S. Census Bureau (U.S. Census Bureau 2010b). The urban area shapefiles define an urban area as a densely developed territory with at least 2500 people (U.S. Census Bureau 2010b). We used the R packages *sp* (version 1.3-1; Bivand et al. 2013), *rgdal* (version 1.3-4; Bivand et al. 2018), and *maps* (version 3.3.0; Becker & Wilks 2018) for this merge. Next, we obtained socioeconomic features for these urban areas using the 2010 Census data from the U.S. Census Bureau (U.S. Census Bureau 2010a). Data were available for the year 2010 and not the full span of our bird observation data. We thus assume that these data have remained comparable. We chose the human population size, median household income, and sprawl/compactness as our general metrics of human social and socioeconomic activity. We calculated a city's sprawl by dividing the human population size in each city by the city's area—a generally recognized measure of sprawl (Huang et al. 2007).

We were interested in species present in an urban area during a breeding season, not the number of observations of each species nor estimates of species abundance, and so our final data set was a list of each species observed in each city during our study period. While questions about abundance are certainly interesting, we considered species-, and city-specific abundance estimates to be beyond the scope of our questions about species-level trait variation. Additionally, our decision to observe the effects of socioeconomics across cities does not enable us to investigate within city variation, although legacy effects due to cultural and political decisions (e.g., racism, economic collapse) may have considerable influence on current biodiversity patterns and processes in cities (Schell et al. 2020; Shackleton & Gwedla 2021). Furthermore, habitat quality and the availability of resources in a city can also change over time as cities age and there is a shift in human behavior and preferences, leading to a change in species responses (Spotswood et al. 2021). For example, our city metric, median household income, is the median household income for each city in 2010. It is thus not possible to look at change over time, although changes in the economic situation could certainly be possible for some cities. We excluded non-native, introduced, and pet traded species, as well as accidental observations from our dataset (see SI Table 2.2). We removed observations that were part of the birds' overwintering range, observations of transient birds, and vagrants from each state.

Data analyses

All analyses were conducted using R version 3.5.0 (R Core Team 2018). To determine whether eBird surveys adequately sampled species using cities during our study period we plotted the accumulation of species in selected cities over the years 2010–2018 (see SI Fig 2.3). These accumulation curves leveled off for the majority of the cities plotted, indicating that the eBird surveys were capturing most species found in a city. Human population size, compactness/sprawl, and median household income were not strongly correlated (SI Fig 2.2) and were treated as independent variables in a series of mixed models that used species traits as dependent variables. These species-level traits were median clutch size, median longevity (the lifespan of an individual in years), median body mass (mass of an individual in grams), and migratory status (migratory or resident). Data were scaled to standardize the range of independent and dependent variables to make model effects comparable.

Clutch size, longevity, and body mass were treated as dependent variables in a series of linear mixed-effects models (LMMs) using the function lmer in R package *lme4* (version 1.1-21; Bates et al. 2014). All urban variables were fit in each model. We also included taxonomic family and U.S. state as random effects allowing intercepts to vary. Random intercepts estimate between-group variation in means, as well as variation within groups in each of our dependent variables. The taxonomic family was added as a random effect to account for variation within Passerine families. Phylogenetic analyses are used to account for the possibility that unreplicated evolutionary events in species evolutionary histories explain contemporary patterns. As we were interested in relationships between species traits and the features of cities, we thus did not control species similarities. This was because urbanization is a contemporary phenomenon—a species that persists in a city is doing so because of the ecology associated with its traits in contemporary timeframes, regardless of its phylogenetic origin. Our aim is to explore city-species trait relationships rather than control for them. Treating family as a random effect allows us to compare like species with like species and then estimate overall general trends across species. This approach allows us to find that phylogenetic signals exist, albeit not estimating it precisely, without statistically controlling for the patterns we were interested in. As socioeconomic variation seemed likely to strongly depend on local governance we included state as a random effect in our models. These hierarchical models account for state-level variation in socioeconomics and statewide and across state variation in climate and can be interpreted as fitting state by state models and then generalizing effects across the states. Model residuals were plotted against the expected values and we saw no strong violations of the models' assumptions, except for body mass. We $\log_{10}$-transformed body mass to ensure the normality of residuals.

Migratory status was a binary variable and so we used a generalized linear mixed-effects model (GLMM) with a binomial error structure and logit link function for this analysis. The model structure was similar to that for LMMs, with family and state treated as random effects and the city traits fit as independent variables. Migratory status was coded 1 for migratory species and 0 for residents.

We tested the residuals of our models for spatial autocorrelation using the R packages *spdep* (Bivand et al. 2013) and *adespatial* (Stéphane et al. 2020). We first computed the maximum distance of the minimum spanning tree, which is the minimum value that keeps all samples connected, and then built a connectivity matrix among sites by identifying the nearest spatial neighbors using this threshold value (Borcard et al. 2011). We then used this spatial weights matrix to calculate the Moran's I correlation coefficient for each of our models. Spatial autocorrelation in model residuals violates the assumption that residuals are independent and identically distributed, which can bias parameter estimates and can increase Type I Error rates (Dormann et al. 2007).

As species with a few sightings in a city may have been incorrect observations or vagrants, we ran our analysis with three different sampling criteria: 1) keeping all observations of species observed at least once in a city (see SI Fig 2.4, Fig 2.5; Table 2.3); 2) keeping only observations of species observed 10 or more times in a city; and 3) keeping only observations of species observed over 20 times in a city (see SI Fig 2.6, Fig 2.7; Table 2.4). For analyses presented in the main text, we chose the second sampling criteria and kept observations of species that were observed 10 or more times in a city—and included both incomplete and complete checklists. We also ran our analyses using complete checklists only (see SI Fig 2.8, Fig 2.9; Table 2.5), which also gave similar results.

Results

Our final dataset was comprised of 13,502 observations of 213 bird species from 29 families identified ten or more times in 551 cities during at least one breeding season (Fig 2.1). We used the position of effect sizes and the breadth of confidence intervals to assess relationships (Nakagawa & Cuthill 2007), and the conditional and marginal R^2 values, and the intra-class correlation coefficients to assess model fit (Nakagawa et al. 2017; Nakagawa & Schielzeth 2013) (Table 2.1 and Table 2.2). Conditional R^2 considers the variance explained by both fixed and random factors, marginal R^2 considers variance explained by fixed factors. The intra-class correlation coefficient calculates the proportion of variance explained by each random effect. City compactness was negatively related to species longevity (-0.056, CI= -0.076, -0.035) and body mass (-0.025, CI= -0.037, -0.013) and positively related to clutch size (0.021, CI= 0.005, 0.037) (Fig 2.2; see Table 2.1 for full model results). This means that as a city became more compact, it supported more species with shorter lifespans, lower body masses, and larger clutches. Median income was negatively related to species body mass (-0.012, CI= -0.021, -0.004) while human population size was positively related to body mass (0.014, CI= 0.003, 0.025) (Fig 2.2; Table 2.1). As migratory status was binary data, where species were classed as migratory (1) or resident (0) we used odds ratios and their confidence intervals to assess support for a relationship. An odds ratio greater than one describes a positive relationship, and therefore our results show that the likelihood of species being migratory increased in more compact cities (1.461, CI= 1.332, 1.602) (Fig 2.3; Table 2.2). There were no other detectable relationships identified in our models (Fig 2.2, Fig 2.3; Table 2.1, Table 2.2).

Spatial autocorrelation

There was no spatial autocorrelation in the model residuals for longevity (P= 0.22, Moran's I= 4.27e-05), clutch size (P= 0.44, Moran's I= -5.14e-05) or body mass (P= 0.26, Moran's I= 2.58e-05) (see Fig S2.10 for maps of spatial variation in Passerine life-history traits and spatial dependency in model residuals for each model across the United States). The model residuals for migratory status were statistically significant, but Moran's I was negligibly low (P= 2.83e-06, Moran's I= 6.26e-04).

Discussion

The lifespans, body masses, clutch sizes, and migratory strategies of species found in different cities all varied with the degree of city sprawl, and the body masses of species varied with median income and population size (Fig 2.3). These relationships presumably reflect differences in the average fitness of a species with these traits given the local features of a particular city. This suggests that phenotype-based differential survival of species—in this case related to environmental factors associated with a city's socioeconomics—plays a role in determining biodiversity at the earliest stage of community formation following the emergence of new environments. Like environmental variation in natural habitats, cities vary in the details of their composition, and different species-level traits and migratory strategies are likely better suited to different types of cities.

Cities are heterogeneous ecosystems that vary in their social, socioeconomic, biological, and physical components (Alberti 2015; Grimm et al. 2008; Schell et al. 2020; Szulkin et al. 2020), and urban ecological and evolutionary processes can be influenced by both past and present human activities (Clarke et al. 2013; Des Roches et al. 2020; Roman et al. 2018). Social inequities in cities, such as relationships between urban vegetation cover, temperature, pollution, and race can influence urban biodiversity patterns and the structure and composition of the urban area as a whole (Clarke et al. 2013; Jesdale et al. 2013; Roman et al. 2018; Schell et al. 2020; Tessum et al. 2019; Watkins & Gerrish 2018). Sprawl is largely a product of such societal and sociopolitical processes (Bullard et al. 2000; Huang et al. 2007). We found that more compact cities with less sprawling suburbs tended to support smaller, short-lived species with larger clutches.

In general, more natural areas with plentiful and stable resources tend to support greater numbers of small-bodied species that prioritize reproduction at the expense of lifespan, perhaps due to high offspring survival rates (Bennett & Owens 2002; Bielby et al. 2007; Sæther & Bakke 2000). Stochastic or resource-poor environments tend to support larger, longer-lived species that prioritize adult survival over producing many offspring. This strategy is thought to buffer against the consequences of reproductive failures by spreading the risk of offspring mortalities across multiple breeding attempts during a long lifetime. In our case, species body mass, clutch size, and lifespan varied together as expected if structurally compact cities were relatively resource-rich and stable environments for birds. Urban sprawl is highly correlated with housing density (SI Fig 2.2): densely housed areas tend to be compact, with housing concentrated in and around city centers. Areas with lower housing density are generally more scattered (Ewing et al. 2002). The remaining natural areas in sprawling low-density cities are generally highly fragmented, degraded, and isolated. Consequently, they might have less vegetation and less connected green space than more compact cities (Marzluff & Ewing 2001; Robinson et al. 2005). Lower levels of sprawl may thus indirectly lead to more resources for birds particularly on a citywide scale.

The results for migratory species support our resource and environmental variability-based interpretation of urban sprawl. More compact urban areas tended to support relatively more migratory species, whereas the median income or human population size of a city were not obviously important. Migratory species generally choose breeding sites with suitable resources for breeding (Dalby et al. 2014; Somveille et al. 2015, 2019). That more migratory species are found and choose to breed in low sprawl cities suggests that they may be choosing these cities, at least in part, based on resource availability (Faaborq et al. 2010; Jenkins et al. 2017; Martin & Karr 1986).

We also found that species body mass was negatively related to median income and positively related to human population size. Body mass is generally thought to correlate with several demographic traits (Sæther 1987; Western & Ssemakula 1982), yet we found no evidence of such covariation with longevity or clutch size when looking at median income and population size (see also Sol et al. 2014).

A recent study exploring species filtering across three cities also found that different cities seemed to filter for slightly different subsets of traits—notably diet guild, habitat preference, and migratory status, suggesting that species filtering in cities is at least partly related to resource availability (Hensley et al. 2019). This finding, in conjunction with our spatially extended analyses, suggests that human actions and socioeconomics can shape trait groups within urban bird communities based on resource availability and variability so that species with similar phenotypic traits tend to co-occur in similar environments. Other factors, such as climate, temperature, the amount of green space, or the amount of open habitat in the city likely also drive species observations in urban areas, as found in previous studies (Filloy et al. 2015, 2019; Lee et al. 2019). These factors are likely in part correlated with the socioeconomic variables we measure and they will also contribute to the unexplained variation in our data.

Cities, and particularly human activities in cities, provide resources and disturb birds in many ways. In more compact cities, housing density and accompanying roads, traffic, and pollution, among other things, are generally more concentrated near city centers. This may lead to more intact less fragmented remnant vegetation patches in those cities, and less noise and light pollution outside the city center. Wealthier areas generally have higher plant diversity and vegetation cover—a well-known pattern called the ‘luxury effect’—and people in wealthier cities may provide more supplemental food, shelter, and nesting habitats for urban birds (Hope et al. 2003; Iverson & Cook 2000; Kinzig et al. 2005; Leong et al. 2018; Talarchek 1990). Wealth effects may present differently across cities, however; wealthier cities generally have more extensive road systems and more sprawling urban areas than poorer cities, which may limit the resources available for birds (Huang et al. 2007). However, wealthier cities also have more open space that corresponds to parks, vegetation, and water (Huang et al. 2007). On the other hand, highly populated and sprawling cities may be the most disturbed in terms of traffic levels, noise, artificial light, and pollution (Isaksson 2018; Luck 2007; McKinney 2001, 2002; Strohbach et al. 2019). This might make these areas most suitable for birds with traits that are advantageous in unstable environments. These habitats might also provide anthropogenic food sources via bird feeders and human food waste (Lepczyk et al. 2004; Tryjanowski et al. 2015) which can influence urban bird community structure and breeding success (Galbraith et al. 2015; Robb et al. 2008). The diverse plant communities found in backyards and gardens in sprawling suburban neighborhoods can also provide important sources of food, cover, and nesting resources for birds able to thrive in human-dominated landscapes (Narango et al. 2018; Smith et al. 2005, 2006; Thompson et al. 2003). Consequently, although cities are generally more similar to each other than to their natural surroundings, our results suggest that from a bird’s eye view, they are heterogeneous habitats supporting different life-history and migratory strategies that in part depend on socioeconomic factors and the built environment. It is worth noting that our method of using species presence in cities as our observations could not distinguish between primarily urban-dwelling species and transient species visiting cities from surrounding areas and so it is possible that not all species are fully urban colonizers (Evans et al. 2011; Sol et al. 2014). Nevertheless, it seems likely that individuals choosing to enter a city are treating it as a resource, especially during energetically taxing breeding seasons, and thus our species are city users if not necessarily urban breeding species.

However, we note that even though cities may provide resources to some birds, cities also harbor considerable threats to birds. Cats, often let to roam free in suburban areas, are the main source of mortality to birds in cities, resulting in 2.4 billion deaths in the U.S. yearly (Loss et al. 2013). Window collisions are also a major source of bird mortality in cities killing millions of birds annually (Calvert et al. 2013; Machtans et al. 2013). Additionally, the food resources found in cities may be of lower quantity or quality than those found in natural areas (Pollock et al. 2017; but see Seewagen et al. 2011). It is, therefore, possible that cities attract birds, but could act as ecological traps—birds might actively choose cities to breed in, and that decision might then reduce their fitness (Hale & Swearer 2016).

Generally, our results highlight the complexity of human-dominated urban ecosystems, where human socioeconomic factors and everyday activities intermix leading to structurally complex environments that support the colonization of some species over others. The proximate causes of urban environmental variation are likely to vary for different cities and species. Nevertheless, species traits can be predicted by the ultimate cause of urban environments—human activities. As cities are the world's most rapidly growing ecosystem, understanding what kinds of species initially colonize cities provides important information about how the distribution of biodiversity will change following rapid, human-caused environmental shifts in an era of global change. Finally, although cities tend to support fewer species than nearby natural areas (Chace & Walsh 2006), on average urban biodiversity is primarily comprised of native species (Aronson et al. 2014). These findings in addition to our results suggest that cities could play a more important role in conservation and management than they currently do.

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Table 2.1 Model summaries for the urban predictors of passerine life-history traits in the United States. One model was fit to data per response variable, including all the urban characteristics: city compactness/sprawl, median income, and human population size. Body mass was $\log_{10}$-transformed. Random effects were specified as (1 | family) and (1 | state), where each level of the grouping factors, family and state, had its own random intercept. We also report the conditional and marginal R^2 values and the intra-class correlation coefficients. Conditional R^2 considers the variance explained by both fixed and random factors, marginal R^2 considers variance explained by fixed factors. The intra-class correlation coefficient calculates the proportion of variance explained by each random effect. The number of observations was n = 13,502 for all the life-history variables.

Response	**Predictors**	**Estimates**	**95% CI**	**SE**	**t value**
Longevity	Intercept	-0.422	-0.713, -0.131	0.15	-2.84
	Compactness	-0.056	-0.076, -0.035	0.01	-5.32
	Median income	-0.004	-0.018, 0.010	0.01	-0.62
	Population size	-0.002	-0.020, 0.016	0.01	-0.22
R^2_c	0.552				
R^2_m	0.033				
$ICC_{[State]}$	0.013				
$ICC_{[Family]}$	0.538				
Clutch size	Intercept	0.400	-0.049, 0.849	0.23	1.75
	Compactness	0.021	0.005, 0.037	0.01	2.55
	Median income	0.005	-0.005, 0.016	0.01	0.98
	Population size	-0.014	-0.028, 0.001	0.01	-1.87
R^2_c	0.826				
R^2_m	0.000				
$ICC_{[State]}$	0.02				
$ICC_{[Family]}$	0.824				
Body mass	Intercept	3.033	2.725, 3.341	0.16	19.30
	Compactness	-0.025	-0.037, -0.013	0.01	-4.09

	Median income	-0.012	-0.021, -0.004	0.00	-2.82
	Population size	0.014	0.003, 0.025	0.01	2.50
R^2_c	0.779				
R^2_m	0.030				
$ICC_{[State]}$	9.851e-04				
$ICC_{[Family]}$	0.778				

SE = Standard error, R^2_c = Conditional R^2, R^2_m = Marginal R^2, ICC = Intra-class correlation coefficient

Table 2.2 Model summaries for the urban predictors of passerine migratory status in the United States. One model was fit to data per response variable, including all the urban characteristics: city compactness/sprawl, median income, and human population size. Body mass was $\log_{10}$-transformed. Random effects were specified as (1 | family) and (1 | state), where each level of the grouping factors, family and state, had its own random intercept. We also report the conditional and marginal R^2 values and the intra-class correlation coefficients. The number of observations was n = 13,298.

Response	**Predictors**	**Odds ratios**	**95% CI**	**SE**	**z value**
Migratory status	Intercept	0.267	0.035, 2.011	1.03	-1.28
	Compactness	1.461	1.332, 1.602	0.05	8.03
	Median income	1.033	0.972, 1.1	0.03	1.04
	Population size	0.930	0.849, 1.020	0.04	-1.54
R^2_c	0.897				
R^2_m	0.003				
$ICC_{[State]}$	0.009				
$ICC_{[Family]}$	0.887				

SE = Standard error, R^2_c = Conditional R^2, R^2_m = Marginal R^2, ICC = Intra-class correlation coefficient

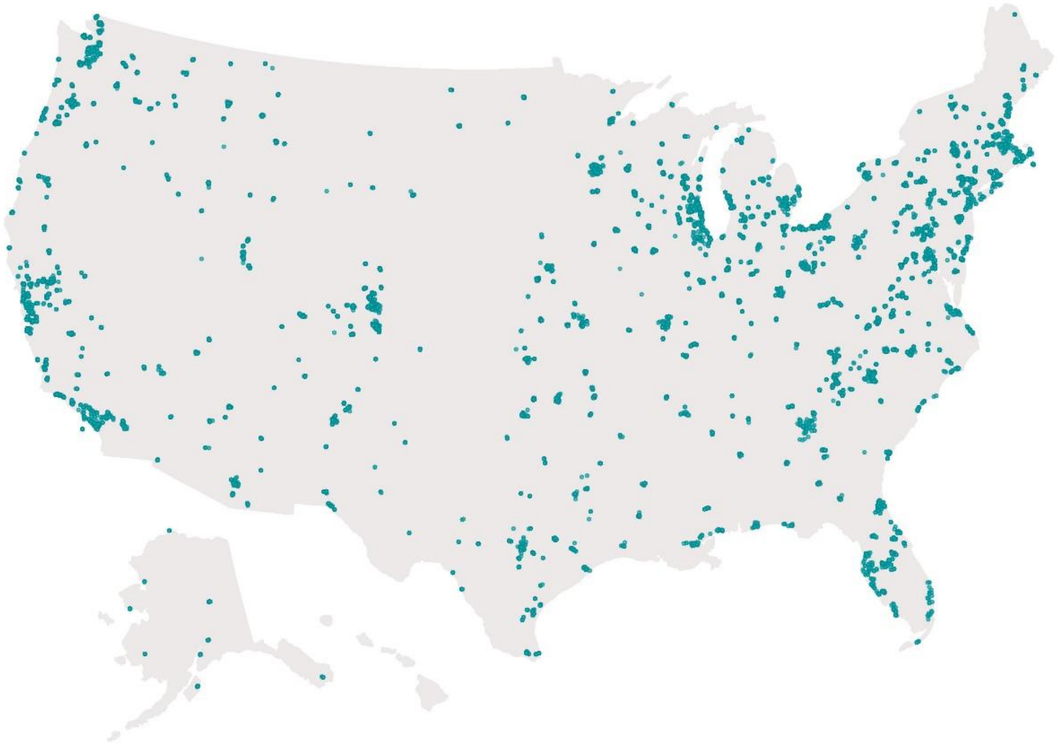

Figure 2.1 Map of 13,502 bird observations of 213 Passerine species in 551 cities across the United States. Circles represent the longitude and latitude coordinates of bird observations.

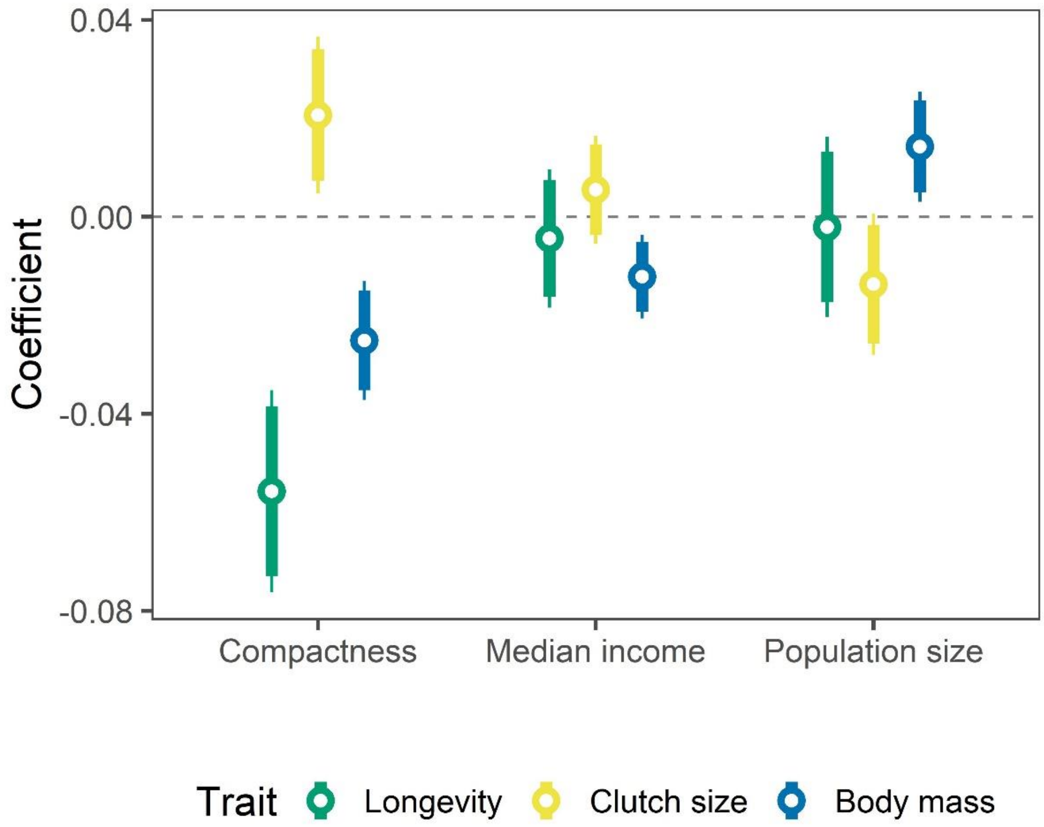

Figure 2.2 Linear mixed-effects model coefficients for urban predictors of passerine life-history traits in the United States. Open circles represent coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. Body mass was $\log_{10}$ -transformed. Sample size is the same for all variables (n = 13,502).

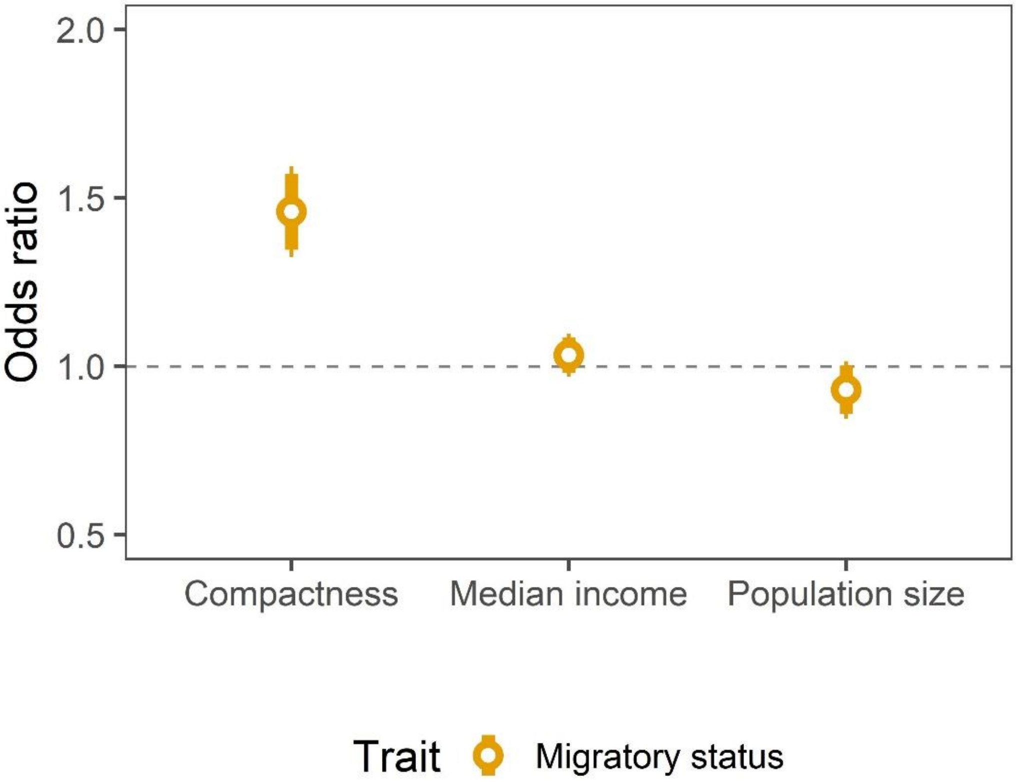

Figure 2.3 Generalized linear mixed-effects model coefficients for the predictors of passerine migratory status in the United States. Open circles are coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. Species are classed as migratory = 1 or resident = 0. An odds ratio greater than one indicates that the chance of finding a migrant bird in the area is higher than a chance of finding a resident, and vice versa. The likelihood of species being migratory increased in more compact less sprawling cities. The sample size is n = 13,298.

Chapter 3: Socioeconomic and structural variation across cities as a driver of migratory bird species richness

RP Kinnunen[1], KC Fraser[1], C Schmidt[1], CJ Garroway[1]

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

Abstract

Some migratory bird species can be found in cities during the breeding season, which implies they find sufficient resources in cities to support them during this energetically costly time. However, we know little about how the species richness of migratory birds varies across different cities. As populations of many migratory bird species are declining knowledge of how they are affected by urbanization is needed as cities could be important resources for management and conservation. Here we ask if socioeconomic variation across cities can predict the species richness of migrants belonging to different breeding habitats found in cities during the breeding season. We hypothesized that the socioeconomic features of cities might attract migrants in ways that are not obvious from the examination of human-associated environmental disturbance alone. To test our predictions, we compiled a dataset from open data sources. Overall, our results show that the socioeconomic features of cities predict the species richness of migratory birds found in cities and that human social effects on migratory birds can diverge from those predicted for human-related environmental disturbance per se. This is likely linked to the human-associated resources available to migrants in these habitats that offset sources of human-associated environmental disturbances. Our results highlight the importance of socioeconomic and structural variation across cities and imply that this should be further considered in studies of urban biodiversity as it can drive patterns in animal ecology. Migratory species are experiencing drastic population declines and natural protected areas are likely insufficient to halt declines. Our results suggest that we may be able to extend conservation and management actions to cities for the benefit of humans and wildlife.

Keywords

anthropogenic disturbance, conservation, migratory birds, socioeconomic variation, species richness, urbanization

Introduction

Land-use change is one of the most important threats to biodiversity worldwide (Sala et al. 2000). Birds seem particularly vulnerable; for example, there have been widespread, drastic population declines over the past 50 years in North America alone (Rosenberg et al. 2019). The estimated net loss of birds on this continent is close to 3 billion, with 2.5 billion of these being migratory (Rosenberg et al. 2019). These declines are comparable to patterns found globally (Gaston et al. 2003; Inger et al. 2015). The decline in the abundance of Nearctic–Neotropical migrants in particular is well documented (Kirby et al. 2008; Robbins et al. 1989; Sauer et al. 2013), but there are population losses across most avifauna and biomes (Gaston et al. 2003; Rosenberg et al. 2019). Migratory birds spend ~ 30% of the year on their migratory journey—enabling them to cope with seasonality and periods of low productivity (Somveille et al. 2015)—and occupy their breeding and overwintering grounds for the rest of the year. Migrants consequently have a comparatively large geographical range and may be particularly susceptible to land-use changes as a result (Runge et al. 2014). Additionally, protected areas alone are not enough to protect the ranges of most migrants that can fly through many different environments during their migratory journey. Therefore, many kinds of habitats and resources need to be considered, at appropriate temporal and spatial scales, if the decline of migrants is to be managed (Runge et al. 2014).

Cities now cover ~ 3% of all habitable land globally (Liu et al. 2014) with urban land growth expected to continue in the coming decades (Seto et al. 2011). Urbanization poses unique challenges to birds as cities can have elevated temperatures (i.e., heat island effect), vast areas with impervious surfaces, and increased levels of traffic, noise, pollution, and artificial light at night compared to more natural areas (Kyba et al. 2017; Leal Filho et al. 2018; Shochat et al. 2006). Additionally, compared to non-urban areas cities also have less vegetation cover, relatively large numbers of non-native species, and altered patterns of connectivity, resource availability, and inter- and intraspecific interactions (Grimm et al. 2008; Groffman et al. 2014). These challenges have led to the extirpation of many species in cities and a decline in biodiversity and species abundance in urban areas (e.g., Aronson et al. 2014; McKinney 2006).

Despite these risks, cities can provide resources for birds and even support some threatened species (Aronson et al. 2014). The warmer climate, abundant anthropogenic food and nesting resources (e.g., bird feeding stations, cultivated plants, nest-boxes, nests under roof awnings), and less dramatic seasonal changes over the year can make cities a desirable habitat for some species (Conole & Kirkpatrick 2011; Jokimäki et al. 2016; Lim & Sodhi 2004). Consequently, cities can be considered new ecosystems that support those species able to live alongside people—the defining feature of urban environments (Pickett et al. 2017)—and take advantage of the resources that cities offer. Many migratory birds are found in cities during breeding and non-breeding seasons (Brawn & Stotz 2001; Hostetler et al. 2005; Partridge & Clark 2018), and urban green areas may offer the only sites available for refueling for numerous migrants crossing large metropolitan areas (Brawn & Stotz 2001; Mehlman et al. 2005). Yet, studies to date have largely overlooked the potential importance of city habitat to migratory birds, and the ways that different migratory species use the habitat are still largely unknown (but see Partridge & Clark 2018; Seewagen et al. 2011; Seewagen & Slayton 2008).

Although cities tend to be more similar to each other than to neighboring rural areas (Groffman et al. 2014), cities vary in their social, socioeconomic, biological, and physical components (Grimm et al. 2008; Schell et al. 2020; Szulkin et al. 2020). Cities are created by humans for humans and are thus aggregates of human activities (Li et al. 2015). Our activities and social structure shape the city environment creating heterogeneity across urban centers (Des Roches et al. 2020; Hobbs et al. 2006; Pickett et al. 2017; Schell et al. 2020). Increasing evidence suggests that human social decision-making can have significant ecological effects on species that differ from those evident in environmental measures of disturbance (Des Roches et al. 2020; Schell et al. 2020). Human activities per se can consequently highly influence the socioeconomic features of cities, and this variation may influence what resources are available to migratory birds in these different types of cities. Yet how and why migratory bird species composition varies in different cities remains largely unknown.

There is some evidence that Neotropical and short-distance migrants are generally underrepresented in urban ecosystems as compared to resident species (Allen & O'Connor 2000; Kluza et al. 2000; Poague et al. 2000), but to the best of our knowledge, no studies have yet explored what types of migrants are found in cities (e.g., migrants from different breeding habitats). A recent continent-wide assessment across the United States found large population losses in many types of birds, including migratory birds, spanning diverse ecological and taxonomic groups (Rosenberg et al. 2019). Shorebirds, grassland, and forest breeding species experienced some of the largest population losses (Rosenberg et al. 2019). Here, we wanted to see how the species composition of migratory birds preferring different breeding habitats might look in different cities by asking if city features can predict the types of migrants found in cities during the breeding season.

The focus of our study was on the effects of human activities on migratory birds per se and we considered city features as general metrics of human social and socioeconomic activity. This is because it is now clear that human activities can have effects on wildlife that are different than those predicted from our effects on environments alone and such effects are not well characterized (Des Roches et al. 2020; Schell et al. 2020). We thus also examined the extent to which a composite variable estimating human-related disturbance (the Human Footprint Index) varied with our socioeconomic features of cities (city age, income, commuting time, or the number of housing units) to guide our interpretation of the effects of human social activities. Answers to these questions will inform us of how migratory birds might be affected by urbanization during an energetically demanding breeding season. As many migratory species are experiencing drastic population declines, this knowledge is important for their conservation and to support the management efforts of migratory birds in cities.

Materials and Methods

Data compilation and variable selection

We compiled data from open data sources (see Supplementary Information for a more detailed data compilation procedure). First, we downloaded the eBird Basic Dataset for the United States (available from https://ebird.org), which is a publicly available large core dataset with >100,000,000 observations worldwide (Sullivan et al. 2009). eBird is an online bird abundance and distribution checklist program that relies on citizen science volunteer observers who submit georeferenced observations of species to a centralized database. Observations are submitted in a checklist format, listing species seen during one bird-watching occasion. Checklists are designated as either complete checklists, where all birds detected and identified were recorded, or incomplete checklists, where some species may have been seen but were not recorded. Regional reviewers verify each species observation based on sighting coordinates identifying outliers (Wood et al. 2011).

We filtered the eBird dataset using Bash scripts and R Statistical Software (version 3.6.3; R Foundation for Statistical Computing, Vienna, Austria). We selected only complete checklists to account for species absences due to reporting (Sullivan et al. 2014); checklists that followed traveling, traveling- property specific, stationary, area, or random protocols (see Supplementary information for more details); and checklists that covered a distance of 5 km or less with a survey time between 5 to 240 minutes (Johnston et al. 2021). This allowed us to refine data and standardize effort between checklists to account for variable survey effort between cities. We selected observations between the years 2010 and 2019. We further filtered data by selecting only migratory birds (full migrants, partial migrants, altitudinal migrants, and nomads) based on BirdLife International classification by Sheard et al. (2020), and discarded data for non-migrant species. We only chose observations of species during the breeding season, as this is an energetically demanding period reflecting reproductive success for migratory birds. We defined the breeding season as beginning from May 27th lasting until July 7th, following the practices of the North American Breeding Bird Survey (available from https://www.pwrc.usgs.gov/bbs/index.cfm). This definition likely removes some observations of breeding migrants, as breeding times may vary between and within species, but our estimations of survey completeness indicate that overall eBird surveys captured most species found in a city (see below). We discarded observations of transient birds likely en route to their breeding grounds, observations that were part of the birds' overwintering range, and vagrants from each state. We note that the migrants in our dataset are species that were detected using cities during the breeding season but may not necessarily breed in cities.

We assigned georeferenced eBird species records to urban areas using U.S. Census-defined urban area maps (see Fig S3.1) (U.S. Census Bureau 2010b). These shapefiles define an urban area as a densely developed territory with a population size of at least 2,500 (U.S. Census Bureau 2010b). We used the R packages *sp* (version 1.3-1; R. S. Bivand et al. 2013), *rgdal* (version 1.3-4; R. Bivand et al. 2018), and *maps* (version 3.3.0; Becker & Wilks 2018) to perform a spatial join between urban areas and eBird records. Next, we obtained socioeconomic features for these urban areas from the 2010 census data and the 2011-2015 American Community Survey 5-year estimates data from the U.S. Census Bureau (U.S. Census Bureau 2010a). The American Community Survey provides current demographic, social, economic, and housing estimates throughout the decade (www.census.gov/acs). The survey randomly samples ~ 3.5 million addresses each year and produces statistics that cover 1-year and 5-year periods for geographic areas in the United States and Puerto Rico. We chose the number of housing units, commuting time, city age, and household income as our general metrics of human social and socioeconomic activity. As our study is observational, we did not want to hypothesize to a large extent what resources our city features may offer to migrants in those cities. However, the number of housing units may, among other things, be correlated with fragmentation and the number of yards and human-provided resources (e.g., bird feeders, number of chimneys) in a city. Commuting time (aggregate travel time to work in minutes) will be correlated with urban sprawl as daily commutes are generally longer in sprawling areas (Sultana & Weber 2007). Sprawl may fragment and isolate any remaining vegetation patches in cities, possibly leading to the loss of open and connected habitat and fewer resources for birds (e.g., grassland birds) (Valiela & Martinetto 2007). The American Community Survey calculates the aggregate travel time to work by adding all commuting times (in minutes) for workers who did not work at home during a reference week. As commuting distances may be longer in larger cities, we standardized commuting time values by dividing travel time by city area (in square miles). We calculated the median age of a housing structure in years (2021 minus the median year when buildings were built) and used it as a proxy for city age as cities with older housing have likely been founded earlier. Older cities likely have older vegetation (e.g., larger trees with more cavities). Median household income can be considered as a proxy for vegetation cover and diversity—as per luxury effect (see review by Leong et al. 2018)—that are important predictors of migratory bird diversity (Hutto 1985; Petit 2000). We also added a city's latitude and longitude as additional covariates in our models to account for spatial variation in species richness as we would expect to find e.g., more boreal forest breeding migrants in the higher latitudes and more eastern forest breeding migrants in the east. We note again that while we suspect that these socioeconomic features will be correlated with environments, we predict that the effects of socioeconomics will differ from those predicted by environments alone.

Migratory bird categories

Data on the primary breeding biomes of each species was based on Rosenberg et al. (Rosenberg et al. 2019), modified from the categories used in the Avian Conservation Assessment Database that provides conservation assessment and species prioritization data for all North American bird species at global and regional scales (available from https://pif.birdconservancy.org/avian-conservation-assessment-database/). Breeding biomes were divided into ten distinct categories: wetlands; coasts; tundra; grasslands; aridlands; boreal forest; eastern forest; western forest; forest generalist; and habitat generalist (see Table 3.1 for descriptions of each category).

Survey completeness

To estimate survey coverage (see Fig S3.2) across our eBird sampling locations we used the R package *KnowBR* (Lobo et al. 2018) (see SI3 for further details). KnowBR uses species accumulation curves and diversity estimators to assess the completeness of species inventories in multiple geographic cells of a size defined by the user.

Data analyses and controlling for spatial variation

All statistical analyses were performed using R Statistical Software (version 3.6.3; R Foundation for Statistical Computing, Vienna, Austria). We analyzed our data by fitting generalized linear models (GLMs) with a negative binomial error distribution and the log link function except in the case of grassland and aridlands species where a poisson distribution and the log link function was most appropriate. Migrant species richness for each breeding biome group (wetlands, coasts, tundra, grasslands, aridlands, boreal forest, eastern forest, western forest, forest generalist, and habitat generalist) was our response variable, and all city features (housing units, median income, city age and commuting time) and city latitude and longitude our explanatory variables. Housing units, median income, city age, and commuting time were not strongly correlated (Fig S3.3). Data were scaled to standardize the range of response and predictor variables to make model effects comparable. The number of housing units and commuting time were log-transformed to ensure the normality of residuals.

We tested the residuals of our models for spatial autocorrelation using the R package *DHARMa* (Hartig 2020). Residuals for all the models were spatially structured (P-value < 0.05) which violates the assumption that residuals are independent and identically distributed. This can bias parameter estimates and can increase Type I Error rates (Dormann et al. 2007). One way to control for spatial variation is to use distance-based Moran's Eigenvector Maps (dbMEMs) (Dray et al. 2006). These capture spatial relationships among data points at all spatial scales and are orthogonal and so can be added to the model as explanatory variables to account for spatial variation (Dormann et al. 2007; Griffith & Peres-Neto 2006). We selected only the smallest subset of spatial eigenvectors as spatial predictors that best minimized the spatial autocorrelation in model residuals (see SI3 for information about MEM selection) and reran our analyses as before, but with dbMEMs added as additional explanatory variables to our model.

Human-related disturbance in each city

The focus of our study was on the effects of human socioeconomics on migratory birds per se, as human social decision-making can have significant ecological effects on species that differ from those evident in environmental measures of disturbance (Des Roches et al. 2020; Schell et al. 2020). We thus quantified the relationships between our city variables and a general measure of extent of human disturbance in each type of city. To do this we extracted the mean Global Human Footprint Index (HFI) for each of our cities as defined by the urban area shapefile maps from the U.S. Census (available from https://sedac.ciesin.columbia.edu/data/set/wildareas-v2-human-footprint-geographic/data-download). The Global Human Footprint Index consists of data generated from nine global data layers representing human population pressure, human land use and infrastructure, and human access, normalized by terrestrial biome and realm (Wildlife Conservation Society & Center for International Earth Science Information Network 2005). The data covers many types of human influences on the environment, namely population density, land use, land cover, built-up areas, nighttime lights, roads, railroads, coastlines, and navigable rivers. Expressed as a percentage, a value of zero on the index represents an area least influenced by humans, and a value of 100 the most disturbed area, or an area most influenced by humans. We then calculated Spearman's rank correlation coefficient ρ to summarise the strength and direction of a relationship between the HFI and our city variables (city age, income, commuting time, or the number of housing units).

Results

Our final dataset had a total of 41 species of eastern forest migrants (Table 3.1; Table S3.1) in 1304 cities; 30 species of habitat generalists in 1556 cities; 71 species of wetland migrants in 1208 cities; 23 species of forest generalists in 1295 cities; 21 species of grassland migrants in 1043 cities; 37 species of western forest migrants in 410 cities; 27 species of aridlands migrants in 473 cities; 36 species of tundra migrants in 223 cities; 19 species of coastal migrants in 226 cities; and 12 species of boreal forest migrants in 278 cities. All species were identified in a city at least once during at least one breeding season.

Survey completeness

To determine if eBird surveys adequately sampled species using cities during our study period (2010–2019), we calculated the final slope of the species accumulation curves and completeness percentage values (see Fig S3.2). Slopes of the accumulation curves leveled off to < 0.19 for the majority of the sampling areas and the completeness percentages of eBird survey effort for each geographic unit were mostly between 64 to 102 percent. This indicates that the eBird surveys were capturing most species found in a city.

Data analyses and controlling for spatial variation

We used the position of effect sizes and the breadth of confidence intervals to assess relationships (Nakagawa & Cuthill 2007) and the coefficient of variation (R^2) and model validation as indicators of model fit (see Table 3.2 for model estimates for the urban predictors and Table S3.2 for estimates for the covariates). City age was negatively related to the species richness of habitat generalists and migratory birds breeding in aridlands and eastern forests, and positively related to the species richness of tundra and coastal migrants (see Fig 3.1). Commuting time (time commuting each day in minutes) was positively related to the species richness of eastern forest, grassland, aridlands, and wetland migrants, and forest and habitat generalists. Median income was positively related to the species richness of eastern, western, and boreal forest migrants, grassland and wetland migrants, and forest and habitat generalists. The number of housing units was positively related to the species richness of all migrants, except for coastal migrants.

Spatial eigenvector maps

Eastern forest migrants were the most spatially structured; other groups had low levels of structure (See Table 3.2 and SI3 for more details on MEMs). The addition of eigenvectors as explanatory variables reduced spatial autocorrelation in our model residuals in all cases.

Human disturbance in each city

Our data to estimate the human disturbance in each city had 1373 observations. The scatterplots (see Fig 3.2) and the associated Spearman's rank correlation coefficients indicate a weak negative correlation between Human Footprint Index and median income ($\rho = -0.16$), suggesting less human disturbance in wealthier cities. The plots also show a positive correlation between the Human Footprint Index and the log-transformed number of housing units ($\rho = 0.26$), and the Human Footprint Index and city age ($\rho = 0.22$). This suggests that highly developed and older cities have more disturbance. We saw no clear association between Human Footprint Index and log-transformed commuting time ($\rho = -0.01$).

Discussion

There were clear correlations between the socioeconomic and structural features of cities and the species richness of migrants generally selecting different habitats as breeding grounds. Patterns of city use by migratory birds did not necessarily follow expectations based on human-related environmental disturbance. For example, species tended to use cities with more housing units but these cities also tended to be more disturbed (higher Human Footprint Index). This suggests that the human social effects on animal ecology can diverge from those expected from human-related disturbance per se. Human-provided resources likely balance the negative effects of human-related disturbance, but this remains to be explored with species-specific field-based studies. Humans are the defining feature of cities, shaping and disturbing the city environment in numerous ways that may not straightforwardly link with measures of environmental disturbance. Our public policies and social decisions, biases and social expectations, and preferences, interests, and management ultimately influence the overall city environment in ways that can influence the resources available to animals in those cities.

Our results show that cities filter migratory birds and the results are surprisingly consistent across species groups that rely on different habitat biomes for breeding. Species generally relying on different habitats during the breeding season are responding to city variation in similar ways. The negative influence of human activities in highly developed cities in conjunction with the positive relationship between the species richness of most migrants and the number of housing units in a city seems to imply that although development can be a substantial threat to some birds (Tilman et al. 2017; Tratalos et al. 2007), the benefit to most migrants in highly developed cities may primarily arise from human-associated habitat and resources. The numerous houses, yards, and diverse plant communities in residential neighborhoods might offer sources of food, cover, and nesting resources for migratory birds (Smith et al. 2005, 2006). Additionally, residential areas can also have a high percentage of forest cover compared to other land use areas (Alberti 2005), and backyards can increase connectivity and ecological function in cities (Sperling & Lortie 2010). Combining greater numbers of residential yards with existing public green spaces and parks may create networks of resource-rich habitats in cities (Goddard et al. 2010) that outweigh the negative consequences of human disturbance as measured by the Human Footprint Index. Tests of this hypothesis would require detailed species-specific field-based studies within cities that are outside the scope of this research.

Older cities had lower species richness of habitat generalists and birds that breed in aridlands and eastern forests, and higher species richness of migrants that breed in tundra and coastal regions. A previous study in Chicago reported a decrease in migratory bird species richness in older areas, albeit at a neighborhood scale, and found a positive relationship between housing age and the intensity of urban development (Loss et al. 2009). Here we found that older cities had higher levels of human-related disturbance but in this instance, city age does not seem to be associated with human-associated resources, except for shorebirds and migrants that generally breed in the tundra. In addition to being more disturbed, older urban habitats tend to have more non-native plant species than recently established habitats (Pyšek & Jarošík 2005), and younger neighborhoods can have more diverse and abundant vegetation than older neighborhoods in cities (Hope et al. 2003; Martin et al. 2004). Some studies also report more invasive species and more non-migrant species in older neighborhoods (Lerman & Warren 2011; Loss et al. 2009), which may negatively influence some migrants. Our results show that these within city patterns may also manifest on a citywide scale.

We found positive relationships between median income and the species richness of forest and habitat generalists, eastern, western, and boreal forest migrants, and grassland and wetland migrants. These findings are likely partly the result of “the luxury effect” (Leong et al. 2018), where plant diversity and vegetation cover are higher in wealthier areas (Hope et al. 2003; Iverson & Cook 2000; Kinzig et al. 2005). Unsurprisingly, wealth was negatively correlated with disturbance. Human preferences, in combination with the local climate, shape and create variation in the diversity and richness of plants found in the yards, gardens, and parks across cities (Pearse et al. 2018; Threlfall et al. 2016). Consequently, the plant species richness and phylogenetic diversity in cities are often greater than in comparable natural areas (Pearse et al. 2018). These effects may be inflated in wealthier cities as the structure and density of the understory and the proportion of native and exotic plant species can all change with the socioeconomic status of the neighborhood (Hope et al. 2003; Martin et al. 2004). However, people in wealthier cities may also directly offer birds more resources like supplementary food in feeding stations, or nesting places in human-built nest-boxes (Hope et al. 2003; Iverson & Cook 2000; Kinzig et al. 2005; Leong et al. 2018; Talarchek 1990), however, the evidence for this is less clear (Davies et al. 2009; Goddard et al. 2013).

Our findings also show that commuting time was positively related to the species richness of eastern forest, forest and habitat generalist, grassland, aridlands, and wetland breeding migrants. Since an increase in commuting time may be associated with more suburban sprawl, this may provide more resources to migratory birds in the form of backyards and landscaping as in the case of city development discussed above. Notably, commuting time was not correlated with human-related environmental disturbance (HFI), again highlighting how human social decisions can act in ways (e.g., provide resources) that are not obviously related to human disturbance.

Recent continent-wide assessment across North America found large population losses in many types of birds, with shorebirds, grassland, and forest breeding species experiencing some of the largest declines (Rosenberg et al. 2019). Although we could not look at population-level differences, we did find that cities supported some of these groups of birds. Human activities—for example, in the form of the number of housing units—seemed to particularly support forest breeding and grassland species. Many species from these two categories are common and generally can be found in urban or highly disturbed habitats, such as the grassland breeding species Eastern and Western Kingbird (*Tyrannus tyrannus*, *T. verticalis*) and Eastern and Western Meadowlark (*Sturnella magna, S. neglecta*) (information on the habitat preferences of these bird species available from https://birdsoftheworld.org). Several of the forest breeding species, like the Rose-breasted

Grosbeak (*Pheucticus ludovicianus*) and Blue Jay (*Cyanocitta cristata*), are common feeder birds. Some, like the Chimney Swift (*Chaetura pelagica*), Vaux's Swift (*Chaetura vauxi*), and the House Wren (*Troglodytes aedon*) use human-associated structures like chimneys or cavities in houses for nesting. The gardening and landscaping practices of homeowners may also provide food and nesting resources for birds. For example, thrushes like the Hermit Thrush (*Catharus guttatus*) and American Robin (*Turdus migratorius*) feed on berries, crab apples, and other fruit provided by people in their yards. Additionally, some hawk species, like the Cooper's Hawk (*Accipiter cooperii*) and Sharp-shinned Hawk (*Accipiter striatus*) commonly eat feeder birds, which might explain their presence in yards. This implies that the resources utilized by migrants in cities can be human-associated, and not necessarily related to the more natural environmental features (e.g., tree cover) often studied in the urban ecology field, although these features are most certainly highly important to wildlife inhabiting cities. Wetland migrants also seemed to thrive in cities. This is perhaps not so surprising, as major cities have generally been built around rivers (Grimm et al. 2008), and wetland bird abundance has been increasing in the past ~ 50 years due to targeted conservation efforts (Rosenberg et al. 2019). Furthermore, previous studies have found more waterbirds in or near urban development than in undeveloped habitats (Andrade et al. 2018; Pearse et al. 2018; Traut & Hostetler 2004), indicating these birds can use even small and isolated wetlands (Pearse et al. 2018).

That various types of migrants that generally select different habitats for breeding are found in cities implies that these birds are choosing to use habitats in cities because cities provide resources needed during the breeding season. However, even though resources found in cities can be used by some migratory birds, cities can also harbor considerable threats to migrants. Cats are the main source of mortality to birds in cities, resulting in 2.4 billion deaths in the U.S. (Loss et al. 2013) and approximately 140 million deaths in Canada (Calvert et al. 2013) yearly. Collisions with houses and low and midrise buildings—the main types found in suburban neighborhoods for example—are also a major contributor to bird mortality in cities killing millions of birds annually (Calvert et al. 2013; Machtans et al. 2013), with long-distance and nocturnal migrants being particularly vulnerable (Arnold & Zink 2011). These major threats could be particularly damaging in highly developed cities where the number of houses also corresponds to a high number of windows that pose a collision risk for birds. Suburban areas are also likely to have many outdoor cats, further intensifying the risks to birds breeding in these areas. There is also some evidence that the food resources that cities provide may be of lower quantity or quality than in more natural areas (Pollock et al. 2017; but see Seewagen et al. 2011). It is thus possible that highly developed cities could act as ecological traps—migrants might actively choose these habitats that might then reduce their fitness (Hale & Swearer 2016). There is, in fact, some evidence that the resources offered by cities may impair the breeding success of some birds (e.g., *Parus major*, Demeyrier et al. 2016; *Falco tinnunculus* Sumasgutner et al. 2014), however, not all species are negatively affected (e.g., *Cardinalis cardinalis*, Leston & Rodewald 2006). If cities with particular features attract migratory birds but act as ecological traps, this could have serious population-level effects on migrants that should be taken into consideration during management and conservation efforts.

Overall, we find results, sometimes counter-intuitive, of city socioeconomics that would not be predictable from human-associated environmental disturbance alone. These results show that human social effects on animal ecology can diverge from those of human-related disturbance per se. We show that the socioeconomic and structural traits of cities—for example, the number of housing units—act as drivers of migratory bird species richness. Our results are surprisingly consistent across species groups, suggesting that species generally relying on different habitats during the breeding season are responding to city variation in similar ways. Cities seem able to support migratory birds even in areas where disturbance levels are high. This is likely linked to human-associated resources (e.g., bird feeders) found in those cities. However, whether the resources offered by city socioeconomics are beneficial to migrants, or whether these cities act as ecological traps to some species still needs further investigation. As many migratory birds are currently facing declines and fragmentation of their preferred natural breeding habitats mainly due to land cover change (Drummond & Loveland 2010), further knowledge of their preferences and their ability to tolerate human-caused disturbance in different cities is of high importance. Management and conservation efforts in urban areas are often focused on preserving larger habitat patches (Müller et al. 2013), but our results show that urban yards, in particular, may also offer benefits to migrants and should be considered in migratory bird management strategies. Our results imply that socioeconomic and structural variation across cities should be further considered in urban biodiversity studies as it can drive patterns in species richness.

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Table 3.1 The total number of migratory species in each breeding biome found in cities across the United States during the breeding season between the years 2010-2019, and the description of each category.

Breeding Biome	Description	# Species
Aridlands	includes arid shrub-dominated communities mainly in southwestern U.S. and northwestern Mexico	27
Boreal Forest	includes boreal forest of Canada and Alaska and birds that use the boreal zone of high mountains in the western and northeastern U.S.	12
Coasts	includes marine zone foragers, and habitats like saltmarsh, beach and tidal estuary, mangroves, and rocky cliffs and islands	19
Eastern Forest	includes all temperate forest types of eastern U.S. and southeastern Canada comprising northern hardwoods, oak-hickory, pine-oak, southern pine, and bottomland hardwood associations	41
Forest Generalist	includes birds that occur in similar abundance in two or more forest biomes	23
Grassland	includes native grassland, prairie, pasture, and grassland birds supporting agriculture	21
Habitat Generalist	includes birds that occur in similar abundance in three or more major habitat	30

	types, usually containing forest and non-forest categories	
Tundra	includes Alpine and Arctic tundra	36
Western Forest	includes all temperate forest types of western U.S. and Canada, the Pacific Northwest rainforest, all western conifer, oak-dominated, and riparian forests, pinyon juniper, juniper-oak woodlands of Edward's Plateau, and pine-oak and high-elevation conifer forests of northwestern Mexico	37
Wetland	includes freshwater and inland wetlands, but not coastal marshes or Arctic tundra	71
Grand Total		318

Table 3.2 Generalized linear model estimates, standard errors (SE), z-values, and 95% confidence intervals for urban predictors of migratory bird species richness for different breeding biome groups in the United States. The commuting time and number of housing units are log-transformed.

Response	Predictors	Estimates	SE	z value	95% CI
Eastern forest	Intercept	-1.60	0.18	-9.03	-1.96, -1.25
	Median income	0.05	0.02	2.70	0.01, 0.09
	City age	-0.08	0.02	-3.40	-0.12, -0.03
	Housing units	0.30	0.02	18.59	0.27, 0.33
	Commuting time	0.10	0.02	4.89	0.06, 0.14
R^2	0.792				
N	1137				
Forest generalist	Intercept	-1.28	0.13	-9.84	-1.54, -1.03
	Median income	0.10	0.01	7.09	0.07, 0.13
	City age	0.02	0.02	1.08	-0.01, 0.05
	Housing units	0.23	0.01	19.72	0.21, 0.26
	Commuting time	0.09	0.01	5.91	0.06, 0.12
R^2	0.726				
N	1130				
Western forest	Intercept	-0.36	0.34	-1.04	-1.01, 0.29

	Median income	0.13	0.03	3.83	0.07, 0.20
	City age	-0.00	0.04	-0.12	-0.07, 0.07
	Housing units	0.20	0.03	6.80	0.15, 0.26
	Commuting time	0.02	0.04	0.57	-0.05, 0.09
R^2	0.612				
N	329				
Boreal forest	Intercept	-1.79	0.39	-4.57	-2.56, -1.02
	Median income	0.10	0.05	1.87	-0.01, 0.20
	City age	0.07	0.06	1.12	-0.05, 0.20
	Housing units	0.25	0.04	6.14	0.17, 0.33
	Commuting time	-0.01	0.05	-0.13	-0.09, 0.08
R^2	0.487				
N	253				
Habitat generalist	Intercept	-0.83	0.13	-6.55	-1.09, -0.58
	Median income	0.07	0.01	5.29	0.04, 0.10
	City age	-0.04	0.02	-2.54	-0.07, -0.01
	Housing units	0.22	0.01	19.77	0.20, 0.24
	Commuting time	0.10	0.01	7.47	0.08, 0.13

R^2	0.610				
N	1311				
Tundra	Intercept	-0.85	0.50	-1.71	-1.81, 0.10
	Median income	0.11	0.06	1.74	-0.01, 0.23
	City age	0.30	0.08	3.87	0.15, 0.45
	Housing units	0.17	0.05	3.70	0.08, 0.25
	Commuting time	0.04	0.05	0.81	-0.06, 0.15
R^2	0.379				
N	206				
Aridlands	Intercept	-1.68	0.26	-6.36	-2.19, -1.16
	Median income	0.02	0.03	0.71	-0.04, 0.08
	City age	-0.07	0.03	-2.16	-0.13, -0.01
	Housing units	0.22	0.02	9.69	0.17, 0.26
	Commuting time	0.05	0.03	1.79	-0.00, 0.11
R^2	0.863				
N	398				
Coasts	Intercept	0.25	0.46	0.54	-0.65, 1.15
	Median income	0.06	0.05	1.22	-0.04, 0.16
	City age	0.13	0.05	2.34	0.02, 0.23

	Housing units	0.05	0.04	1.23	-0.03, 0.13
	Commuting time	0.01	0.05	0.12	-0.09, 0.11
R^2	0.50				
N	209				
Grassland	Intercept	-2.80	0.19	-14.78	-3.17, -2.43
	Median income	0.06	0.02	2.53	0.01, 0.10
	City age	-0.03	0.03	-1.29	-0.08, 0.02
	Housing units	0.24	0.02	14.59	0.21, 0.27
	Commuting time	0.15	0.02	6.64	0.10, 0.19
R^2	0.659				
N	901				
Wetland	Intercept	-2.56	0.22	-11.78	-2.97, -2.14
	Median income	0.06	0.02	2.39	0.01, 0.10
	City age	-0.00	0.03	-0.17	-0.06, 0.05
	Housing units	0.33	0.02	17.14	0.30, 0.37
	Commuting time	0.15	0.02	6.15	0.10, 0.19
R^2	0.650				
N	1074				

R^2 = Nagelkerke's pseudo-R2, N = the number of observations

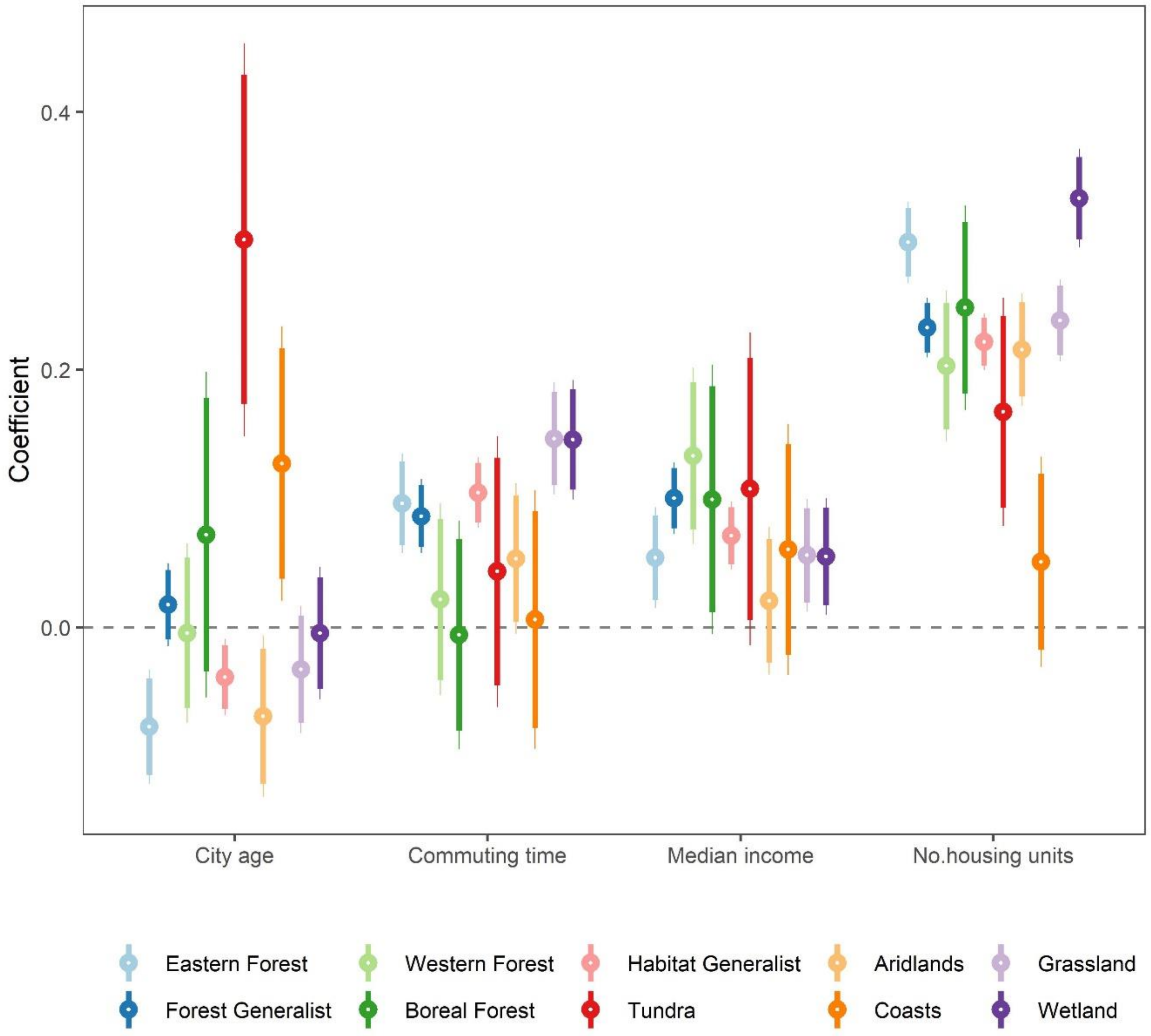

Figure 3.1 Generalized linear model coefficients for urban predictors of migrant species richness for different breeding biome groups in the United States. The circles represent coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. There is a relationship between variables when confidence intervals do not overlap zero. The commuting time and number of housing units are log-transformed.

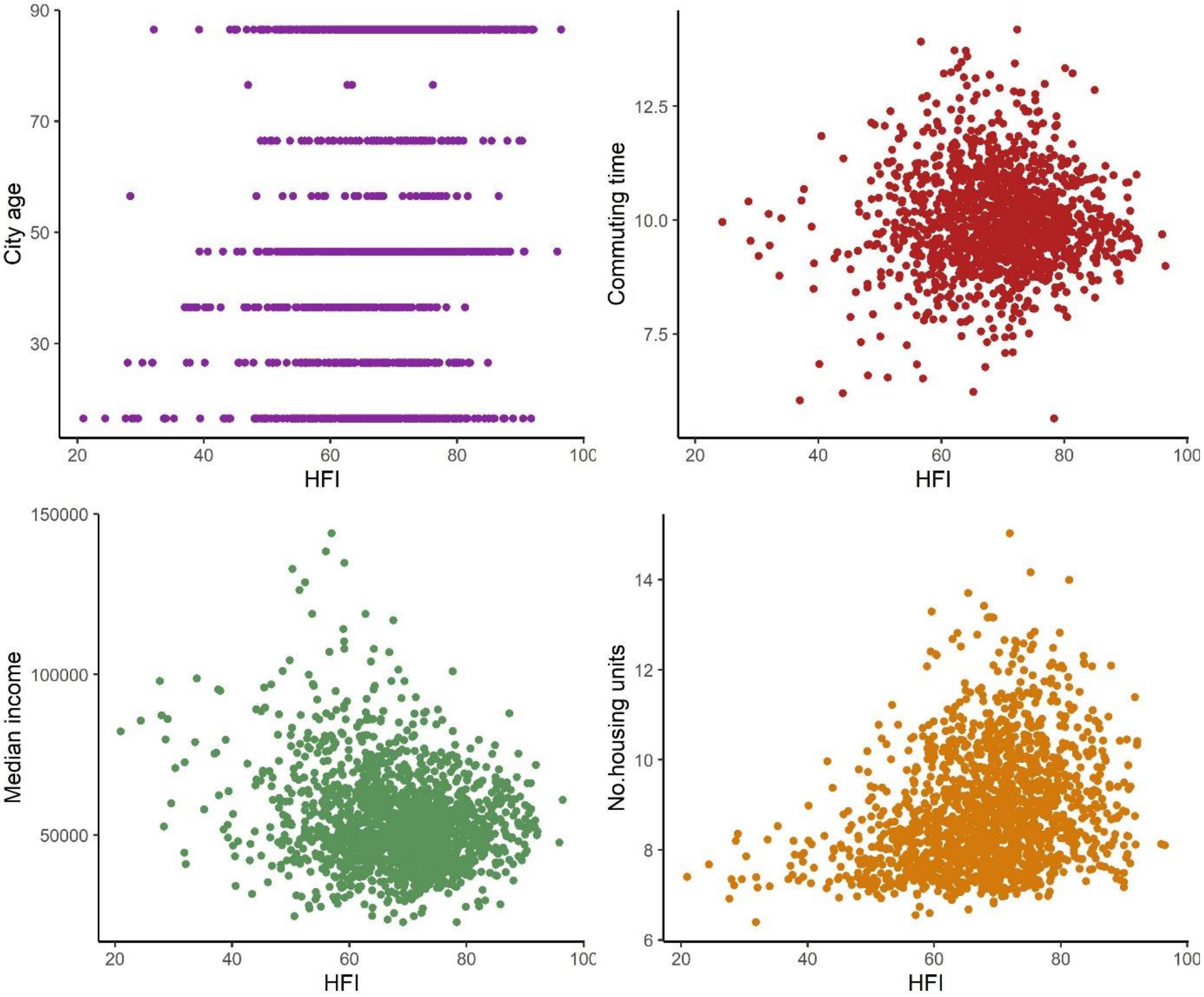

Figure 3.2 Scatterplots showing the correlation between the Human Footprint Index (HFI) and the city variables city age, commuting time (in minutes), median income, and the number of housing units. The Human Footprint Index is expressed as a percentage, where a value of zero represents an area least influenced by humans, and a value of 100 an area most influenced by humans. The commuting time and number of housing units are log-transformed.

Chapter 4: Squirrels alter their activity in cities in response to competition and artificial light

RP Kinnunen[1], CJ Garroway[1]

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

Abstract

Urbanization can permanently alter the natural environment and greatly influence wildlife inhabiting cities. Changing their behavior in response to new selection pressures in cities can make individuals more successful and may enable species persistence in urban habitats. Not much is known about the ways animals modify their behavior in cities, however. We wanted to investigate this by studying the differences in the activity patterns of Eastern grey squirrel (*Sciurus carolinensis*) and North American red squirrel (*Tamiasciurus hudsonicus*) populations between urban and rural areas. To do this we used data simultaneously acquired from Passive Integrated Transponder (PIT) tag data loggers from urban and rural sampling sites. Our final dataset had 3024 recordings, after limiting data to one record per individual per hour, of 36 individual grey squirrels and 26 individual red squirrels. We found differences in the overall and temporal activity patterns of squirrels, likely caused by the environmental differences between urban and rural areas and competition between species. We saw evidence of high overlap in activity between red and grey squirrels in both cities and rural areas, however, the peaks in activity for each species mainly occurred when the other species were less active, suggesting that grey squirrels likely adjusted the timing of their daily activities to avoid the more territorial and aggressive red squirrels. Consequently, temporal segregation may facilitate the coexistence of grey and red squirrels in our study region. Additionally, we found that the hourly light levels varied between our urban and rural sampling areas, and this variation may have partly accounted for the differences in activity in these habitats. Overall, our results show that behavioral adjustments likely help squirrel populations to persist in cities and that both abiotic and biotic mechanisms alter the temporal activity of animals in cities.

Keywords

activity patterns, behavioral flexibility, competition, Sciuridae, squirrel, urbanization

Introduction

Urbanization leads to rapid, often irreversible alterations to the natural habitat, and the wildlife inhabiting cities are subjected to new challenges never before encountered (McDonald et al. 2020). Changing their behavior in response to new selection pressures in cities can make individuals more successful and may enable species persistence in urban habitats (Lowry et al. 2013). The timing of daily activities—i.e., activity patterns—can be used to investigate the behavioral changes of wildlife, for example, in species foraging or predator avoidance strategies (Downes 2001; González-Solís et al. 2002), inter- or intraspecific competition or niche differentiation (Bu et al. 2016; Ikeda et al. 2016; Lewis et al. 2015; Niedballa et al. 2019), or the ways animals adjust their temporal activity in response to human-caused disturbance (Cruz et al. 2018; Presley et al. 2009). The multitude of uses makes activity estimation an attractive method for urban-rural comparisons, yet it is currently largely underutilized in urban research (but see Dominoni et al. 2013; Parker et al. 2014; Thomas et al. 2018).

Human actions shape the city environment in ways that can have a considerable effect on the wildlife inhabiting cities. Compared to more natural areas cities have increased levels of traffic, pollution, artificial light, and noise, as well as modified distribution of food and nesting resources (Grimm et al. 2008). Cities can also have altered biotic interactions, as urbanization can influence the circadian cycles of animals (Łopucki & Kiersztyn 2020), create or decouple predator-prey associations (Haskell et al. 2001; Rodewald et al. 2011), and influence inter-and intraspecific competition patterns within communities (Lewis et al. 2015). These can all influence the ecology and evolution of animals (Johnson & Munshi-South 2017; Shochat et al. 2006). The wildlife that ultimately colonizes and persists in cities needs to adjust to the new selection pressures in urban environments—the species unable to do so will become extirpated from the area (McDonald et al. 2020). Some species thrive in urban habitats and have successfully colonized cities all over the world (e.g., Norway rat, *Rattus norvegicus;* Eastern grey squirrel, *Sciurus carolinensis*). These species generally have traits that enable population persistence in urban environments. The first response of animals to human-caused disturbance is often behavioral, and behavioral flexibility under changing environmental conditions is an important phenotypic response for succeeding in urban environments (Lowry et al. 2013; Sol et al. 2013). Previous studies have identified several behavioral differences between urban and non-urban animals (reviewed in Ritzel & Gallo 2020), for example in exploratory behavior (Atwell et al. 2012; Miranda et al. 2013; Thompson et al. 2018), aggression (Grunst et al. 2018), and fear response (Van Donselaar et al. 2018). Being a faster, bolder, more aggressive explorer in a new environment might enable animals to quickly obtain information about predators or competing individuals and gain access to better food and nesting resources (Smith & Blumstein 2008; Sol et al. 2013). Additionally, alterations to diet or foraging patterns, the timing and duration of breeding, or inter-, and intraspecies interactions can also facilitate species persistence in cities, yet research investigating these types of behavioral changes in cities is still relatively rare (Bonier et al. 2007; Lowry et al. 2013; but see Partecke et al. 2006; Randa & Yunger 2006). Studies on birds currently represent the majority of these investigations, and further research on mammals is needed to get a more comprehensive picture of how wildlife has modified their behavior in response to the urban environment (Ritzel & Gallo 2020; Santini et al. 2019). We investigated this by studying the activity patterns of two squirrel species, the Eastern grey squirrel (*Sciurus carolinensis*) and the North American red squirrel (*Tamiasciurus hudsonicus*), between urban and rural areas. We were particularly interested in differences in daily activity patterns between urban and rural areas and differences in activity between grey and red squirrels. However, we also investigated whether light and temperature levels varied between our urban and rural study areas to help us with our conclusions.

Differences between urban and rural areas

We chose the Eastern grey squirrel and the North American red squirrel as our two study organisms, as both species are commonly found in urban and rural areas in our study location. The activity patterns of squirrels are likely to differ between urban and rural areas, as squirrels generally have higher population densities in cities compared to rural areas (Parker & Nilon 2008; Sarno et al. 2015). This could lead to increased inter- or intra-specific competition—and increased aggression between individuals—(Parker & Nilon 2008), or promote increased tolerance between squirrels in cities (Haigh et al. 2017; Łopucki et al. 2021). Squirrels also eat a less diverse selection of natural food items in cities, compared to natural areas (Robinson & Cowan 1954), however urban squirrels supplement their diet with human food waste and other human-provided food sources, such as birdseed (Reher et al. 2016; Thomas et al. 2018). Urban squirrels also have to adjust to living amongst humans and their pets, and previous studies found city squirrels to be either more (*Sciurus carolinensis*, Sarno et al. 2015) or less (*Sciurus niger*, McCleery 2009) vigilant in urban areas compared to non-urban areas, which can lead to a trade-off with time spent foraging. Differences between habitats can thus influence the behavior of squirrels and, consequently, we predicted that the foraging activity of squirrels would differ between urban and rural areas.

Differences between species

Grey and red squirrels might also differ in their temporal and social activities because of their differing foraging and social systems (Steele 1998). Grey squirrels are non-territorial scatter-hoarders that in their natural environment rely on characteristics associated with deciduous forests for food, nesting, and shelter from predation (McPherson & Nilon 1987; Riege 1991). North American red squirrels, in turn, are territorial larder-hoarders that generally inhabit boreal and northern temperate forests, primarily coniferous forest habitats (Holloway and Malcolm 2007). Despite these differences, the two species have high overlap in their diets. Both species are common in the city of Winnipeg, Canada, where their ranges overlap. As the timing of daily activity of both the red and grey squirrels is altered based on food availability and quality interspecific competition is likely to occur (Wauters et al. 2002). This might lead to red and grey squirrels living in the same habitat being active at different times of the day to avoid direct competition. As red squirrels are highly aggressive throughout the year in both breeding and non-breeding seasons (Boonstra et al. 2008), we hypothesized that grey squirrels would adjust the time of their daily activities to avoid red squirrels, leading to temporal segregation between red and grey squirrels in both habitats. We predicted we would see less overlap in activity between the two species in cities, as it is generally believed that the higher population densities in cities encourage more aggressive behavior between urban animals compared to animals in more natural areas (however see Haigh et al. 2017; Łopucki et al. 2021; Lowry et al. 2013; e.g., Parker & Nilon 2008; Tuomainen & Candolin 2011).

Differences in temperature and light

Cities tend to have elevated temperatures and higher levels of artificial light at night compared to neighboring more natural areas (Arnfield 2003; Kyba et al. 2017; Leal Filho et al. 2018). Artificial light at night can affect animal behavior and ecological interactions in many taxa by impacting foraging, reproduction, and communication (Longcore & Rich 2004). There is also some evidence that peak times in the daily activity of squirrels can be related to daily temperature (Pauls 1979). Temperature and light can thus affect animals in a multitude of ways, yet both are often neglected in urban studies (Hölker et al. 2010). Consequently, if temperature and light levels are altered in our urban sampling area these differences could alter the activity of squirrels between urban and rural areas. We predicted that as cities are generally warmer and have more artificial light than nearby rural areas, urban squirrels would start to forage earlier than rural squirrels and that we would see an increase in foraging activity closer to dawn and dusk. We predicted that rural squirrels would be more active throughout the day, as temperatures might be more constant in a forest environment. Alternatively, these patterns might be reversed if the higher density of squirrels in cities leads to more competition for resources, driving urban squirrels to forage throughout the day (Parker et al. 2014).

To test our predictions, we asked whether 1) activity patterns of squirrels differed between urban and rural areas, 2) the activity patterns between grey and red squirrels differed and if there were differences between cities and rural areas, and 3) if there were differences in light and temperature levels between our urban and rural study areas. To answer our questions, we used data acquired from Passive Integrated Transponder (PIT) tag data loggers placed in urban and rural sampling sites between May and October in 2019-2020 in Winnipeg, Manitoba, Canada.

Materials and Methods

Study sites and trapping

We conducted live trapping of red and grey squirrels in one urban and one rural site between May and September 2017-2020. The urban site is located in the city of Winnipeg, Manitoba, Canada, and consists of a ~10 ha park located on the University of Manitoba campus and a suburban neighborhood next to the park. The study site is bordered by the Red River and two major highways. Winnipeg is the largest city in the province of Manitoba with a population of 778,489 and a total land area of 464,33 km^2 (Statistics Canada 2016). Winnipeg lies 239 meters above sea level and has high seasonal climatic variation, with temperature varying from the extremes of around −24 °C to −33 °C between January to March to around +30 °C to +35 °C between June to September (Environment Canada 2020). The rural site is a ~34 ha forest patch next to an active honey-farm and bordered by agricultural land, near the cities Morden and Winkler in southern Manitoba (49°24'01.1"N, 98°00'29.2"W).

We used live traps (Tomahawk Live Trap Co., Tomahawk, WI, USA) to capture squirrels at study sites. Between 20-40 traps in total were set to the urban site and between 80-100 traps to the rural site each trapping day. The number of traps was different for the two sites to ensure approximately the same number of squirrels was captured at both sites. Traps were baited with peanut butter and checked regularly. Traps were placed in sheltered locations under vegetation cover or covered with canvas to provide shade and to calm the animals when inside the trap. After capture, squirrels were detained in a canvas capture bag and we recorded the weight (g), body and tail length (cm), skull width (cm), age (adult or juvenile), reproductive status, and sex for each individual. Each squirrel was pit tagged between the shoulder blades with passive integrated transponder (PIT) tags. All efforts were made to minimize handling time. Squirrels were then released at the place of capture. Our protocol (Protocol Number: f16-003) was approved by the University of

Manitoba animal care and use committee following Canadian Council on Animal Care guidelines.

Data collection

We used data from PIT tag data loggers to assess the activity patterns of squirrels between urban and rural sites. These data loggers are high-performance, ISO-compliant stationary RFID transceivers specifically designed for long-lasting detection, storage, and transmission of PIT tag IDs (Biomark Inc., Boise, Idaho, USA). They are designed for projects that need low power consumption, a large detection area, and high adaptability to environmental changes. We placed these PIT tag data loggers inside a wooden box that acted as a feeding station and placed the boxes in suitable locations in one urban and one rural study site. The feeder boxes were scattered across our sampling sites and settled within a forest patch to keep them from being directly disturbed by people. We placed six data loggers at the urban site and eight at the rural site (n =14) between 31st July and 31st October in 2019, and eight loggers at the urban and seven at the rural sites (n =15) between 5th May and 26th October in 2020. The data loggers transmit and receive PIT tag IDs when a squirrel with an injected PIT tag is in close proximity to the data logger box, with the PIT tag ID, date, and time to the nearest second recorded by the reader. The sampling effort (the sum of the number of hours each data logger was active during our sampling period) totalized 1766 logger hours. The data loggers are powered by two 24-volt DC (Direct Current) batteries, which were replaced weekly. A small amount of birdseed was placed on the feeder box when batteries were replaced to mimic a random food source for the squirrels. The added food attracted squirrels to the data logger but it was depleted quickly (~ in less than a day), after which the squirrels still visited the logger when foraging. The additional food should therefore not have a large overall impact on the activity times of squirrels regarding our analyses.

Environmental variables

One HOBO Pendant Temperature/Light 8K Data Logger (Onset Computer Corporation, Bourne, MA, USA) was placed on a tree branch ~1 meter from a PIT tag data logger. The loggers were programmed to record one light intensity (Lux) and one temperature (°C) reading once per hour at each study site. The measurement range for temperature is between -20 °C to +70 °C and between 0 to 320,000 lux for light intensity. The accuracy of the temperature measurement is ±0.53 °C from 0° to +50 °C. The light intensity measures relative light levels. We placed seven HOBO loggers at the rural study site and six loggers at the urban site in 2019, and six loggers each on both urban and rural sites in 2020.

Data analyses

All analyses were conducted using R version 3.6.3 (R Core Team 2018). The data loggers recorded the time and the date, and the pit tag ID of each individual squirrel. We compiled the data from the urban and rural data loggers for our study period for our two study species. We only kept recordings of the same individual—identified by its pit tag ID—from the same data logger recorded one hour apart to limit autocorrelation (Linkie & Ridout 2011). We used the R package *overlap* (Version 0.3.3; Ridout & Linkie 2009) to quantify the overlap between urban and rural squirrel activity or between grey and red squirrel activity for July, August, September, and October, independently. We first estimated each activity pattern separately as a probability density function, using kernel density estimates. This was done by considering our data as a random sample from the underlying distribution that describes the probability of a pit tag being read by a data logger within a specified time (Ridout & Linkie 2009). We then quantified the degree of overlap between the two densities estimated before, for example, grey squirrels between urban and rural areas in a particular month. This was done by calculating the coefficient of overlapping (Δ) (described in Ridout & Linkie 2009), which varies from 0 to 1—from no overlap to complete overlap. We used estimator Δ_1 for sample sizes <50, and estimator Δ_4 for sample sizes >50, as these are the most reliable estimators for small and large sample sizes, respectively (Ridout & Linkie 2009). We used the nonparametric Mardia-Watson-Wheeler statistical test (Batschelet 1981)—commonly used in activity analyses (Frey et al. 2017)—to determine if activity distributions between populations and species vary significantly, and the Mann-Whitney U test to compare differences in temperature and light levels between urban and rural areas.

Results

Our final dataset had 3024 PIT tag data-logger recordings at a rate of one recording per hour (see Table 4.1 for the numbers of recordings in each month) of 36 individual grey squirrels (25 urban, 11 rural) and 26 individual red squirrels (13 urban, 13 rural) that visited our feeding stations from July to October between 2019 and 2020. 1703 of those recordings in total were grey squirrels (811 urban, 892 rural), 1321 were red squirrels (450 urban, 871 rural). We had 193 data collection days in total.

Do the activity patterns of squirrels differ between urban and rural areas?

Overall, the activity of squirrels differed significantly between urban and rural areas during our study period, July to October 2019-2020 (grey squirrel, $W = 7.5$, $p = 0.02$; red squirrel, $W = 18.8$, $p < 0.0001$). The activity of grey squirrels peaked around 6.30 am and between 4 and 6 pm in the city, and between 8 and 10 am and around 7 pm in the rural area; the activity of red squirrels peaked between 9 am and 12 pm and around 5 pm in the city, and was constant between 8 am and 3 pm, and peaked around 7 pm in the rural area. The degree of overlap in daily activity (i.e., the coefficient of overlapping Δ) was 0.89 (Δ_4) for grey squirrels and 0.85 (Δ_4) for red squirrels (Fig 4.1). Monthly, between urban and rural areas (Fig 4.2) the activity of grey squirrels significantly differed in July ($W = 15.7$, $p = 0.0004$; the degree of overlap $\Delta_4 = 0.72$), with the activity peaking around 7 am, 3 pm and 7 pm in the city, and around 11 am and 7 pm in the rural area. The activity did not significantly differ in August ($W = 2.6$, $p = 0.27$, $\Delta_4 = 0.84$), September ($W = 1.8$, $p = 0.40$, $\Delta_4 = 0.86$), or October ($W = 4.9$, $p = 0.09$, $\Delta_4 = 0.89$). The activity of red squirrels was significantly different between urban and rural areas (Fig 4.2) in September ($W = 14.3$, $p = 0.0008$, $\Delta_4 = 0.78$), with the activity peaking around 9 am and 5 pm in the city, and around 3 pm and 6 pm in the rural area. The activity did not significantly differ in July ($W = 1.9$, $p = 0.39$, $\Delta_1 = 0.80$), August ($W = 4.9$, $p = 0.09$, $\Delta_1 = 0.85$), or October ($W = 1.5$, $p = 0.46$, $\Delta_4 = 0.93$).

Do the activity patterns between grey and red squirrels differ and are there differences between cities and rural areas?

The activity of grey and red squirrels was significantly different in cities ($W = 10.3$, $p = 0.006$) between July to October 2019-2020, with grey squirrels most active around 7.30

am, and between 3 to 6 pm, and red squirrels between 8.30 and 11 am, and 4 pm. The activity was not significantly different in rural areas ($W = 5.6$, $p = 0.06$). The degree of overlap in daily activity (i.e., the coefficient of overlapping Δ) was 0.90 (Δ_4) between grey and red squirrels in urban areas and 0.92 (Δ_4) in rural areas (Fig 4.3). Monthly, the activity between grey and red squirrels in cities differed significantly in July ($W = 8.0$, $p = 0.02$, $\Delta_4 = 0.79$) (Fig 4.4), with grey squirrels most active around 7 am, and 3 and 7 pm, and red squirrels between 8 and 9 am, with the activity subsequently declining. The difference in activity was not significant in August ($W = 1.5$, $p = 0.5$, $\Delta_1 = 0.85$), September ($W = 3.6$, $p = 0.2$, $\Delta_4 = 0.90$), or October ($W = 4.5$, $p = 0.1$, $\Delta_4 = 0.84$). The activity between grey and red squirrels in the rural area differed significantly in July ($W = 19.1$, $p < 0.0001$, $\Delta_1 = 0.63$), with grey squirrels most active around 11 am and 7 pm, and red squirrels around 8 am and 9 pm, in August ($W = 10.6$, $p = 0.005$, $\Delta_4 = 0.81$), with grey squirrels most active around 8.30 am and 7 pm, red squirrels pretty consistently active all day, and in September ($W = 12.2$, $p = 0.002$, $\Delta_4 = 0.84$), with grey squirrels most active around 10 am and 7 pm, and red squirrels between 3 to 6 pm. There were no significant differences in October ($W = 0.13$, $p = 0.9$, $\Delta_4 = 0.96$).

Are there differences in temperature and light levels between urban and rural areas?

There were significant differences in the mean hourly light intensity (Lux) ($W = 4531110.5$, $p = 0.008$) and the mean hourly temperature (°C) ($W = 4970931$, $p = 0.0002$) levels between urban and rural areas from May to October 2019-2020 (Fig 4.5). The average mean hourly light intensity was 9362.1 lux in the city and 7777.4 lux in the rural area. In general, the mean hourly light levels were higher in the city between 5 am and 8 pm, compared to the rural area. The average mean hourly temperature was 17.3 °C in the city and 18.2 °C in the rural area, and in general, the mean hourly temperature levels were higher in the rural area between 2 am and 2 pm, compared to the city. We saw no significant differences between the mean daily temperature (°C) ($W = 14956$, $p = 0.3$) and light intensity ($W = 13095$, $p = 0.3$) levels between urban and rural sampling areas (Fig 4.5).

Discussion

We investigated differences in the activity patterns of grey and red squirrel populations between urban and rural areas to gain a better understanding of how behavioral changes may facilitate the persistence of wildlife in cities. We found differences in the overall and temporal activity patterns of squirrels, likely caused by the environmental differences between urban and rural areas and competition between species. We saw evidence of high overlap in activity between red and grey squirrels in both cities and rural areas, however, the peaks in activity for both species mainly occurred when the other species were less active, indicating that grey squirrels might adjust the time of their daily activities to avoid the more territorial and aggressive red squirrels. Additionally, both urban grey and urban red squirrels started foraging earlier than their rural counterparts, perhaps due to altered light levels between urban and rural areas, but this varied during different months and between species.

Differences in activity between urban and rural areas

Research investigating differences in the activity patterns of wildlife generally uses either behavioral observations collected by an observer or uses radio-tracking or camera-trap data (Cruz et al. 2018; Łopucki & Kiersztyn 2020; e.g., Parker et al. 2014; Wassmer & Refinetti 2019). These data are often laborious to collect and obtaining simultaneous observations across different sampling sites may in some cases be unattainable. By utilizing PIT tag dataloggers, we were able to simultaneously collect large quantities of data on multiple individuals belonging to different species in several locations in our urban and rural sampling sites. Overall, we saw significant differences in the foraging activity of squirrels between urban and rural areas. Although there were generally high levels of overlap in activity there were also some monthly differences; sometimes activity was more continuous over the day, at other times it peaked at specific periods. Similar to a study conducted in six urban parks in Baltimore, Maryland (*Sciurus carolinensis*, Parker et al. 2014*)*, we saw some evidence that urban grey squirrels were generally more continuously active throughout the day than their rural conspecifics, however, the differences in activity were only significant in July. The activity peaks for urban grey and red squirrels primarily occurred at different times than for their rural conspecifics but were highly variable between months. Parker et al. (2014) attributed their results to increased intraspecific competition due to higher population density and reduced wariness and predation pressure in cities (Parker & Nilon 2008) which may also partly be contributing factors to our results. We did not calculate squirrel densities in this study, however, we had more individuals visiting our loggers in the city (n=38) than in the rural area (n=24). However, our between species results (see below) might indicate that cities may have more resources available for squirrels than rural areas. It is, therefore, possible that the differences seen are more the result of interspecific competition than lack of resources due to high population density in cities.

The differences between urban and rural activity patterns can also reflect differences in predation risk and predator avoidance. Predation risk is one of the largest costs associated with foraging in small mammals, inflated by even the smallest changes in the habitat (Brown & Kotler 2004). As cities are highly modified environments, the risk of predation is likely to differ between urban and rural areas. Previous studies have indeed found differences, mainly reduced predation rates in urban areas compared to more rural areas, particularly in birds (Eötvös et al. 2018). Fox squirrel (*Sciurus niger*) mortalities due to predation were also lower in cities; <5% of the fatalities in the urban area and >60% in the rural area were caused by predation in Texas, USA (Mccleery et al. 2008). Urban areas may thus offer a release from predation for many animals, and squirrels likely have predators in rural areas that are not often seen in cities. However, raptors, like hawks and owls, and species like coyotes (*Canis latrans*) and red foxes (*Vulpes vulpes*) can be found in cities (Baker et al. 2007; Boal & Dykstra 2018; Riley et al. 2003) and are known predators of squirrels. If predation risk would be the underlying cause for the different patterns of activity, however, we would expect to see more overlap in peak activity between the red and grey squirrels in cities and rural areas, as both species would likely avoid foraging when predators are most active. Our results, therefore, suggest that competition between red and grey squirrels is likely a stronger driver of squirrel activity patterns.

Differences in activity between grey and red squirrels

Supporting our predictions, we found that the highest levels of activity for red and grey squirrels living in the same habitat tended to be at different times of the day both in urban and rural habitats. This implies that grey and red squirrels living in the same habitat are choosing different times of the day to forage and be active—i.e., there is temporal segregation between species—highlighting the importance of interspecific competition for these species. As red squirrels are territorial and highly aggressive throughout the year (Boonstra et al. 2008) grey squirrels likely adjust the timing of their daily activities to avoid them. However, there were also high levels of overlap in activity between the two species. The differences in activity between species were significant mainly in the rural area, and there was more overlap in activity between grey and red squirrels in the city compared to the rural area. This is consistent with there being less competition for resources in cities that may result in increased tolerance between the two species (Haigh et al. 2017). Humans can provide a multitude of food and nesting resources (e.g., human food waste, direct feeding, bird feeding stations, cultivated plants, bird nest-boxes) to wildlife in cities that can be utilized by squirrels and not commonly found in rural areas (Kostrzewa & Krauze-Gryz 2020). Previous studies have shown that human food subsidies can reduce competition within and between populations as well as the tendency of individuals to dominate resources (Łopucki et al. 2021; Oro et al. 2013). The overlap in activity between the two species was highest during the late summer and fall months when squirrels start preparing for winter by scatter-hoarding food or establishing middens (i.e., overwinter food storages) and when juveniles disperse to try and find territories of their own. As adequate food storage and the possession of territory and a suitable tree den are likely to ensure survival over winter (Kemp & Keith 1970; Larsen & Boutin 1994), high levels of activity during this time are likely to be expected.

Differences in temperature and light levels between urban and rural areas

Overall, the activity patterns of squirrels followed hourly light levels, particularly in the morning. Our results showed that the light levels generally started to increase just after 5 am in the city, and approximately an hour later in the rural area, and reflecting this pattern, urban squirrels generally started to forage earlier than squirrels in the rural area. This is particularly interesting, as it implies that artificial light can alter the temporal activity patterns of animals in cities. Similar patterns have been seen in urban birds, for example, whereby species advance their activity in cities to early morning and night hours compared to more natural areas (Dominoni et al. 2013; Spoelstra et al. 2018). Contrary to our predictions, our results showed that the mean hourly temperatures were higher in the rural area, compared to the city. These differences are likely attributable to latitudinal differences between the sampling sites, as the rural sampling location was further south compared to the urban location. In fact, the mean temperature during 2019-2020 was ~ 0.98-1.23 °C higher in Morden (rural) than in Winnipeg (urban) (Government of Canada, available from https://climate.weather.gc.ca/). The mean daily temperature and light differences between the sites were negligible and did not seem to influence the foraging activity of squirrels.

Conclusions

Urbanization can permanently alter the natural environment and influence the wildlife inhabiting cities. Behavioral flexibility may enable population persistence in cities and manifest in differences in temporal and spatial activity patterns of species. We found evidence of such behavioral flexibility in squirrels in cities, possibly the result of the biotic and abiotic differences between urban and rural areas. Although red and grey squirrels had a high overlap in the timing of their activities, the peaks in activity for each species mainly occurred when the other species were less active, suggesting that grey squirrels likely adjusted the time of their daily activities to avoid the more territorial and aggressive red squirrels. Consequently, temporal segregation between species may help facilitate the coexistence of Eastern grey squirrels and North American red squirrels in our study region. We also found that variation in hourly light levels between urban and rural areas may have partly accounted for the different activity patterns of squirrels in these habitats. Overall, our findings show that squirrels alter their activities in urban areas, likely as a result of the environmental differences between urban and rural areas and competition between species. This implies that behavioral adjustments likely facilitate the persistence of squirrel populations in cities.

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Table 4.1 Numbers of PIT tag data-logger recordings for Eastern grey squirrel (*Sciurus carolinensis*) and North American red squirrel (*Tamiasciurus hudsonicus*) in urban and rural sampling areas during our study period, July to October 2019-2020, and in each month.

	Grey squirrel		**Red squirrel**	
Month	Urban	Rural	Urban	Rural
July-October 2019-2020	811	834	395	868
July	122	107	60	33
August	165	162	48	331
September	203	175	113	229
October	123	258	78	214

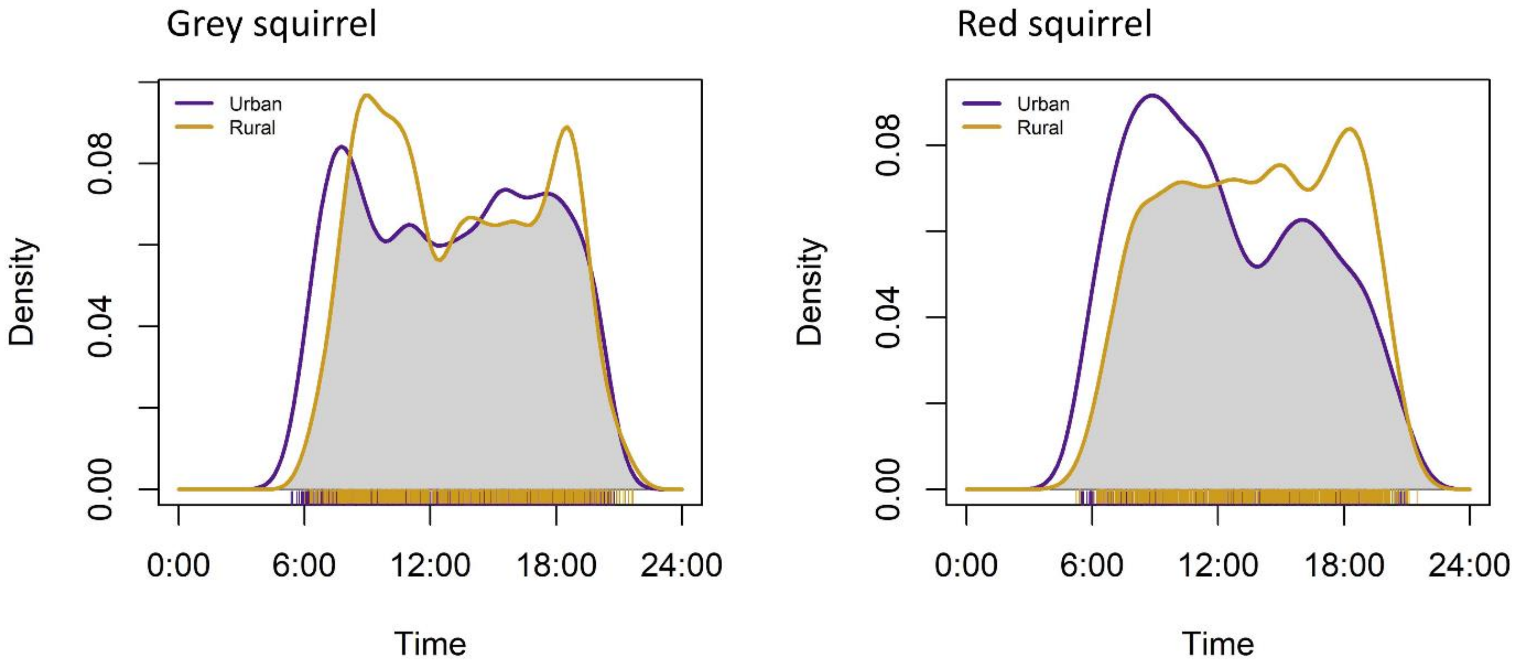
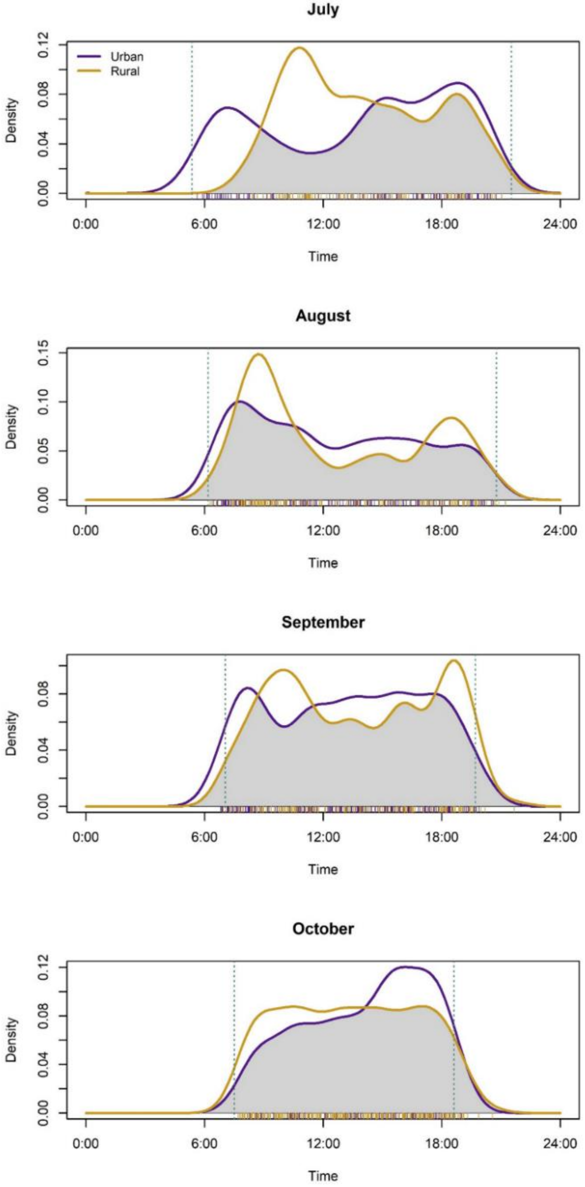


Figure 4.1 Temporal activity and the degree of overlap (grey shaded area) between urban and rural areas for grey (*Sciurus carolinensis*) (left) and red squirrels (*Tamiasciurus hudsonicus*) (right). The coefficient of overlapping (Δ) among urban and rural areas was 0.89 for grey squirrels and 0.85 for red squirrels. The short vertical lines at the bottom of the plots indicate the times of day at which the species pit tag was read at a datalogger.

Grey squirrel



Red squirrel

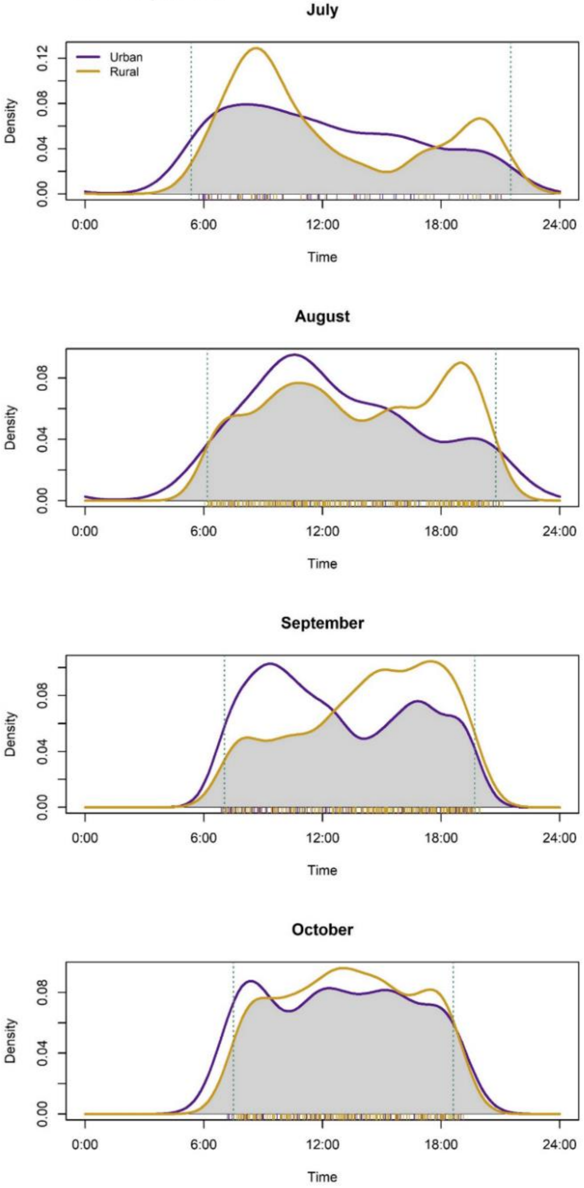


Figure 4.2 Temporal activity and the degree of overlap (grey shaded area) between urban and rural areas for grey (*Sciurus carolinensis*) (left) and red squirrels (*Tamiasciurus hudsonicus*) (right) in July, August, September, and October. In July, the coefficient of overlapping (Δ) among urban and rural areas was 0. 72 for grey squirrels, 0. 80 for red squirrels; in August Δ = 0.84 for grey, Δ = 0.85 for red squirrels; in September Δ = 0.86 for grey, Δ = 0.78 for red squirrels; and in October Δ = 0.89 for grey, Δ = 0.93 for red squirrels. The short vertical lines at the bottom of the plots indicate the times of day at which the species pit tag was read at a datalogger. The purple line represents the urban area, the yellow line the rural area. The vertical dotted lines mark the sunrise and sunset on the 15th of each respective month in 2019.

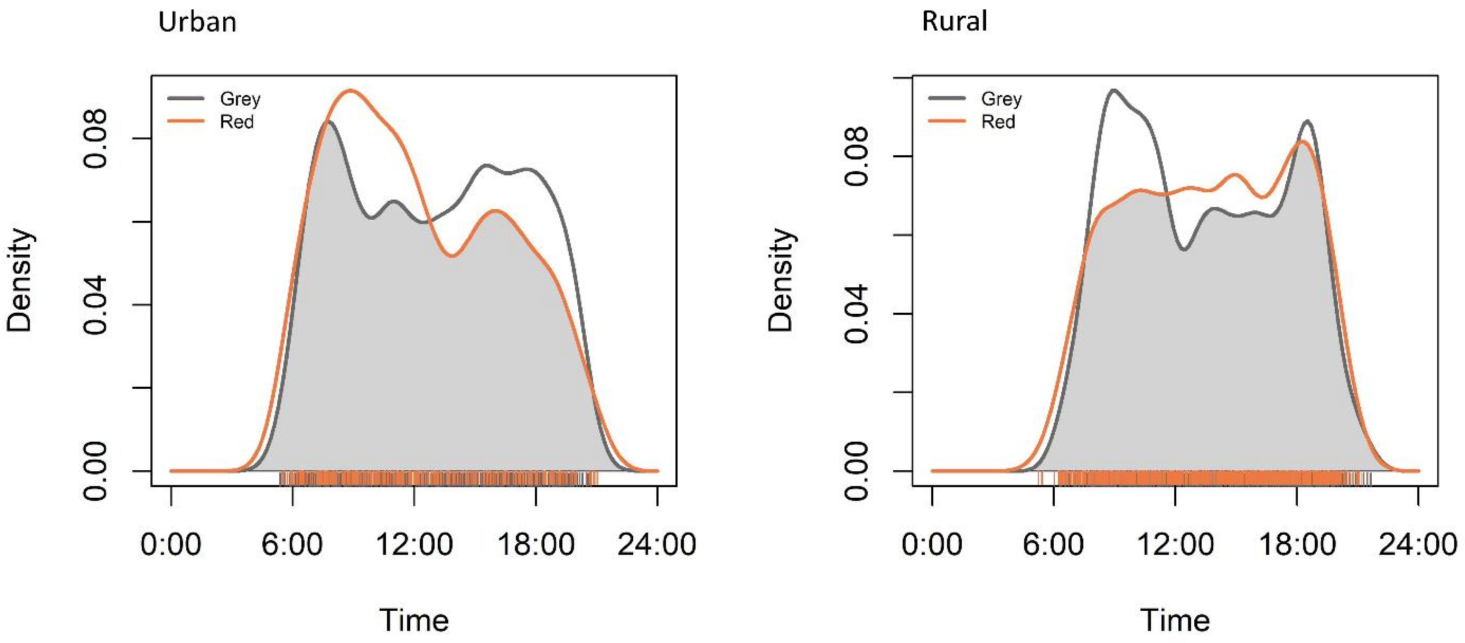


Figure 4.3 Temporal activity and the degree of overlap (grey shaded area) between grey (*Sciurus carolinensis*) and red squirrels (*Tamiasciurus hudsonicus*) in urban (left) and rural (right) areas. The coefficient of overlapping (Δ) was 0.90 between grey and red squirrels in urban areas and 0.92 in rural areas. The short vertical lines at the bottom of the plots indicate the times of day at which the species pit tag was read at a datalogger.

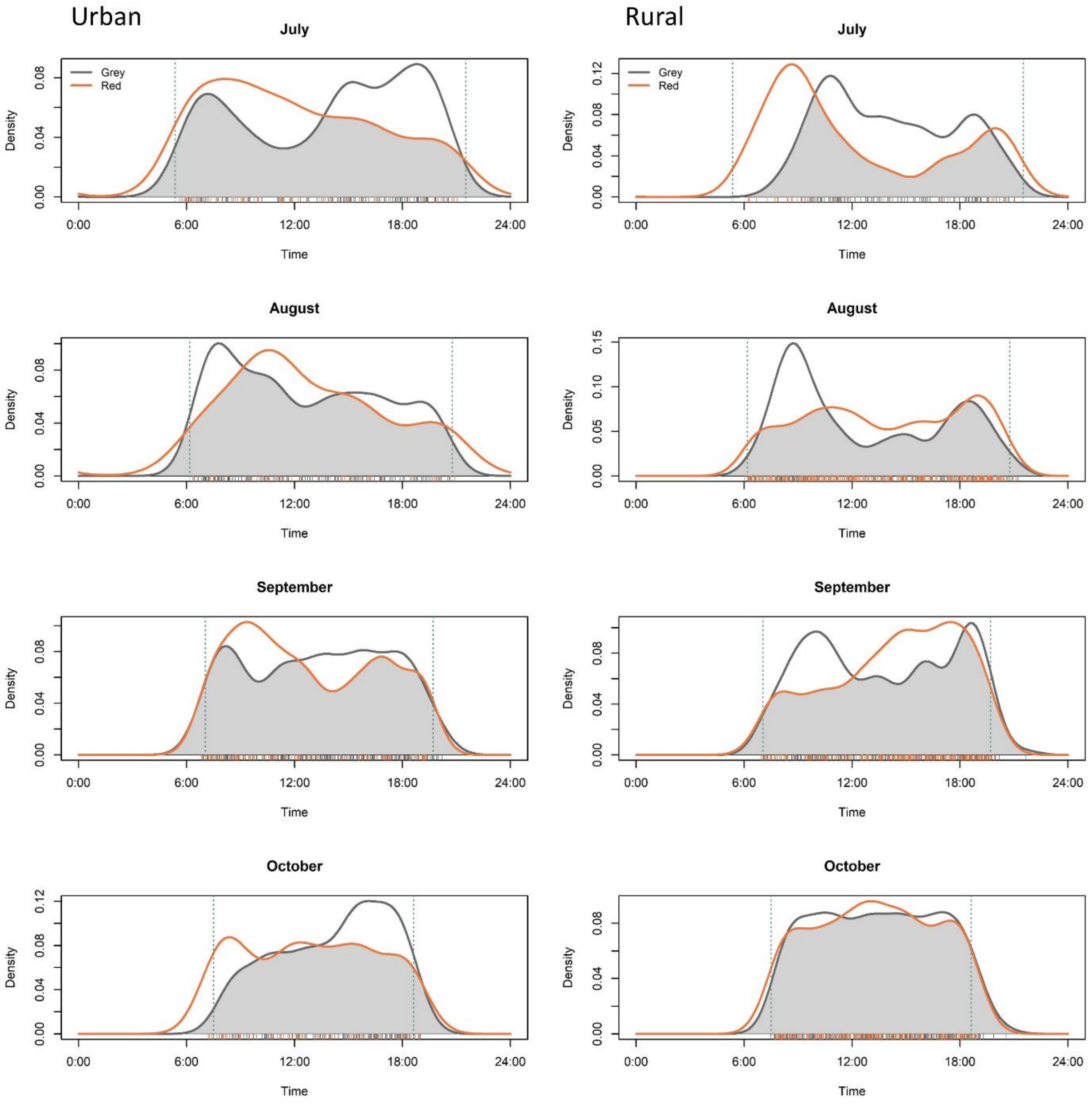

Urban
Rural
July
August
September
October
Grey
Red
Density
Time
0:00
6:00
12:00
18:00
24:00
0.00
0.04
0.08
0.12
0.05
0.10
0.15

Figure 4.4 Temporal activity and the degree of overlap (grey shaded area) between grey (*Sciurus carolinensis*) and red squirrels (*Tamiasciurus hudsonicus*) in urban (left) and rural (right) areas in July, August, September, and October. In July, the coefficient of overlapping (Δ) among grey and red squirrels was 0.79 in urban and 0.63 in rural areas; in August Δ = 0.85 in urban, Δ = 0.81 in rural areas; in September Δ = 0.90 in urban areas and Δ = 0.84 in rural areas; and in Δ = October 0.84 in urban and Δ = 0.96 in rural areas. The short vertical lines at the bottom of the plots indicate the times of day at which the species pit tag was read at a datalogger. The grey line represents grey squirrels, the orange line red squirrels. The vertical dotted lines mark the sunrise and sunset on the 15th of each respective month in 2019.

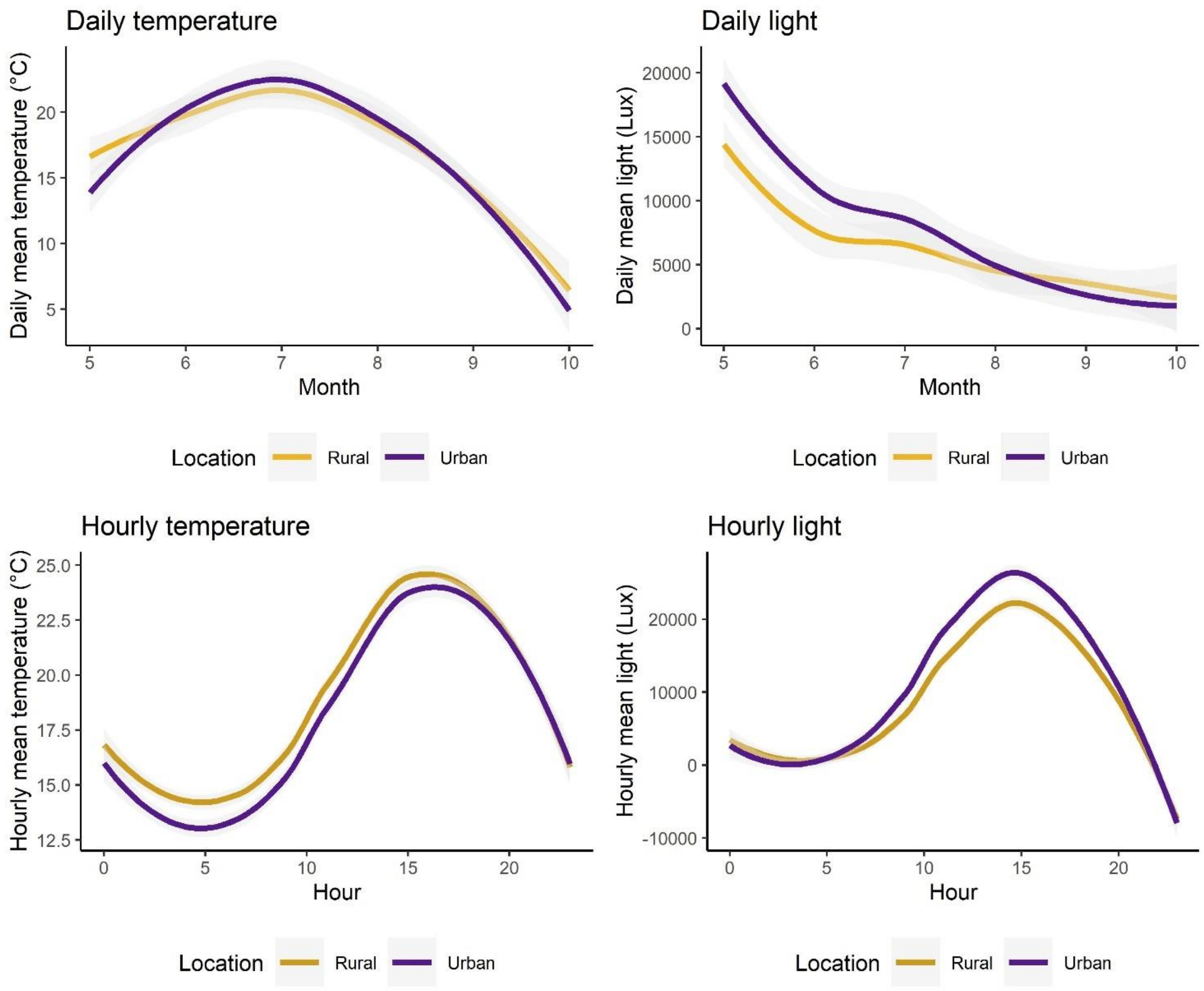


Figure 4.5 Differences between mean daily temperature (°C) and light intensity (Lux) levels (above) and mean hourly temperature and light levels (below) with 95 % confidence intervals (in light grey) between rural and urban sampling areas during May to October 2019-2020.

Chapter 5: No evidence of *Toxoplasma gondii* infection in urban and rural squirrels

RP Kinnunen[1], C Schmidt[1], A Hernández-Ortiz[2], CJ Garroway[1]

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

[2] Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, S7N 5B4 Canada

Abstract

Wildlife in cities can act as reservoirs for parasites that can infect domestic pets and have implications for human health. For example, the Coccidian parasite *Toxoplasma gondii* can infect any mammalian species, with cats and other Felidae acting as the only definitive hosts. Rodents, such as squirrels, could play an important role in urban infection dynamics of *T. gondii*, as squirrels occur at higher densities in cities than in natural environments and regularly share their territories with cats. In urban and suburban areas squirrels can encounter infectious oocysts shed in cat feces in contaminated soil or in the food they eat. They are also preyed upon by cats making them a potentially important species for completing the *T. gondii* life cycle in cities. We hypothesized that due to increased exposure to cats, urban squirrels would be more susceptible to *T. gondii* infection relative to squirrels in more natural areas. We investigated this using molecular and serological methods on samples collected from American red squirrels (*Tamiasciurus hudsonicus*), Eastern grey squirrels (*Sciurus carolinensis*), Northern flying squirrels (*Glaucomys sabrinus*), and Least chipmunks (*Tamias minimus*) in and around the city of Winnipeg, Manitoba, Canada. We tested a total of 230 tissue samples from 46 squirrels for *T. gondii* DNA using quantitative PCR and supplemented these data with analyses of 13 serum samples from grey squirrels (*Sciurus carolinensis*) testing for *T. gondii* antibodies by indirect ELISA. We found no evidence of *T. gondii* infection in any squirrel. This suggests that squirrels are not

important intermediate hosts of *T. gondii* in cities and do not need to be considered as sources of infection to cats.

Introduction

Wildlife in cities can act as reservoirs for parasites that can infect domestic pets and have implications for human health (Mackenstedt et al. 2015). Indeed, previous work has found increased levels of wildlife parasitism in cities compared to rural areas (Deplazes et al. 2004; Giraudeau et al. 2014; Lehrer et al. 2010; Reperant et al. 2009). This increased level of parasitism could reflect both the increased population density of urban host species and higher within and between species contact rates in response to resource provisioning in cities relative to rural areas (Bradley & Altizer 2007; Gliwicz et al. 1994). Contrasting this pattern, in some cases the overall species richness and diversity of parasites can be reduced in cities as urbanization can lead to a decline in host species richness. With reduced access to hosts, parasites with one or a few host species may then also become extirpated (Bradley & Altizer 2007). Parasites that spread through direct contact or oral-fecal routes are likely to be favored in urban areas, (Bradley & Altizer 2007) but general knowledge underlying the ways host-parasite interactions operate in cities is still limited (Mackenstedt et al. 2015).

The Coccidian parasite *Toxoplasma gondii* is an interesting parasite within the context of urban ecosystems. Domestic cats—numerous in cities as pets (Baker et al. 2008; Sims et al. 2008)—and other Felidae are the only known definitive hosts of *T. gondii* (Elmore et al. 2010). When infected, cats can shed millions of infectious *T. gondii* oocysts into the environment daily in their feces for a duration of one to two weeks, making them an important part of the parasite's life cycle (Dubey 2001; Fayyad et al. 2016). Humans can acquire infection from cats by accidentally ingesting oocysts, for example when cleaning cat litter or not washing hands after gardening (Centers for Disease Control and Prevention 2019). Other mammals can act as intermediate hosts for *T. gondii*, acting as reservoirs for the parasite and, in some cases, as possible sources of infection for cats in cities. Cats and intermediate hosts can become infected by consuming carcasses infected by *T. gondii* tissue cysts, or by coming into contact with oocyst contaminated soil, water, or food. A significant proportion of the human population globally is infected with *T. gondii,* but most healthy people do not experience symptoms of infection (Tenter et al.

2000). However, immunocompromised people and pregnant women require medical intervention to avoid serious health issues (Dixon 1992; Hill et al. 2005; Tenter et al. 2000). Consequently, further knowledge of *T. gondii* infection dynamics in cities is needed.

Urbanization can increase the risk of an animal being exposed to *T. gondii* (Ballash et al. 2015; Conrad et al. 2005; Lehrer et al. 2010). Many squirrel species (Sciuridae) are ubiquitous in cities, where they are commonly found at much higher densities than in natural environments (Parker & Nilon 2008). In urban and suburban areas squirrels regularly share their territories with domestic cats and collect and cache their food in backyards and gardens where they can encounter infectious oocysts shed in cat feces in contaminated soil or in the food they eat. This may make urban squirrels particularly susceptible to parasite infection compared to their rural counterparts. After being infected squirrels act as intermediate hosts for the parasite, and the parasite can remain within the host body in tissue cysts for the rest of the host's life. The infection can be asymptomatic or develop into the disease toxoplasmosis (Dubey et al. 2006; Jokelainen & Nylund 2012). Many *T. gondii* strains isolated from nature are of low virulence, leading to subclinical toxoplasmosis that does not kill the animal, but can make prey—such as a squirrel—susceptible to predation by cats (Dubey & Frenkel 1973), thus enabling the parasite to complete its life cycle (Dubey et al. 2006; Dubey & Frenkel 1973). Squirrels may thus act as a source of infection to cats, in a similar way to other prey species (Afonso et al. 2007). As such squirrels may play a role in *T. gondii* population and infection dynamics in cities.

Our aim in this paper was to survey the prevalence of *T. gondii* infection in squirrel (Sciuridae) populations in and around the city of Winnipeg, Manitoba, Canada. We specifically asked whether squirrels are important intermediate hosts of *T. gondii* and whether *T. gondii* infection is more common in a city than in more natural habitats. We hypothesized that, due to high population densities of both squirrels and cats in cities (Parker & Nilon 2008; Sims et al. 2008), urban squirrels may act as intermediate hosts of *T. gondii* and that urban squirrels will have a higher prevalence of *T. gondii* infection than rural squirrels. We tested these hypotheses by using molecular and serological methods on samples collected from four squirrel species in and around the city of Winnipeg, Manitoba, Canada.

Materials and Methods

To investigate *T. gondii* prevalence in squirrels whether in an acute or chronic stage we followed recommendations from previous studies and used molecular and serological methods (Galeh et al. 2020). Quantitative PCR was done on a wide-ranging sample from four squirrel species (American red squirrel, *Tamiasciurus hudsonicus*; Eastern grey squirrel, *Sciurus carolinensis*; Northern flying squirrel, *Glaucomys sabrinus*; and Least chipmunk, *Tamias minimus*) in and around the city and serological testing on focal study populations of grey squirrels (*Sciurus carolinensis*).

Study Sites

We conducted live trapping of grey squirrels in one urban and one rural site between 5th June and 1st August 2019 (Fig 5.1). The urban site is located in the city of Winnipeg, Manitoba, Canada, and consists of an ~10 ha park located on the University of Manitoba campus and a suburban neighborhood next to the park. The study site is bordered by the Red River and two major highways with high amounts of car traffic. Winnipeg is the largest city in the province of Manitoba with a population of 778,489 and a land area of 464,33 km^2 (Statistics Canada 2016). Winnipeg lies 239 meters above sea level and has high seasonal climatic variation, with temperature varying from the extremes ranging between −24 °C to −33 °C between January to March to around +30 °C to +35 °C between June to September (Environment Canada 2020b). The rural site is a ~34 ha forest patch next to an active honey-farm, near the twin cities Morden and Winkler in southern Manitoba (49°24'01.1"N, 98°00'29.2"W), bordered by agricultural land.

Collection of Samples

We collected the carcasses of 25 red squirrels, 16 grey squirrels, four flying squirrels, and one chipmunk from trappers, wildlife rehabilitation centers, and pest control companies during the years of 2017 to 2019 to collect tissue samples for PCR. We did not obtain the carcasses close enough to death to collect usable serological samples.
We also used live traps (Tomahawk Live Trap Co., Tomahawk, WI, USA) to capture grey squirrels at the study sites. Between 20-40 traps in total were set at the urban site and between 80-100 traps were set at the rural site each trapping day. Traps were baited with peanut butter and checked regularly. The number of traps was different for the two

sites to ensure approximately the same number of squirrels was captured at both sites. Traps were placed in sheltered locations under vegetation cover or covered with canvas to provide shade and calm the animals inside the trap. After capture, squirrels were handled in a canvas capture bag and we recorded the weight (g), body and tail length (cm), skull width (cm), age (adult or juvenile), reproductive status, and sex for each individual. We collected a minimum of 500 µL of blood from the femoral vein of each grey squirrel and stored the sample on ice until processing. Each squirrel was pit-tagged between the shoulder blades with passive integrated transponder (PIT) tags. All efforts were made to minimize suffering. We then released the squirrels at the place of capture. Our protocol (Protocol Number: f16-003) was approved by the University of Manitoba animal care and use committee following Canadian Council on Animal Care guidelines.

Molecular Methods

Upon necropsy, we collected the entire liver, spleen, brain, heart, kidneys, and lungs, and stored tissues separately at −20 °C until further analysis. As *T. gondii* is a cyst-forming parasite, the detection probability of *T. gondii* can differ between organs (Elmore et al. 2016). Consequently, we tested multiple samples per individual from two to six different organs to maximize the probability of detecting the parasite.

Cell lysis

Cell lysis was done by first adding 3 ball bearings to each 2 mL screw-cap tube containing 0.6 mL of ATL buffer and adding 100 mg of frozen tissue or pipetting 0.1 mL of sample (if liquid such as a thawed brain) to the tube. We placed the samples in a BeadBeater for 3 minutes after which they were quickly centrifuged. We added 70 µL of Proteinase K to the ATL lysate. We incubated the lysate at +56 °C for 1-3 hours during which the tubes were intermittently inverted several times. We centrifuged the lysate quickly and added 0.6 mL of AL buffer. We inverted the tubes several times and incubated them at +70 °C for 10-30 minutes in a dry block. We then again inverted the tubes intermittently several times. The samples were then centrifuged for 3 minutes at 10,000 x *g*. After the completion of the cell lysis, we used 100 µL of the ATL/ProtK/AL lysate to continue the extraction.

Nucleic acid extraction and real-time PCR

We did nucleic acid extraction for *T. gondii* using 5X MagMAX viral Isolation kit (Applied Biosystems AMB1836-5). Primers and probes for real-time PCR were designed according to the protocol of De Craeye et al. (2011) (see Table S5.1 for primers and probes). PCR product size was 106 bp. We used cellular r18S (ribosomal RNA gene) as an internal control of all PCRs. Real-time PCR was conducted using TaqMan Fast Advanced Master Mix (Applied Biosystem) in Applied Biosystems™ 7500 Real-Time PCR System. Each PCR reaction had concentrations of 10 µM of T2 /F primer, 10 µM of T3/R primer, and 5 µM of probe in the master mix. Thermo cycling Program: Initial denaturation and activation of the Taq polymerase at +95 °C for 2 minutes, followed by 45 cycles at +95 °C for 5 seconds and +60 °C for 33 seconds. We analyzed the results using 7500 System SDS Software.

Serological Methods

We collected a minimum of 500 µL of blood from 15 individual grey squirrels and stored samples on ice. All samples were processed within 12 hours. We centrifuged the collected blood samples at 3500 rpm for 15 minutes and froze the serum at −20 °C until used for testing.

Enzyme-linked immunosorbent assay

We used enzyme-linked immunosorbent assays (ELISA) to detect serum antibodies (IgG) against *T. gondii.* As species-specific conjugates are not available for squirrels, we used a commercially available ELISA kit for testing the samples (Multi-species ID Screen Toxoplasmosis Indirect kit, IDVet, Grabels, France) following the manufacturer's instructions. We read the optical density values at 450 nm in a spectrophotometer and calculated results using these values and kit controls expressed as S/P (Sample to Positive Ratio) percentage (S/P%). We considered samples with S/P% less or equal to 40% negative; samples with S/P% between 40 and 50% doubtful or inconclusive; and samples with an S/P% higher than 50% positive, following the kit's protocol.

Results

We tested a total of 230 tissue samples from 46 squirrels from four squirrel species for *T. gondii* DNA using quantitative PCR (Table 5.1). Twenty-six of the carcasses were from urban locations within Winnipeg and 20 were from rural locations between 30 and 250 km from Winnipeg (Fig 5.1). We had 25 American red squirrels (*Tamiasciurus hudsonicus*); 16 Eastern grey squirrels (*Sciurus carolinensis*); four Northern flying squirrels (*Glaucomys sabrinus*); and one Least chipmunk (*Tamias minimus*). *T. gondii* DNA was not detected in any of the 230 tissue samples (liver; heart; brain; lung; spleen; kidney).

We also tested a total of 13 (out of 15) samples of blood sera from grey squirrels for *T. gondii* antibodies (IgG) by indirect ELISA. Two of the collected samples did not have enough volume for testing. These results were negative—no *T. gondii* antibodies were detected in any sample (see Table S5.2).

Discussion

We hypothesized that, due to higher population densities of both squirrels and cats in cities (Parker & Nilon 2008; Sims et al. 2008), urban squirrels may act as important intermediate hosts of *T. gondii* and have a higher prevalence of *T. gondii* infection than rural squirrels. However, we found no evidence of *T. gondii* infection in squirrels.

T. gondii has been found in many wild animals including on farms and natural areas e.g., Gray fox (*Urocyon cinereoargenteus*) (Lindsay et al. 2001; Smith & Frenkel 1995; Tizard et al. 1978), Red fox (*Vulpes vulpes*) (Wanha et al. 2005). However, studies investigating *T. gondii* infection dynamics in cities are still relatively rare (but see e.g., Ballash et al. 2015; Conrad et al. 2005; Dubey et al. 2014; Frenkel et al. 1995; Lehrer et al. 2010; Mercier et al. 2013; Murphy et al. 2008) considering the parasite can infect most mammalian species, including domestic cats and humans. Rodents can act as intermediate hosts for the parasite and as sources of infection to cats if consumed when infected by *T. gondii*. Squirrels are among the many species capable of getting a *T. gondii* infection, but our knowledge is limited, and studies have mostly focused on acute, fatal cases with little information existing on host-parasite dynamics during chronic, latent infection (Jokelainen & Nylund 2012).

The prevalence of *T. gondii* in Sciurids in urban areas is not well known. An earlier study from Guelph, Ontario, Canada found no evidence of infection across nine locations within and around the city from Eastern grey squirrels (n= 16, the number of urban captures not specified) and chipmunks (*Tamias striatus*, n= 6) using the Sabin-Feldman dye test (Tizard et al. 1978). In natural areas, toxoplasmosis has been found in the Eastern grey squirrel (Dubey et al. 2006; Jacobs et al. 1962; Smith & Frenkel 1995; Walton & Walls 1964), Western grey squirrel (*Sciurus griseus*) (Soave & Lennette 1959), and Eurasian red squirrel (*Sciurus vulgaris*) (Fayyad et al. 2016; Jokelainen & Nylund 2012). However, *T. gondii* prevalence in squirrels has generally been low (indirect hemagglutination test 1 of 265 positive individuals (Burridge et al. 1979); PCR 3 of 19 (Jokelainen & Nylund 2012); Sabin-Feldman dye test 2 of 11 (Smith & Frenkel 1995)).

Using serological techniques with bioassay and PCR together can give a more reliable estimation of infection rate (Galeh et al. 2020), as *T. gondii* is a cyst-forming parasite, and the distribution of *T. gondii* tissue cysts can be uneven and vary between organs (Elmore et al. 2016). This can lead to detection difficulties using PCR-based methods (Opsteegh et al. 2010). The sensitivity and specificity of antibody detection methods can also vary, which can lead to misinterpretation of results and possible false negatives and positives (Gilbert et al. 2013). The multi-species ELISA kit we used has been successfully used to detect *T. gondii* antibodies in wildlife (Roqueplo et al. 2011; Sharma et al. 2019), has high sensitivity and specificity compared to other serological tests such as the modified agglutination test (Sharma et al. 2019), and does not cross-react with other coccidian parasites—a factor known to limit the specificity of serological assays (Hirota et al. 2010). As we sampled several different organs and tissues per individual by PCR and used serological methods as an additional test to survey *T. gondii* prevalence in squirrels our results are less likely to be false negatives.

T. gondii prevalence in Manitoba, in general, is not well known and no previous survey of *T. gondii* prevalence in squirrels exists from the province. Serological testing in 1981 reported that of 55,527 pregnant women 129 showed signs of a recent *T. gondii* infection (Sekla et al. 1981). The same study also reported that 19 of 72 cats and one polar bear tested positive for *T. gondii* but results from 28 other species were all negative. An earlier study from Manitoba in 1976 found that *T. gondii* prevalence in pregnant women in urban areas was 8.17 %, and 6.29 % in rural areas (Shettigara et al. 1976). Moreover, the Canadian prairies generally seem to harbor a low prevalence of *T. gondii* in domestic sheep, pigs, and cattle (Nation and Allen 1976; Smith 1991).

The extreme climatic variations in the region may decrease the viability or infectivity of oocysts and tissue cysts in carcasses, therefore decreasing overall *T. gondii* prevalence in the area (Nation & Allen 1976). The temperature in Winnipeg between June to September can reach extremes of +30 °C to +35 °C (Environment Canada 2020b) with daily average temperatures varying from approximately +20 °C to +13 °C (Environment Canada 2020a). *T. gondii* oocysts are highly resistant to environmental variation but sporulation (i.e. infectivity) is dependent on fixed temperatures and factors such as soil moisture that can influence the time oocysts can survive at high temperatures (Dubey et al. 1970). Additionally, winters in Winnipeg can be cold and windy with extreme temperatures ranging between −24 °C and −33 °C between January to March. Oocysts cannot sporulate and become infective after exposure to −21 °C for 1 day or −6 °C for 7 days (Frenkel et al. 1975). After sporulation, oocysts can withstand lower temperatures better, being able to survive at −21 °C for 28 days (Frenkel et al. 1975), yet oocysts can not sporulate if the conditions are unfavorable (Dubey et al. 1970). It is thus possible that the combination of hot summers and cold winters reduces the viability of oocysts in Manitoba. Contact rates between domestic cats and *T. gondii* intermediate hosts may also be lower during the winter if people keep their pets indoors in cold weather. Winnipeg has an average yearly precipitation of 521 mm, but the years 2018 and 2019 were unusually dry in southern Manitoba (Government of Manitoba 2020). This could have influenced oocyst survival in the area during data collection, as oocysts survive better in moist than in dry conditions (Frenkel et al. 1975; Lélu et al. 2012).

We note that *T. gondii* antibodies have been found in skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*) from Saskatchewan (modified agglutination test: average seroprevalence in skunks 15.6%; in raccoons 20.8% in 1999, 12.5% in 2000) and Manitoba (in skunks 28%; in raccoons 27.5%) (Hwang et al. 2007), which implies that although climatic variation may lower the prevalence of *T. gondii* in the prairie provinces of Canada, *T. gondii* is still present in the area. It is also possible that our negative findings are the result of infected individuals dying from the disease. Nonetheless, our results in conjunction with previous studies suggest that squirrels are not important intermediate hosts of *T. gondii* and likely do not act as reservoirs for *T. gondii* in cities. This knowledge is important as squirrels tend to occur at higher densities in cities than in more natural habitats which creates the possibility for increased parasite transmission between squirrels and cats, and further, cats and humans. When wildlife parasites are a human health concern, such as with *T. gondii*, then management actions are warranted. Our results suggest that squirrels do not need to be considered as possible sources of infection to cats. In general, our results shed light on the prevalence of *T. gondii* in Sciurids in urban areas. This is important as *T. gondii* infection dynamics are still relatively unknown in cities.

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Table 5.1 The number of individuals and the number of tissue samples from each squirrel species from rural and urban sampling locations tested for *Toxoplasma gondii* DNA using quantitative PCR. The common and scientific names of species are listed, as are the total numbers of PCR samples tested for each species, and the total number of individuals and samples in each sampling location.

Species	**Scientific name**	**#squirrels**	**#squirrels**	**#samples**	**#samples**	**Total PCR**
		Rural	Urban	Rural	Urban	
Red squirrel	*Tamiasciurus hudsonicus*	12	13	72	77	149
Grey squirrel	*Sciurus carolinensis*	4	12	8	44	52
Least chipmunk	*Tamias minimus*	0	1	0	5	5
Northern flying squirrel	*Glaucomys sabrinus*	4	0	24	0	24
Total		**20**	**26**	**104**	**126**	**230**

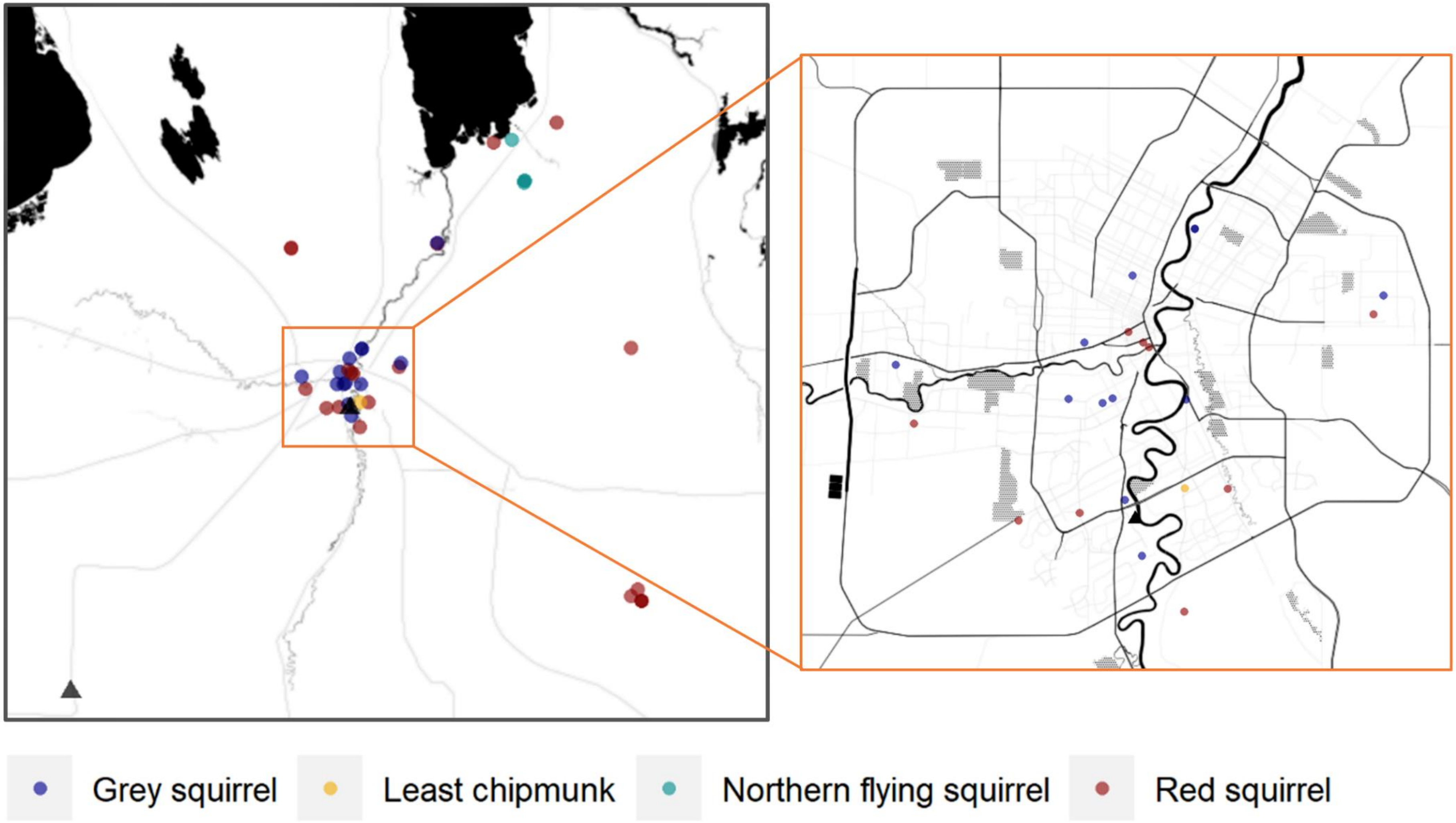
● Grey squirrel
● Least chipmunk
● Northern flying squirrel
● Red squirrel

Figure 5.1 Map showing the urban and rural study sites (black triangle) where serological samples were collected from grey squirrels (*Sciurus carolinensis*) for testing of *Toxoplasma gondii* antibodies, and the locations of squirrel (Sciuridae) carcasses (dots) used to collect tissue samples for quantitative PCR detection of *T. gondii*. The map on the left shows the locations of squirrels from in and around the city of Winnipeg, Manitoba, Canada, and the smaller map (framed orange) is a close-up of the urban locations within the city perimeter. The map was created using an R package *ggmap* (Kahle & Wickham 2013). Map tiles by Stamen Design, under CC BY 3.0. Data by OpenStreetMap, under ODbL.

Chapter 6: Discussion

"It must be admitted that the ecologist is something of a chartered libertine; he roams at will over the preserves of the plant and animal biologists, the physiologist, the behaviorist, the meteorologist, the geologist, the physicist, the chemist, and even the sociologist. He poaches from all these and from other established and respected disciplines. It is indeed a major problem for the ecologist, in his own interest, to set bounds to his divagations."

― Ayman Macfayden, *Animal Ecology: Aims and Methods*

The four data chapters of this thesis present an overview of the ecology of wildlife in cities furthering our knowledge of how species and populations are responding to rapid environmental change. My use of both synthetic macroecological analyses (Chapters 2 and 3) and local-scale focal species studies (Chapters 4 and 5) has enabled me to uncover some of the mechanisms that enable wildlife to colonize and persist in cities. I first showed which species, in general, colonize new environments after rapid environmental change by looking at both the phenotypic traits of Passerines and the species richness of migratory birds across structurally and socioeconomically varying cities (Chapters 2 and 3). I then determined if behavioral modifications may facilitate population persistence in urban environments by investigating differences in the activity patterns of Eastern grey squirrels (*Sciurus carolinensis*) and North American red squirrels (*Tamiasciurus hudsonicus*) between urban and non-urban areas (Chapter 4). Finally, I explored the possible parasite-related ecological consequences of urban colonization by squirrels (Sciuridae) for domestic cats, and possibly their owners, in the newly assembled urban ecosystem (Chapter 5).

The socioeconomic and structural complexity in cities drives patterns in species traits and species richness

Land-use change is one of the biggest threats to biodiversity and ecosystem services worldwide (Sala et al. 2000). Urbanization is one of the most pervasive examples of land-use change and can have significant ecological effects on the environment and colonizing wildlife (Alberti et al. 2003). Conservation and management strategies addressing, for example, the tolerance of wildlife to human-related threats like urbanization need to consider many temporal and spatial scales if effective solutions to this multidimensional, multidisciplinary crisis are to be found (Kerr et al. 2007). Although there has been an increasing interest in urban ecology in the past decades, research to date has mainly considered cities as homogenous environments with most biodiversity comparisons conducted between urban and rural areas, largely overlooking variation across cities—a defining feature of contemporary urban landscapes across the globe (Huang et al. 2007). Global or continent-wide macroecological investigations investigating how biodiversity responds to urbanization have also been largely lacking (but see e.g., Aronson et al. 2014; Callaghan et al. 2021). Synthetic data analyses that combine raw open-source data from multiple sources provide a data-intensive way to assess broad-scale spatial biodiversity patterns by including ecological data with explanatory and possibly spatial variables into models to address scientific questions and hypotheses. Using these methods, I show that cities that vary in their structural and socioeconomic features (e.g., median household income, sprawl, city age) seem to filter for birds with different subsets of traits (Chapter 2) and act as drivers of migratory bird species richness (Chapter 3).

In Chapter 2 I show that heterogeneity across cities—created by human social decision-making processes, socioeconomic factors, and everyday activities—seems to select for phenotypes that confer tolerance to the particular human-associated environmental conditions in each city and support the colonization of some species over others. The proximate causes of urban environmental variation are likely to vary for different cities and species, for example, some cities will have more traffic and pollution than other cities but may have more green backyards that offer resources for wildlife. These patterns are likely to vary across cities depending on each city's history and the political and social activities and decisions made by the dominant social groups in those cities (Des Roches et al. 2020; Schell et al. 2020). How each colonizing species responds to the citywide variation will depend on the tolerance level and phenotypic traits that that species has. Overall, my results suggest that environmental variation across cities, in this instance related to the environmental factors associated with a city's socioeconomics, is acting as a selective force at the species level— species with generally similar phenotypic traits tend to cluster in similar urban environments as only species with phenotypic traits that confer tolerance to the conditions in each city will survive and successfully establish populations. Ecological selection thus plays a role in determining patterns of biodiversity when communities are first formed after the emergence of new environments.

In Chapter 3 I show that the socioeconomic and structural traits of cities drive patterns in migratory bird species richness. My results are generally consistent across birds that rely on different habitat biomes for breeding. This is particularly interesting as it implies that these differing birds are responding to city variation in similar ways, which suggests that migrants may be relying on human-provided resources (like bird feeding stations or nests under roof awnings) in cities, not just the more natural city elements (e.g., tree cover or vegetation diversity). As I wanted to focus on the effects of human activities on migratory birds, I quantified the relationships between the city variables and the Human Footprint Index—a general measure of the extent of human-related disturbance—in each city. My results show that cities with certain features may attract migratory birds even if human-associated disturbance levels in the area are high. This implies that human social effects on animal ecology can differ from the effects of human-related disturbance per se. This further strengthens our above-mentioned interpretation that species filtering in socioeconomically varying cities is likely linked to human-associated resources found in those cities. However, whether the resources offered by different cities are beneficial to migrants, or whether these cities act as ecological traps to some species still needs further investigation at the local level.

Overall, my findings from these two chapters suggest that future research on biodiversity patterns in cities should consider cities as complex heterogeneous ecosystems and aggregates of human activities, where the social, cultural, and political actions of humans largely modify the city environment in ways that may not be equivalent to disturbance. My research shows—at a broad macroecological scale—that the social decisions people make within cities can capture characteristics of the urban environment in ways that are generally predictive of the species found in cities. Understanding what kinds of species initially colonize cities improves our knowledge of how the distribution of biodiversity will change following rapid environmental change. In addition, although cities are often overlooked in bird management and conservation efforts, cities may offer benefits to wildlife and could play a more important role in conservation and management than they currently do.

Behavioral flexibility facilitates population persistence in cities

Although research focused on urban wildlife is on the rise, investigations that focus on behavioral change in urban mammals are still rare (Ritzel & Gallo 2020). In Chapter 4 I used data obtained from specifically designed PIT tag data loggers to study differences in the temporal and spatial activity patterns of Eastern grey squirrels (*Sciurus carolinensis*) and North American red squirrels (*Tamiasciurus hudsonicus*) between urban and non-urban areas. The data loggers allowed the passive collection of large quantities of data throughout the day across a period of several months, which allowed me to draw robust biological inferences about behavioral changes between urban and rural squirrels. As the environment can filter species traits both by environmental filtering and through competition between similar species—as has been the topic of many discussions and some debate in the community ecology field (Kraft et al. 2015)—my investigations in Chapter 4 shed some light on these underlying patterns that may enable species persistence after rapid environmental change. I show that behavioral flexibility may enable species persistence of squirrels in cities and is possibly the result of both biotic (competition) and abiotic (light intensity) differences between urban and rural areas. Although I saw high levels of overlap in activity between red and grey squirrels in both cities and rural areas, the peaks in activity for each species mainly occurred when the other species were less active, indicating that grey squirrels might adjust the time of their daily activities to avoid the more territorial and aggressive red squirrels. Consequently, temporal segregation seems to allow the coexistence of Eastern grey squirrels and North American red squirrels in the study region. I also found that light intensity levels varied between cities and rural areas and that this variation may have partly accounted for the different activity patterns of squirrels in these habitats. Overall, in Chapter 4 I show that behavioral adjustments likely allow squirrel populations to persist in cities and that both abiotic and biotic mechanisms alter the temporal activity patterns of animals in cities.

Ecological consequences of urban colonization by squirrels

As the urban area keeps expanding at an unprecedented rate more and more natural areas will be lost, and as wildlife increasingly moves to cities existing host-parasite dynamics may shift and evolve. These changes may have impacts on humans or their pets, as wildlife commonly forage or nest in people's backyards. In Chapter 5 I explored these kinds of possible parasite-related ecological consequences of urban colonization by squirrels in cities by surveying the prevalence of *Toxoplasma gondii* infection in squirrel (Sciuridae) populations in and around the city of Winnipeg, Manitoba, Canada. My approach was particularly strong as I used both molecular and serological methods, where previous studies have mainly used one or the other (Galeh et al. 2020). I found no evidence of *T. gondii* infection in squirrels. My results in conjunction with previous studies suggest that squirrels are not important intermediate hosts of *T. gondii* and likely do not act as reservoirs for *T. gondii* in cities. This knowledge is important as squirrels tend to occur at higher densities in cities than in more natural habitats which creates the possibility for increased parasite transmission between squirrels and cats, and further, cats and humans. When wildlife parasites are a human health concern, such as with *T. gondii*, then management actions are warranted. My results suggest that squirrels do not need to be considered as sources of infection to cats. In general, my results shed light on the prevalence of *T. gondii* in Sciurids in urban areas. This is important as *T. gondii* infection dynamics are still relatively unknown in cities.

Using synthetic analyses and focal species-specific studies to investigate species response to urbanization

I used both synthetic analyses (Chapters 2 and 3) and focal species studies (Chapters 4 and 5) to investigate the mechanisms that enable wildlife to colonize and persist in cities. This approach meant that I got to deepen my skills in ecological modeling and get my hands dirty in the field, and additionally, I was able to investigate both continental-scale species level and local-scale population-level biodiversity patterns.

I first conducted synthetic analyses of publicly available census and citizen science data to determine which species, in general, colonize new environments after rapid environmental change. Ecological data is famously complex, noisy, and messy. With citizen-science data becoming ever more popular in recent decades (Dickinson et al. 2012) the opportunities for large macroecological data explorations have multiplied. However, the large size and variability of these data often present its challenges both at the organizational and analysis stage, and more complex data often requires more complex methods of data analysis (Harrison et al. 2018; Kelling et al. 2009). Mixed-effects models (Chapter 2), namely linear mixed-effects models (LMMs) and generalized linear mixed models (GLMMs), offer flexibility for analyzing complex ecological data that can often be non-independent, non-normal, have many grouping variables (e.g., sites, species, populations), and low sample sizes (Bolker et al. 2009; Harrison et al. 2018). These models broaden linear models by including both fixed and random effects as predictor variables. The random effects typically are a grouping variable (e.g., U.S. state, taxonomic family; Chapter 2) and allow the estimation of variance in the dependent variable within and between these groups.

Ecological data can also be spatially structured and it may be necessary to account for spatial autocorrelation in the residuals (Chapter 3). Spatial structure in the model residuals violates the assumption that residuals are independent and identically distributed. This can bias parameter estimates and can increase Type I Error rates (Dormann et al. 2007). In Chapter 3 I used distance-based Moran's Eigenvector Maps (dbMEMs) (Dray et al. 2006) to account for spatial variation in my residuals. These capture spatial relationships among data points at all spatial scales and are orthogonal, and can be added to the model as explanatory variables to account for spatial autocorrelation (Dormann et al. 2007; Griffith & Peres-Neto 2006; Legendre, Pierre & Legendre 2012).

Both above-mentioned statistical tools are still relatively under-utilized in ecological investigations (although the use of mixed models seems to be gaining more traction). However, mixed-effects models offer a flexible approach to analyzing complex and noisy open-source data at a macroecological scale, and Eigenvector mapping techniques can be used to describe and control for spatial patterns in the data.

I then used field research methods to determine if behavioral modifications facilitate population persistence in urban environments. Conducting fieldwork in an urban setting is hugely different from conducting fieldwork in a natural habitat and comes with its own set of challenges. Probably the biggest challenges are the need to gain access to private properties and being prepared for many types of encounters with the public that can vary from a small child worrying about the squirrels, to homeowners curiously observing every step of the data collection procedure, to people being aggressive towards you. However, after tackling these challenges when trapping and PIT tagging squirrels, automating the data collection process using PIT tag data loggers provided an efficient way of collecting large quantities of high-quality data simultaneously from multiple sampling sites, often unattainable with traditional field methods. These kinds of field collection methods are likely to become ever more inventive and important in the future.

Overall, by using these diverse methods I gained a broad overview of the ecology of wildlife in cities, and how species and populations are responding to rapid environmental change.

Afterword

Like Ayman MacFayden's description of ecologists at the start of this chapter, I did in fact, to a degree, feel like a "chartered libertine" during my thesis research. As I moved from one chapter to another, I shifted from birds to squirrels to parasites, went from continent-wide explorations to more local-scale investigations, and collected data sitting at the computer, being in the field catching squirrels, and collecting organ samples in a veterinary lab. To try and interpret my results for each of my chapters I indeed had to refer to (or poach) ideas from plant and animal biologists, population geneticists, parasitologists, physiologists, behaviorists, and sociologists. Furthermore, setting boundaries became important when honing my conclusions, and tying together my ideas for my thesis Introduction and Discussion. However, even though the interdisciplinary nature of ecology, and my thesis, at first seemed complicated, unconnected, and messy, I can now appreciate how everything in ecology really is connected to everything else.

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Appendix

Supplementary Information 2

The socioeconomic status of cities covaries with avian life-history strategies

Contents:

Tables S2.1-S2.5
Figures S2.1-S2.9

Data compilation

eBird

We compiled our dataset by combining passerine life-history data and urban characteristics data from open source and citizen science sources, and by georeferencing this data by overlaying observations with US Census Bureau's urban area shapefiles (see Fig S2.1 for a flowchart of the data compilation procedure). We first downloaded the eBird Basic Dataset (EBD) from eBird.org (Sullivan et al. 2009) to gain access to bird observation data across the United States. eBird is a real-time, online, bird abundance and distribution checklist program jointly coordinated by the Cornell Laboratory of Ornithology and the National Audubon Society. The eBird project relies on citizen science volunteer observers who submit either bird count of species, or the presence of a species at a particular location to a centralized database. Observers may use a location of their own choice or alternatively, specific eBird nominated birding "hotspots". Regional reviewers will then identify outliers and verify each species observation based on the sighting coordinates (Wood et al. 2011). The EBD is a large core dataset (text file with >100,000,000 observations) that allows access to all raw eBird observations worldwide and is publicly available to use to answer many types of ecological questions. The eBird observations are in the global coordinate system World Geodetic System (WGS 84).

Avian life-history data

To access bird life-history data (body mass, clutch size, longevity) we downloaded an Amniote life-history database (Myhrvold et al. 2016) from The Dryad digital repository (datadryad.org). This database is a systematically compiled database of life-history traits for birds, mammals, and reptiles, that is publicly available to use in comparative life-history analyses (Myhrvold et al. 2015). The median value is reported for species' traits with multiple raw data points. To link our observations of passerines with their life-history traits we merged bird species observations from eBird with life-history data in R. We compared the species lists from eBird and the Amniote Life-History database to see what species were excluded by the merge and found 17 species of Passerines that were present in the eBird dataset that were not found in the Amniote database (listed in Table S2.1). After the merge we had a species trait dataset of observations of 431 species of passerines recorded during their breeding season across the United States.

Spatial join of bird data with urban area shapefiles

We only wanted to keep observations of breeding passerines in urban areas across the United States. To do this we spatially joined our previously compiled species trait data to urban area shapefiles provided by the U.S. Census Bureau. The urban area shapefiles used are one of the relationship files associated with the 2010 Census TIGER/Line Shapefiles (U.S. Census Bureau 2010a). The core TIGER/Line Files and Shapefiles do not include demographic data, but the geographic entity codes (GEOIDs) that can easily be linked to the U.S. Census Bureau's demographic data, available on American FactFinder (https://factfinder.census.gov). 2010 Census geography reflects the boundaries of governmental units in force as of January 1, 2010, and statistical area boundaries outlined for the 2010 Census. The shapefiles match the 2010 Census data that uses 2010 geography. The urban area shapefiles define an urban area as a densely developed territory with at least 2500 people (U.S. Census Bureau 2010a) and can be used to define and georeference urban areas. All Census Bureau generated shapefiles are in the global coordinate system North American Datum of 1983 (NAD83). The shapefile can be downloaded from https://www.census.gov/cgi-bin/geo/shapefiles/index.php?year=2010&layergroup=Urban+Areas. We conducted the spatial join of our two spatial datasets – Species trait dataset observations with points (individual x-y locations) and urban area shapefiles with polygons, and associated attribute data using the R packages *sp* (version 1.3-1; Bivand et al. 2013), *rgdal* (version 1.3-4; Bivand et al. 2018) and *maps* (version 3.3.0; Becker and Wilks 2015). R package *sp* provides S4 classes and methods such as points, lines, polygons, and grids for importing, manipulating, and exporting spatial data in R. *Raster* is used to manipulate raster and vector data and *rgdal* to read or write spatial data files. As the first step of spatially joining our bird and city data, we had to re-project the eBird coordinate reference system (CRS) from WGS 84 to NAD83, so that both datasets had the same CRS. After this, we conducted the spatial join using the over command from the *sp* library. The over command conducts spatial overlay by returning the attributes from spatial object y (urban area shapefile) at the spatial locations of object x (eBird latitude and longitude). After the spatial join, we had a georeferenced dataset of urban Passerines.

U.S. Census data

Next, we downloaded census data from the U.S. Census Bureau (U.S. Census Bureau 2010b) to access structural and socioeconomic city characteristics data. As the U.S. census data did not contain coordinates for the cities, we got latitude and longitude for each city by merging city trait data with U.S. Census Gazetteer files (U.S. Census Bureau 2010c) by using city name and state as common elements. The Gazetteer files list all geographic areas for designated geographic area categories. Information such as geographic identifier codes, names, and latitude and longitude coordinates are included in the files.

Spatial join of city data with urban area shapefiles

We merged our dataset with the urban area shapefiles provided by the U.S. Census Bureau as before, using the R packages sp, *rgdal* and *maps*. After these steps, we had a georeferenced dataset of city characteristics from a total of 2,738 cities.

Merging of spatially joined city and eBird data

We then merged the georeferenced urban Passerine data and city characteristics data in R by using the name of the urban area and state as the common elements. After these steps, we had a dataset of 260 species of Passerines in 1,980 urban areas.

Adding migratory status of each species to the dataset

As the next step of data manipulation, we added the migratory status of each passerine to our dataset. For this, we classified each bird species as either resident or migratory. Birds were classified as residents if they are present in a state year-round, even if the species is generally classified as a migrant. Birds were classified as migrants if they do not have a year-round presence in a state and migrate from elsewhere to breed there. We deleted any duplicated rows (same species multiple times in the same urban area) in R and any observations from Hawaii as island ecosystems can differ from that of mainland communities. We only kept bird observations between 2010-05-27 and 2018-07-07. We excluded any non-native, introduced, or pet trade species, as well as accidental observations from our dataset (Table S2.2), and removed observations that were part of the birds overwintering or migration range, and vagrants from each state. For some U.S. states, a species could be both a breeding-migrant and a breeding-resident depending upon what part of the state was considered. We deleted those observations from our dataset. We also deleted any observations from urban areas that had an average population size of under 2,500 people, to keep within the definition of an urban area by the U.S. Census Bureau.

We were also interested in the possible effect of urban sprawl/city compactness on bird traits and calculated this by dividing the population size of a city by its area (Sprawl = Population size/Area). After these steps, we had our final dataset of 213 species of Passerines in 551 urban areas in 48 states (Fig S2.1).

Selection of variables and accounting for sampling effort

Six urban characteristics suspected to be correlated underwent Spearman rank correlation analysis (Fig S2.2). We chose three largely uncorrelated urban characteristics; human population size, compactness/sprawl, and median household income as our predictor variables. We chose clutch size, longevity (the lifespan of an individual in years), body mass (mass of an individual in grams), and migratory status (migratory or resident) as our response variables.

We were interested in species presence in an urban area during a breeding season, not the number of observations of each species, and so our final data set was made up of presence data for each species observed in each city. To estimate the adequacy of eBird surveys in cities of different human population sizes (New York, New York, population size 8175133; San Francisco, California, 805235; Denver, Colorado, 600158; Albuquerque, New Mexico, 545852; Toledo, Ohio, 287208; Portland, Maine, 66194; Flagstaff, Arizona, 65870; Park City, Utah, 7558) and to add a measure of effort into our analyses when we were not interested in species abundance, we plotted the accumulation of species in selected cities over the years 2010 -2018 (see Fig S2.3). These accumulation curves leveled off for the majority of the cities plotted, indicating that the Ebird surveys were capturing most species found in a city.

Data analyses and results with different criteria

As species with a low number of sightings in a city may have been accidental observations or vagrants, we ran our analysis with three different criteria: 1) keeping all observations of species observed at least once in a city (see Fig S2.4, Fig S2.5; Table S2.3); 2) keeping only observations of species observed 10 or more times in a city (see the main paper Fig 2.2, Fig 2.4; Table 2.1); and 3) keeping only observations of species observed over 20 times in a city (see Fig S2.6, Fig S2.7; Table S2.4). The results were all quantitatively similar, so we chose the second option to ensure we had multiple observations of each species in a city. The eBird observations are submitted in a checklist format, listing species seen during one bird-watching occasion. Checklists are either complete checklists where all birds detected and identified were recorded, or incomplete checklists where some species that were seen were not recorded. To ensure that we had the most complete species list possible for our analysis and that some species were not missing due to lack of recording we also ran our analyses using complete checklists only (see Fig S2.8, Fig S2.9; Table S2.5), which led to quantitatively almost identical results to using all the checklists. Therefore, for our analyses, we used all observations, including incomplete and complete checklists.

Table S2.1 The species that were excluded by merging bird species observations from eBird and the Amniote Life-History database.

Scientific name	**Common name**
Ammospiza caudacuta	Saltmarsh Sparrow
Ammospiza maritima	Seaside Sparrow
Ammospiza nelsoni	Nelson's Sparrow
Centronyx bairdii	Baird's Sparrow
Ammodramus henslowii	Henslow's Sparrow
Corvus monedula	Eurasian Jackdaw
Griseotyrannus aurantioatrocristatus	Crowned Slaty Flycatcher
Pterorhinus pectoralis	Greater Necklaced Laughingthrush
Leiothlypis celata	Orange-crowned Warbler
Leiothlypis crissalis	Colima Warbler
Leiothlypis luciae	Lucy's Warbler
Leiothlypis peregrina	Tennessee Warbler

Leiothlypis ruficapilla	Nashville Warbler
Leiothlypis virginiae	Virginia's Warbler
Spizelloides arborea	American Tree Sparrow
Sporophila morelleti	Morelet's Seedeater

Table S2.2 The species that were excluded from our analysis, and the reason why they were excluded. Both the scientific and common names of each species are included.

Scientific name	**Common name**	**Reason excluded**
Sturnus vulgaris	European starling	Introduced
Passer domesticus	House sparrow	Introduced
Carduelis carduelis	European goldfinch	Introduced
Corvus cornix	Hooded crow	Eurasian species
Corvus splendens	House crow	Non-native, Asian origin
Fringilla montifringilla	Brambling	Eurasian species
Lonchura punctulata	Scaly-breasted munia	Pet trade, Introduced
Motacilla alba	White wagtail	Old World species

Parus major	Great tit	Old World species
Pycnonotus cafer	Red-vented bulbul	Introduced
Pycnonotus jocosus	Red-whiskered bulbul	Asian species, Introduced
Sporophila torqueola	Cinnamon-rumped seedeater	Accidental, Mexican species
Taeniopygia guttata	Zebra finch	Introduced, Australian species
Turdus grayi	Clay-colored thrush	Central American species
Zosterops japonicas	Japanese white-eye	Asian species, Introduced
Chloris chloris	European greenfinch	Eurasian species
Icterus pectoralis	Spot-breasted oriole	Introduced
Acridotheres tristis	Common myna	Introduced

Table 2.3 Model summaries and the number of observations for the urban predictors of passerine life-history traits and migratory status in the United States when all observations of species observed at least once in a city were kept. One model was fit to data per response variable, including all the urban characteristics: city compactness/sprawl, median income, and human population size. Body mass was log10-transformed. For the migratory status category species are classed as migratory = 1 or resident = 0. An odds ratio greater than one indicates that the chance of finding a migrant bird in the area is higher than a chance of finding a resident and vice versa. The coefficient of variation is an indicator of model fit. Random effects were specified as (1 | family) and (1 | state), where each level of the grouping factors, family and state, had their own random intercept. The symbol σ2 is the residual variance; τ00 is the variance among the random effects; and N the total number of groups. The number of observations is the same for all the variables (n = 42,186).

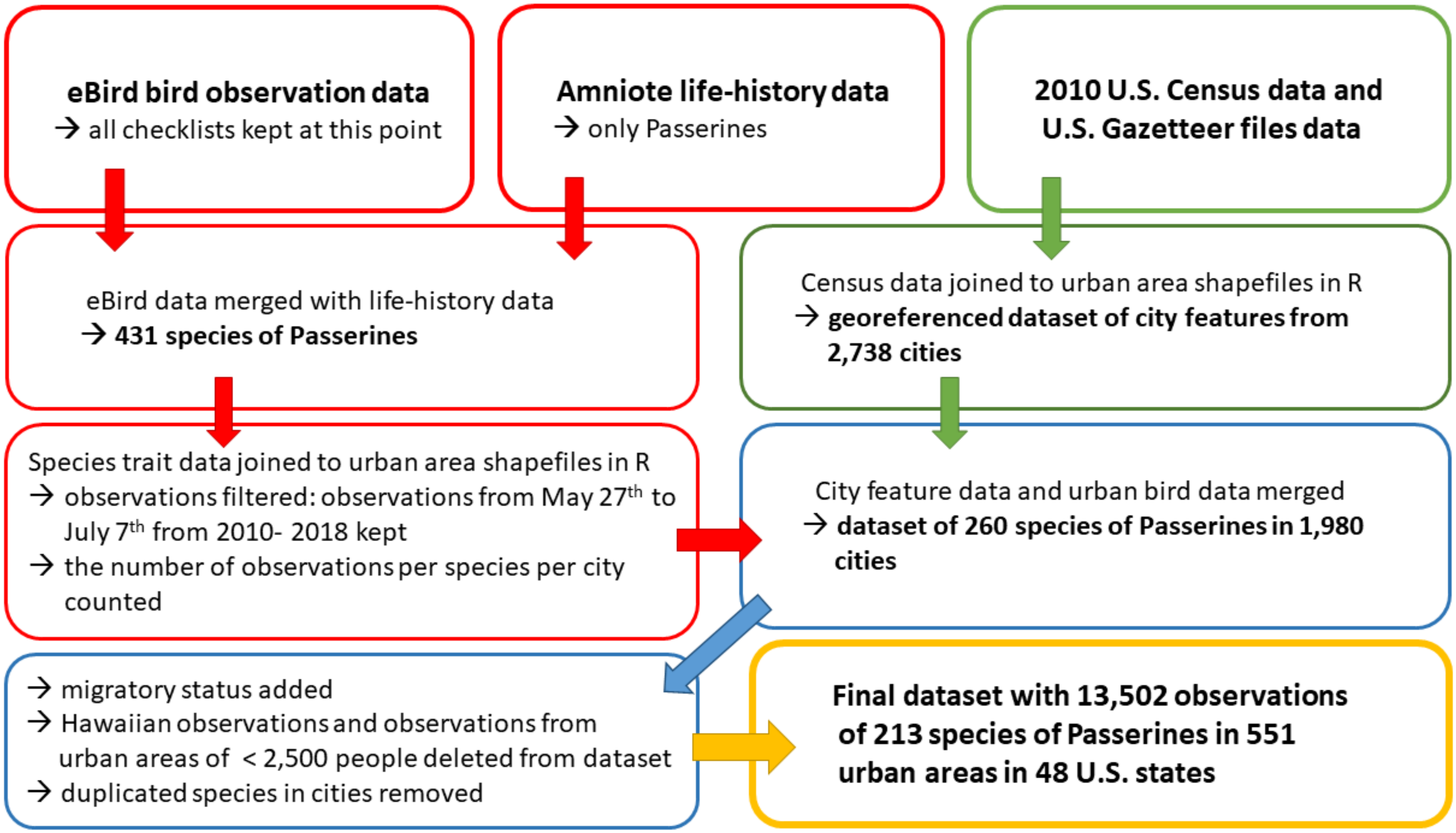
	Longevity		**Clutch size**		**Body mass**		**Migratory status**	
Predictors	*Estimates*	*95 % CI*	*Estimates*	*95 % CI*	*Estimates*	*95 % CI*	*Odds Ratios*	*95 % CI*
Intercept	-0.397	-0.693, -0.102	0.420	-0.026, 0.867	3.026	2.722, 3.329	0.284	0.030, 2.693
Compactness	-0.048	-0.058, -0.038	0.003	-0.005, 0.011	-0.013	-0.019, -0.006	1.216	1.162, 1.273
Median income	-0.006	-0.014, 0.001	0.004	-0.002, 0.010	-0.010	-0.015, -0.005	1.026	0.992, 1.061
Population size	0.001	-0.008, 0.010	-0.003	-0.010, 0.004	0.004	-0.002, 0.009	0.992	0.947, 1.039
Random Effects								
σ^2	0.51		0.33		0.20		3.29	
τ_{00}	0.01_{State}		0.00_{State}		0.00_{State}		0.31_{State}	
	0.64_{family}		1.49_{family}		0.69_{family}		37.58_{family}	
	29_{family}		29_{family}		29_{family}		29_{family}	
	48_{State}		48_{State}		48_{State}		48_{State}	
Observations	42816		42816		42816		42816	

Table S2.4 Model summaries and the number of observations for the urban predictors of passerine life-history traits and the predictors of passerine migratory status in the United States when only species with > 20 observations per city were kept. One model was fit to data per response variable, including all the urban characteristics: city compactness/sprawl, median income, and human population size. Body mass was log10-transformed. For the migratory status category species are classed as migratory = 1 or resident = 0. An odds ratio greater than one indicates that the chance of finding a migrant bird in the area is higher than a chance of finding a resident and vice versa. The coefficient of variation is an indicator of model fit. Random effects were specified as (1 | family) and (1 | state), where each level of the grouping factors, family and state, had their own random intercept. The symbol σ2 is the residual variance; τ00 is the variance among the random effects; and N the total number of groups. The number of observations is the same for all the variables (n = 8,882).

	Longevity		Clutch size		Body mass		Migratory status	
Predictors	*Estimates*	*95 % CI*	*Estimates*	*95 % CI*	*Estimates*	*95 % CI*	*Odds Ratios*	*95 % CI*
Intercept	-0.440	-0.744, -0.135	0.364	-0.092, 0.820	3.030	2.712, 3.348	0.293	0.035, 2.471
Compactness	-0.072	-0.098, -0.045	0.024	0.004, 0.044	-0.029	-0.045, -0.014	1.511	1.343, 1.701
Median income	-0.014	-0.032, 0.004	0.002	-0.012, 0.015	-0.012	-0.023, -0.002	0.991	0.917, 1.071
Population size	0.001	-0.022, 0.025	-0.017	-0.035, 0.001	0.016	0.001, 0.030	0.926	0.826, 1.038
Random Effects								
σ^2	0.50		0.30		0.20		3.29	
τ_{00}	0.01 $_{\text{State}}$		0.00 $_{\text{State}}$		0.00 $_{\text{State}}$		0.32 $_{\text{State}}$	
	0.63 $_{\text{family}}$		1.49 $_{\text{family}}$		0.72 $_{\text{family}}$		30.51 $_{\text{family}}$	
ICC	0.56		0.83		0.78		0.90	
N	28 $_{\text{family}}$		28 $_{\text{family}}$		28 $_{\text{family}}$		28 $_{\text{family}}$	
	48 $_{\text{State}}$		48 $_{\text{State}}$		48 $_{\text{State}}$		48 $_{\text{State}}$	
Observations	8882		8882		8882		8882	

Table S2.5 Model summaries and the number of observations for the urban predictors of passerine life-history traits and the predictors of passerine migratory status in the United States when only complete checklists were kept. One model was fit to data per response variable, including all the urban characteristics: compactness/sprawl, median income, and human population size. Body mass was log10-transformed. The coefficient of variation is an indicator of model fit. Random effects were specified as (1 | family) and (1 | state), where each level of the grouping factors, family and state, had their own random intercept. The symbol σ2 is the residual variance; τ00 is the variance among the random effects; and N the total number of groups. The number of observations is the same for all the variables (n = 13,040).

	Longevity		**Clutch size**		**Body mass**		**Migratory status**	
Predictors	*Estimates*	*95 % CI*	*Estimates*	*95 % CI*	*Estimates*	*95 % CI*	*Odds Ratios*	*95 % CI*
Intercept	-0.414	-0.715, -0.113	0.371	-0.086, 0.828	3.029	2.712, 3.345	0.214	0.028, 1.654
Compactness	-0.053	-0.074, -0.032	0.022	0.006, 0.038	-0.025	-0.037, -0.012	1.444	1.316, 1.585
Median income	-0.005	-0.020, 0.009	0.005	-0.006, 0.016	-0.013	-0.021, -0.004	1.028	0.966, 1.094
Population size	-0.004	-0.023, 0.015	-0.015	-0.029, -0.000	0.014	0.003, 0.025	0.939	0.857, 1.030
Random Effects								
σ^2	0.50		0.31		0.20		3.29	
τ_{00}	0.02_{State}		0.00_{State}		0.00_{State}		0.29_{State}	
	0.62_{family}		1.50_{family}		0.72_{family}		28.22_{family}	
ICC	0.56		0.83		0.78		0.90	
N	28_{family}		28_{family}		28_{family}		28_{family}	
	48_{State}		48_{State}		48_{State}		48_{State}	
Observations	13040		13040		13040		13040	



eBird bird observation data
→ all checklists kept at this point

Amniote life-history data
→ only Passerines

2010 U.S. Census data and U.S. Gazetteer files data

eBird data merged with life-history data
→ **431 species of Passerines**

Census data joined to urban area shapefiles in R
→ **georeferenced dataset of city features from 2,738 cities**

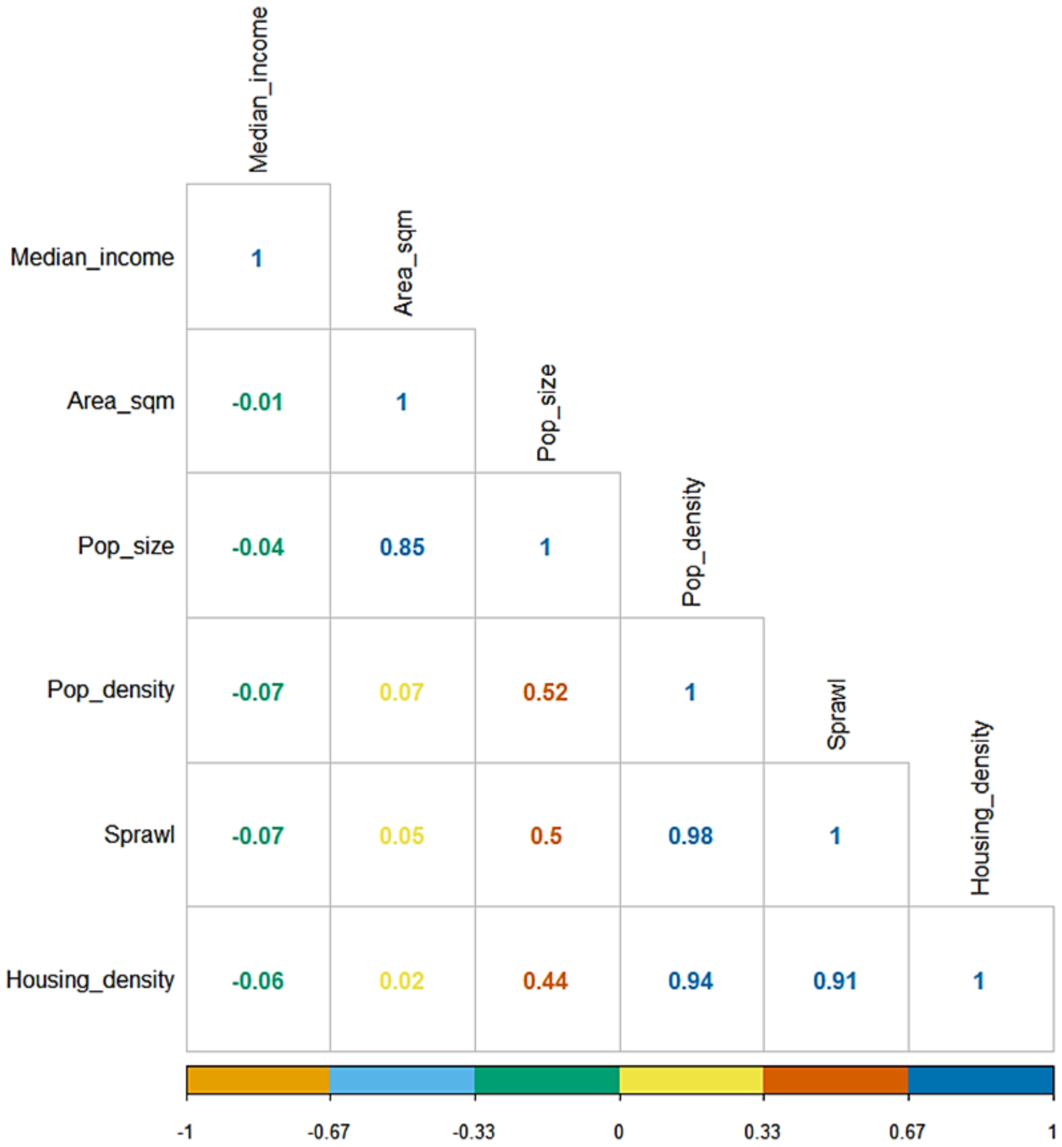
Species trait data joined to urban area shapefiles in R
→ observations filtered: observations from May 27th to July 7th from 2010- 2018 kept
→ the number of observations per species per city counted

City feature data and urban bird data merged
→ **dataset of 260 species of Passerines in 1,980 cities**

→ migratory status added
→ Hawaiian observations and observations from urban areas of < 2,500 people deleted from dataset
→ duplicated species in cities removed

Final dataset with 13,502 observations of 213 species of Passerines in 551 urban areas in 48 U.S. states

Figure S2.1 Flow chart describing the data compiling procedure.



	Median_income	Area_sqm	Pop_size	Pop_density	Sprawl	Housing_density
Median_income	1					
Area_sqm	-0.01	1				
Pop_size	-0.04	0.85	1			
Pop_density	-0.07	0.07	0.52	1		
Sprawl	-0.07	0.05	0.5	0.98	1	
Housing_density	-0.06	0.02	0.44	0.94	0.91	1

Figure S2.2 Pearson correlation coefficients between predictor variables.

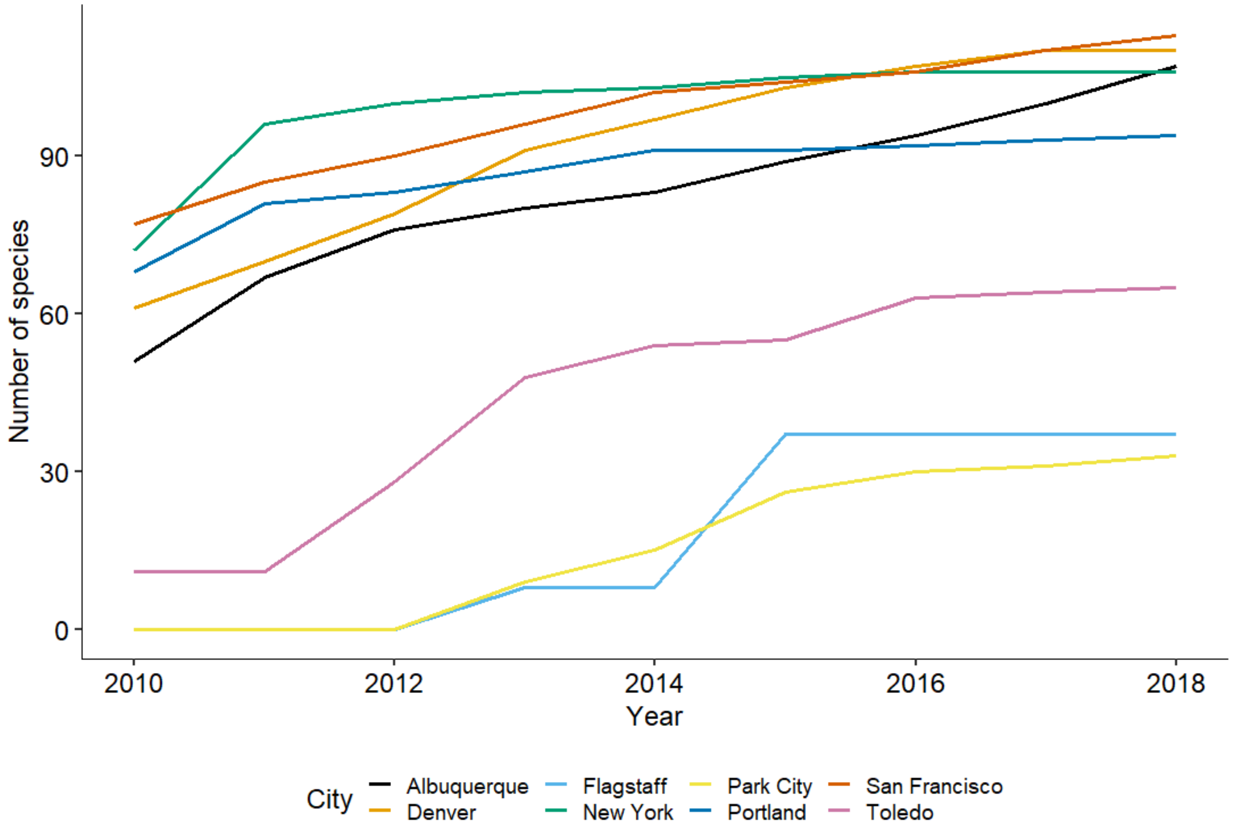


Figure S2.3 The accumulation curves of Passerine species richness for selected cities in the United States between the years 2010 - 2018.

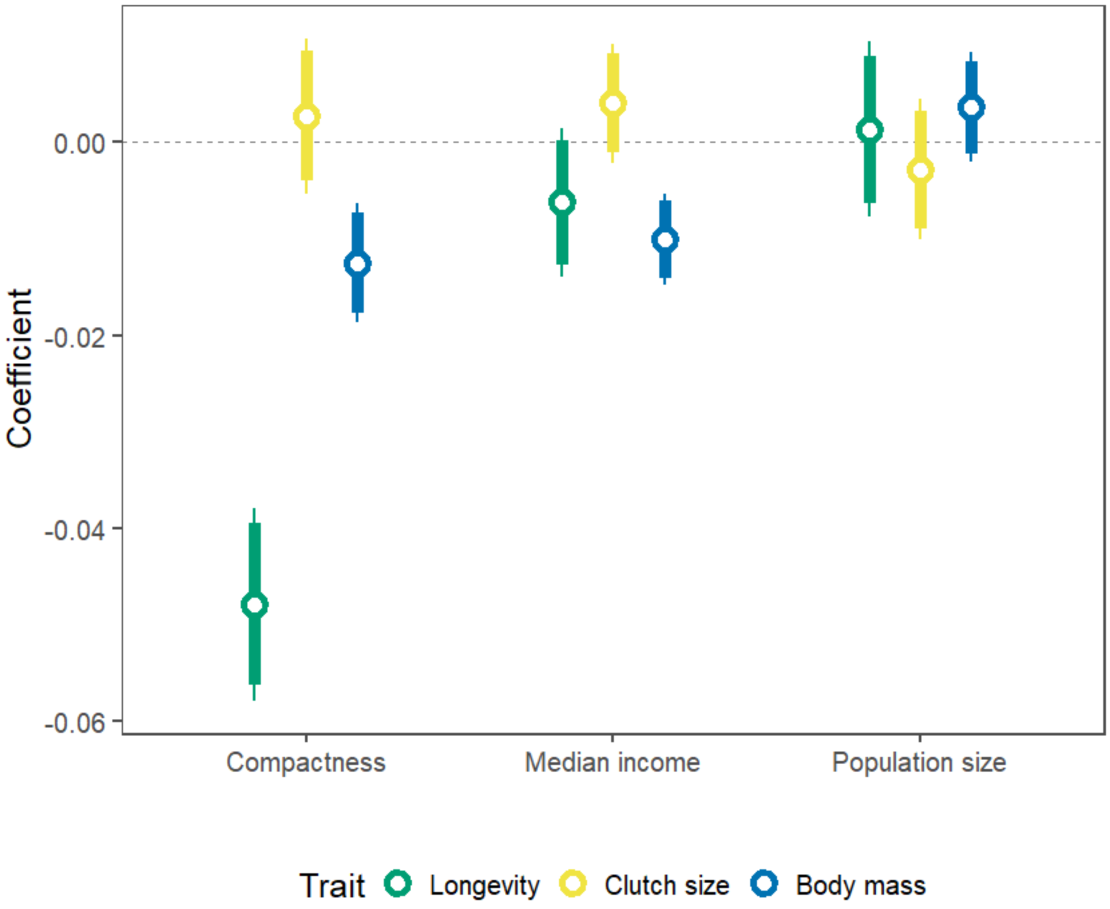


Figure S2.4 Linear mixed-effects model coefficients for urban predictors of passerine life-history traits in the United States when all observations of species observed at least once in a city were kept. Open circles represent coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. Body mass was $\log_{10}$ -transformed. Sample size is the same for all variables (n = 42,186).

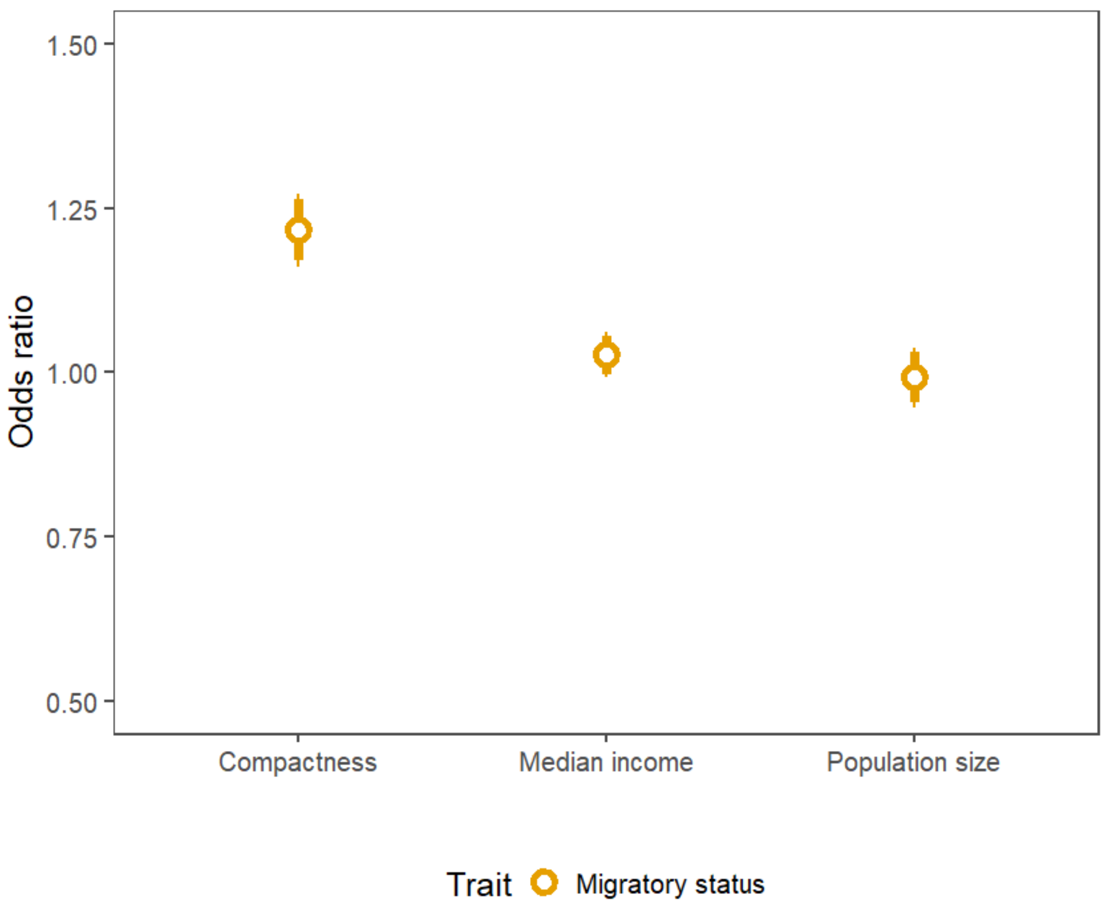


Figure S2.5 Generalized linear mixed-effects model coefficients for the predictors of passerine migratory status in the United States when all observations of species observed at least once in a city were kept. Open circles are coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. Species are classed as migratory = 1 or resident = 0. An odds ratio greater than one indicates that the chance of finding a migrant bird in the area is higher than a chance of finding a resident and vice versa. The sample size is (n = 42,186).

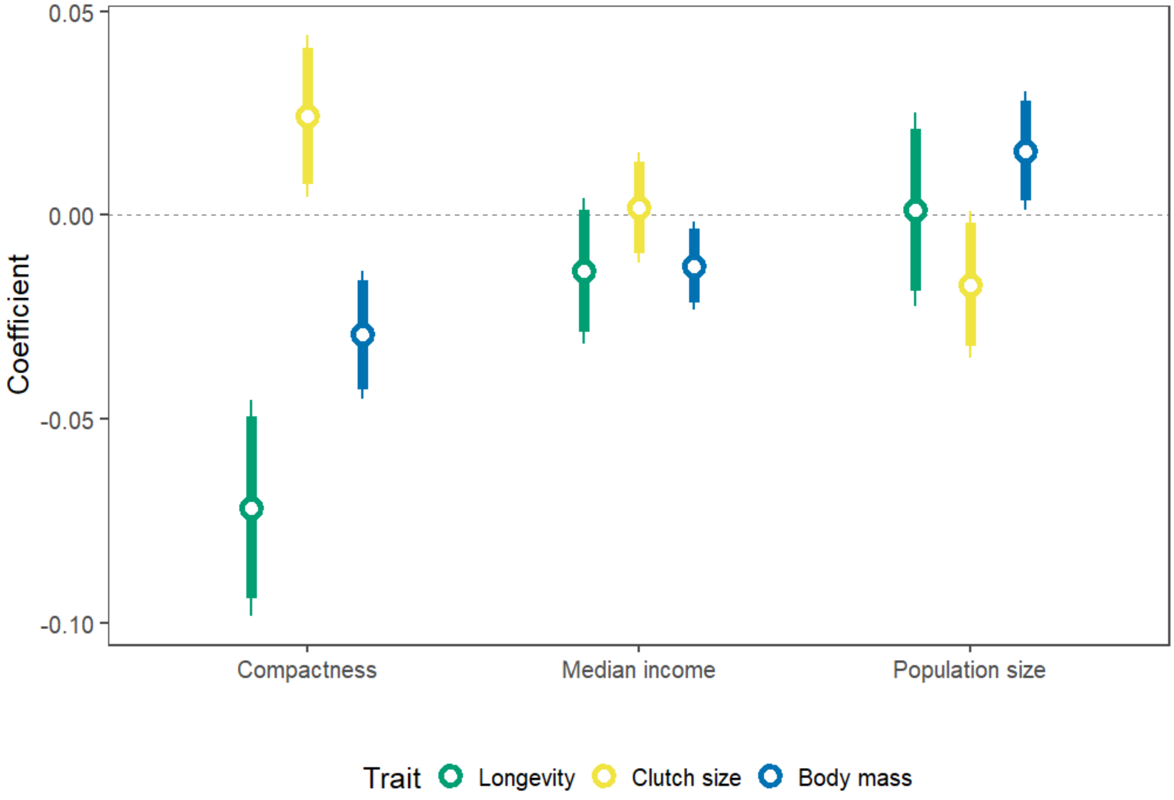


Figure S2.6 Linear mixed-effects model coefficients for urban predictors of passerine life-history traits in the United States when only species with > 20 observations per city were kept. Open circles represent coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. Body mass was $\log_{10}$-transformed. Sample size is the same for all variables (n = 8,882).

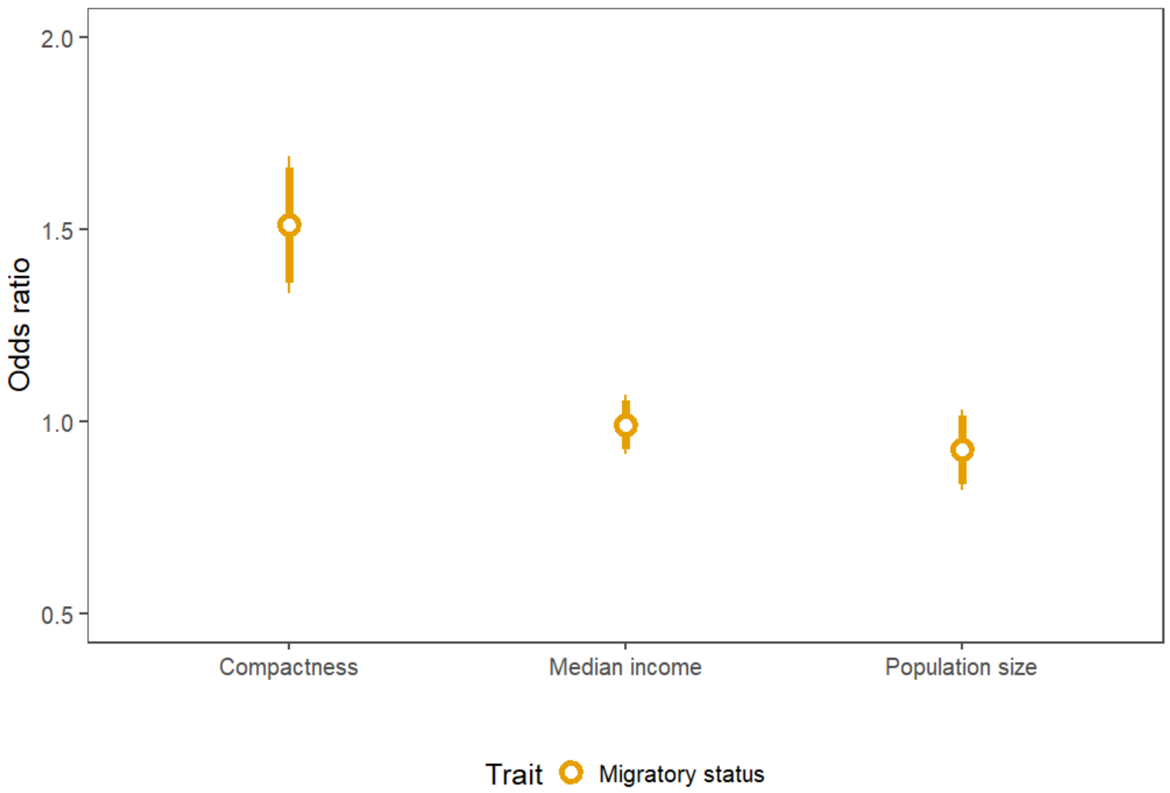


Figure S2.7 Generalized linear mixed-effects model coefficients for the predictors of passerine migratory status in the United States when only species with > 20 observations per city were kept. Open circles are coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. Species are classed as migratory = 1 or resident = 0. An odds ratio greater than one indicates that the chance of finding a migrant bird in the area is higher than a chance of finding a resident, and vice versa. The sample size is (n = 8,882).

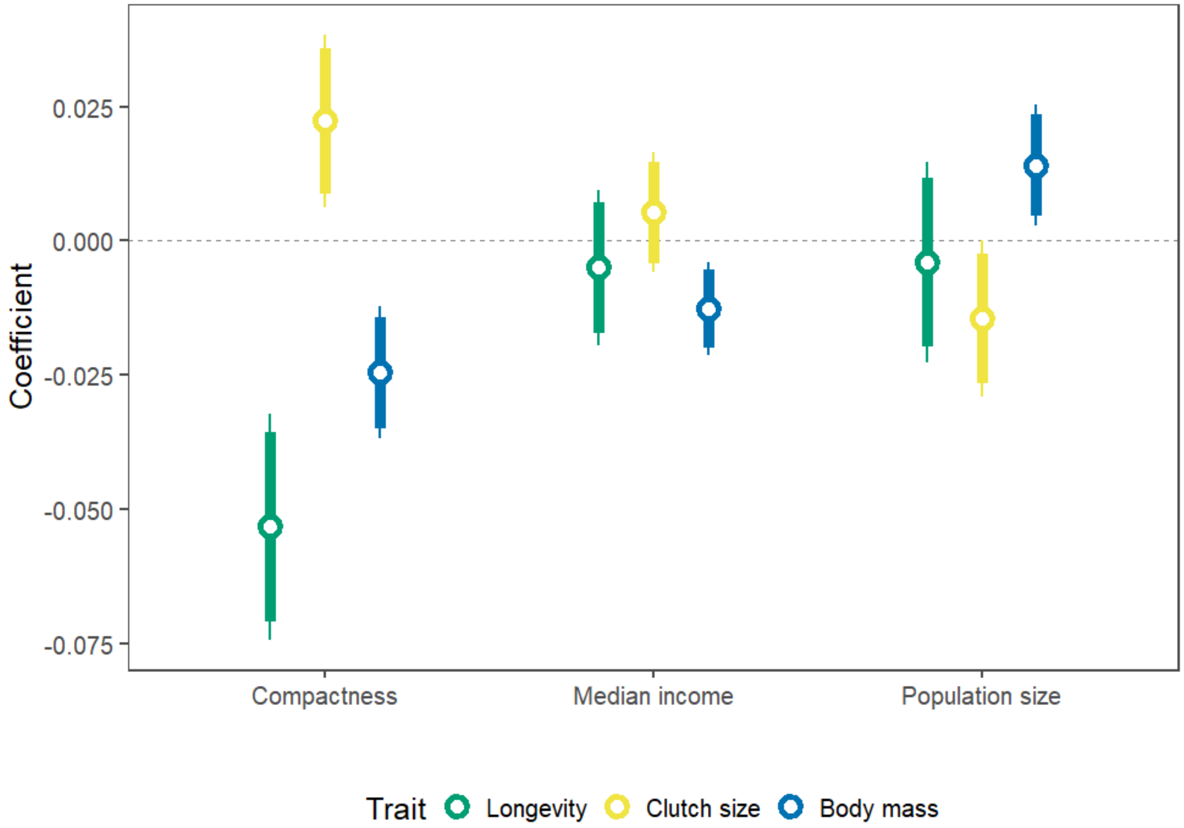
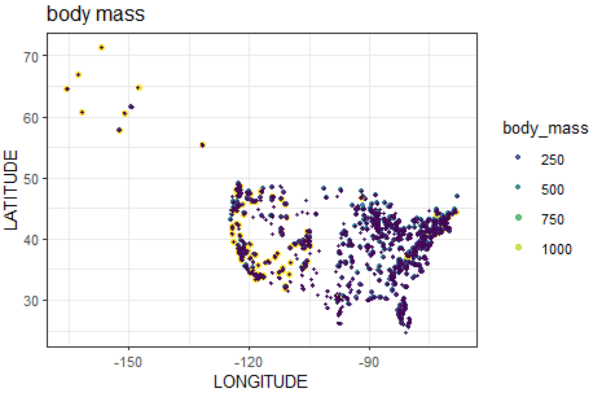
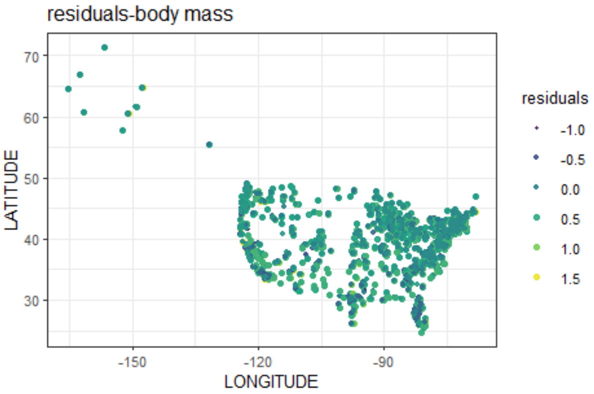
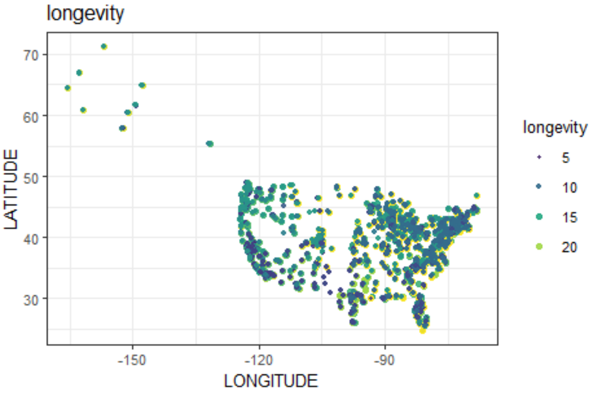


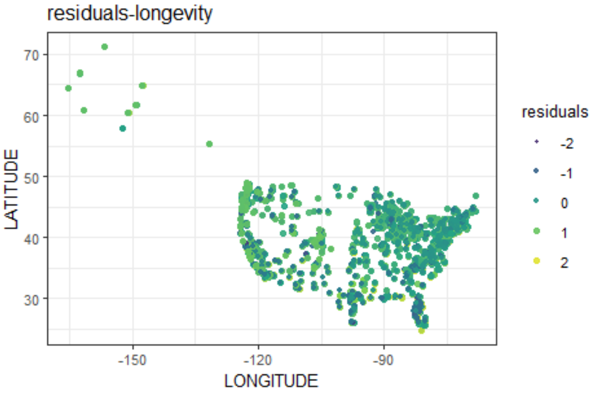
Figure S2.8 Linear mixed-effects model coefficients for urban predictors of passerine life-history traits in the United States when only complete checklists were kept. Open circles represent coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. Body mass was $\log_{10}$ -transformed. Sample size is the same for all variables (n = 13,040).

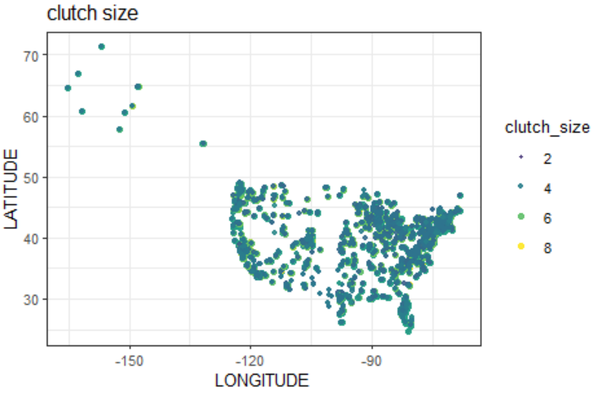
Figure S2.9 Generalized linear mixed-effects model coefficients for the predictors of passerine migratory status in the United States when only complete checklists were kept. Open circles are coefficient estimates, bold whiskers are 90% confidence intervals, and narrow whiskers are 95% confidence intervals. Species are classed as migratory = 1 or resident = 0. An odds ratio greater than one indicates that the chance of finding a migrant bird in the area is higher than a chance of finding a resident, and vice versa. The sample size is the same for all the variables (n = 13,040).

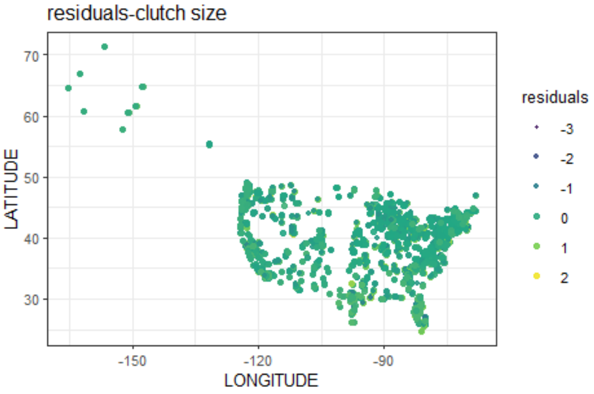

body mass

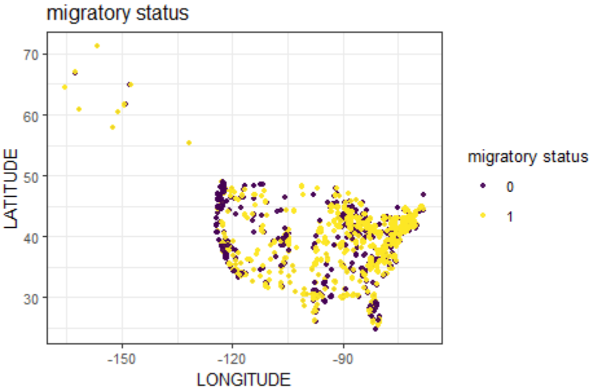

residuals-body mass


longevity


residuals-longevity


clutch size


residuals-clutch size


migratory status

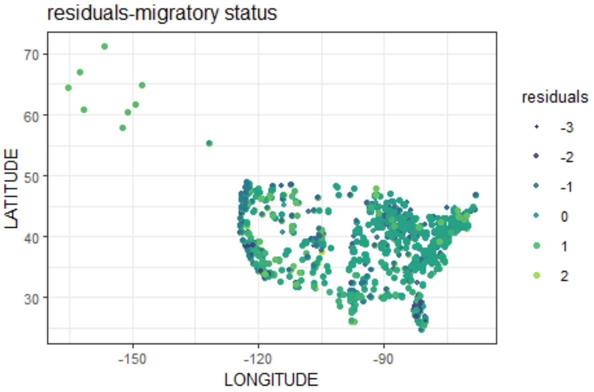

residuals-migratory status

Figure S2.10 Spatial variation in Passerine body mass (g), longevity (years), clutch size, and migratory status (1 = migratory, 0 = resident) across the United States (left), and spatial dependency in model residuals for each model (right). One model was fit to data per response variable (body mass, longevity, clutch size or migratory status), including all the urban characteristics: compactness/sprawl, median income, and human population size. Body mass was log10-transformed. Random effects were specified as (1 | family) and (1 | state), where each level of the grouping factors, family and state, had their own random intercept.

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Data sources

American FactFinder: https://factfinder.census.gov

An Amniote Life-History Database: https://doi.org/10.6084/m9.figshare.3563457.v1

eBird: https://ebird.org/data/download

Urban area shapefile: https://www.census.gov/cgi-bin/geo/shapefiles/index.php?year=2010&layergroup=Urban+Areas.

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Supplementary Information 3

Socioeconomic and structural variation across cities as a driver of migratory bird species richness

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Materials and Methods

Data compilation

We compiled our dataset by combining data of migratory bird observations and city features that were obtained from open source and citizen science sources, and by georeferencing this data by overlaying observations with U.S. Census Bureau's urban area shapefiles. We first downloaded the eBird Basic Dataset (EBD) from http://ebird.org/data/download (Sullivan et al. 2009) to access bird observation data from across the United States. eBird is a real-time, online, bird abundance and distribution checklist program jointly coordinated by the Cornell Laboratory of Ornithology and the National Audubon Society that relies on citizen science volunteer observers who submit georeferenced observations of species to a centralized database. Observations are submitted in a checklist format, listing species seen during one bird-watching occasion. Checklists are designated as either complete checklists, where all birds detected and identified were recorded, or incomplete checklists, where some species may have been seen but were not recorded. Regional reviewers verify each species observation based on sighting coordinates identifying outliers (Wood et al. 2011). The downloaded dataset was filtered to include birds across the United States from between the years 2010 to 2019.

We filtered the eBird dataset to a more manageable size by discarding data not needed for the analysis using custom Bash scripts. We applied further filtering protocols to refine data and standardize effort between checklists to account for variable survey effort between cities using R Statistical Software (version 3.6.3; R Foundation for Statistical Computing, Vienna, Austria). Only complete checklists were selected to account for species absences due to reporting (Sullivan et al. 2014). We selected checklists that followed traveling (observer traveled more than 30 meters away from the starting point of their checklist), traveling-property specific (observer stayed inside a single property boundary), stationary (the whole birding activity occurred at a single, fixed location), area (targeted, specialized surveys that thoroughly search a specific area for birds), or random (observations made at a randomly selected location over a period of at least five minutes) protocols, and checklists that covered a distance of 5 km or less with a survey time between 5 to 240 minutes (Johnston et al. 2021). As data filtering is a data-hungry process, and we were interested in investigating species richness in cities (not species abundances in certain areas that would require information about the presence or absence of species), we allowed for a slightly larger selection range for our protocol filters as this allows for the inclusion of more species in each city to our dataset.

We only selected migratory bird species (full migrants, partial migrants, altitudinal migrants, and nomads based on BirdLife International classification used by Sheard et al. (2020)) using Bash scripts, and discarded data for non-migrant species. We only chose observations of species during the breeding season, as this is an energetically demanding period reflecting reproductive success for migratory birds. We defined the breeding season as beginning from May 27th lasting until July 7th, following the practices of the North American Breeding Bird Survey (available from https://www.pwrc.usgs.gov/bbs/index.cfm). This definition likely removes some observations of breeding migrants, as breeding times may vary between and within species, but our estimations of survey completeness (see below) indicate that overall eBird surveys captured most species found in a city (see Fig S3.2).

We assigned georeferenced eBird species records to urban areas using U.S. Census-defined urban area maps (see Fig S3.1) (U.S. Census Bureau 2010b). These shapefiles define an urban area as a densely developed territory with a population size of at least 2,500 (U.S. Census Bureau 2010b). We conducted a spatial join of our two spatial datasets – eBird observations with points (individual x-y locations) and urban area shapefiles with polygons and associated attribute data using the R packages *sp* (version 1.3-1; R. S. Bivand et al. 2013), *rgdal* (version 1.3-4; R. Bivand et al. 2018), and *maps* (version 3.3.0; Becker & Wilks 2018). R package *sp* provides S4 classes and methods such as points, lines, polygons, and grids for importing, manipulating, and exporting spatial data in R. *Raster* is used to manipulate raster and vector data and *rgdal* to read or write spatial data files. As the first step of spatially joining our eBird and city data, we re-projected the eBird coordinate reference system (CRS) from WGS 84 to NAD83, so that both datasets had the same CRS. Then we conducted the spatial join using the over command from the *sp* library. The over command conducts spatial overlay by returning the attributes from spatial object y (urban area shapefile) at the spatial locations of object x (eBird latitude and longitude). After the spatial join, we had a georeferenced dataset of urban migrants.

Next, we obtained socioeconomic features for these urban areas from the 2010 census data and the 2011-2015 American Community Survey 5-year estimates data from the U.S. Census Bureau (U.S. Census Bureau 2010a). As the U.S. Census data did not contain coordinates for the cities, we got latitude and longitude for each city by merging census data with U.S. Census Gazetteer files (U.S. Census Bureau 2010c) by using city name and state as common elements. The Gazetteer files list all geographic areas for designated geographic area categories. Information such as geographic identifier codes, names, and latitude and longitude coordinates are included in the files. We merged our city feature dataset with the urban area shapefiles provided by the U.S. Census Bureau as before, using the R packages sp, *rgdal* and *maps*. After these steps, we had a georeferenced dataset of city characteristics. We then merged the georeferenced urban eBird data and city features data in R by using the name of the urban area and state as the common elements. Next, we counted the number of eBird checklists in each city using R and Excel's Pivot Table function. We did a further check of the data and discarded observations of transient birds likely still en route to their breeding grounds, observations that were part of the birds' overwintering range, and vagrants from each state. We then added the primary breeding biomes of each migratory species to our data that we obtained from a recently published manuscript (Rosenberg et al. 2019). Breeding biomes were divided into 11 distinct categories: wetlands; coasts; tundra; grasslands; aridlands; boreal forest; eastern forest; western forest; forest generalist; introduced: and habitat generalist (see Table 3.1 in the main paper for descriptions of each category). Introduced species were removed during data analysis as the group only contained observations from two species. We counted the total number of observations per city (used in survey completeness analyses) and the total number of species per breeding biome per city (that is used as our response variable in our analyses) using R.

Next, we calculated city age using the median age of a housing structure in years as our proxy. This required several steps as our raw data was the total number of houses in the year the structure was built that was divided into several groups per city (Two example columns: YEAR STRUCTURE BUILT!!Total housing units!!Built 2014 or later; YEAR STRUCTURE BUILT!!Total housing units!!Built 2010 to 2013). We first selected the housing age group that had the most houses (housing units) in each city and then calculated the median housing age (2021 minus the median year when buildings were built). To calculate commuting times in each city we used the aggregate travel time to work (in minutes) obtained from the 2011-2015 American Community Survey 5-year estimates data from the U.S. Census Bureau (U.S. Census Bureau 2010a). The American Community Survey provides current demographic, social, economic, and housing estimates throughout the decade (www.census.gov/acs). The survey randomly samples ~ 3.5 million addresses each year and produces statistics that cover 1-year and 5-year periods for geographic areas in the United States and Puerto Rico. The American Community Survey calculates the aggregate travel time to work by adding all commuting times (in minutes) for workers who did not work at home during a reference week. As commuting distances may be longer in larger cities, we standardized commuting time values by dividing travel time by city area (in square miles). We then added these two remaining city variables to our dataset.

Survey completeness

To estimate survey coverage across our eBird sampling locations we used the R package *KnowBR* (Lobo et al. 2018). KnowBR uses species accumulation curves and diversity estimators to assess the completeness of species inventories across different geographical extents and an unrestricted number of geographic cells of a size defined by the user. The final slope of the species accumulation curves and completeness percentages (i.e., the percentage of observed species with respect to predicted species) are used to categorize and map the level of survey effort for each geographic unit (Lobo et al. 2018). We used the number of database records from each city for all years as a surrogate of survey effort and assumed that this number correlates positively with the probability of recording a species within that city. The species accumulation curves were calculated using the exact estimator (Ugland et al. 2003) and the Rational function (Ratkowsky 1990) to adjust the data. We defined the geographical extent across which the final slopes and completeness values were calculated at a grid cell resolution of 26 minutes (i.e., resolution of the spatial units in minutes on which calculations were carried out) (see Fig S3.2).

Variable selection and controlling for spatial variation

Nine city features suspected to be correlated underwent Spearman rank correlation analysis (Fig S3.3). We chose four not strongly correlated city variables, the number of housing units, median income, city age, and travel time, as our predictor variables for our analyses.

Spatial eigenvector maps

We tested the residuals of our models for spatial autocorrelation using the R package *DHARMa* (Hartig 2020). Residuals for all the models were spatially structured (P-value <

0.05) which violates the assumption that residuals are independent and identically distributed. This can bias parameter estimates and can increase Type I Error rates (Dormann et al. 2007). One way to control for spatial variation is to use distance-based Moran's Eigenvector Maps (dbMEMs) (Dray et al. 2006). These capture spatial relationships among data points at all spatial scales and are orthogonal and so can be added to the model as explanatory variables to account for spatial variation (Dormann et al. 2007; Griffith & Peres-Neto 2006). We chose all models with significant Moran's I value (< 0.05) for dbMEM analysis. Response variables (e.g., migrant species richness for each breeding group) were detrended before dbMEM analysis (Borcard et al. 2004). We computed dbMEMs using the R package *adespatial* (Stéphane et al. 2020) by computing eigenvectors of a truncated geographic distance matrix among sites. These connectivity matrices were weighted by a weighting function decreasing linearly with the distance (Stéphane et al. 2020). The dbMEM eigenvalues are proportional to Moran's I coefficient of spatial correlation computed on the eigenfunctions using the sites that are still connected after truncation (Borcard et al. 2011; Dray et al. 2006). We selected only the smallest subset of spatial eigenvectors as spatial predictors that best minimized the spatial autocorrelation in model residuals using the MIR approach, or the minimization of Moran's I in the residuals (Griffith & Peres-Neto 2006) following recommendations of Bauman et al. (2018). The addition of the least amount of MEM variables to the model ensures the independence of the residuals and helps to avoid model overfitting or a loss of statistical power to detect the contribution of our predictor variables to the variability of the response data (Bauman et al. 2018). We performed a global significance test before eigenvector selection as recommended by Blanchet et al. (2008). We then reran our analyses as before, but with dbMEMs added as additional explanatory variables to our model.

Eastern forest migrants had 8 distance-based eigenvectors (dbMEMs) (see Table S3.2) with positive eigenvalues that were retained as spatial descriptors; wetland migrants had 3 dbMEMs; forest generalists 1 dbMEM; grassland migrants 3 dbMEMs; western forest migrants 2 dbMEMs; aridlands migrants 4 dbMEMs; tundra migrants 2 dbMEMs; habitat generalists 1 dbMEM; and coastal migrants 2 dbMEMs. The addition of eigenvectors as explanatory variables reduced spatial autocorrelation in our model residuals in all cases. Although for some models the autocorrelation was still significant, the observed Morans I values (compared to the expected) were all less than 0.1.

Table S3.1. The species names and the total number of migratory birds in each breeding biome found in cities across the United States during the breeding season.

Breeding Biome	**Common Name**	**Scientific Name**
Tundra	American Golden-Plover	*Pluvialis dominica*
	American Pipit	*Anthus rubescens*
	Arctic Tern	*Sterna paradisaea*
	Arctic Warbler	*Phylloscopus borealis*
	Baird's Sandpiper	*Calidris bairdii*
	Black-bellied Plover	*Pluvialis squatarola*
	Brant	*Branta bernicla*
	Cackling Goose	*Branta hutchinsii*
	Dunlin	*Calidris alpina*
	Golden-crowned Sparrow	*Zonotrichia atricapilla*
	Greater Scaup	*Aythya marila*
	Greater White-fronted Goose	*Anser albifrons*
	Harris's Sparrow	*Zonotrichia querula*
	King Eider	*Somateria spectabilis*
	Lapland Longspur	*Calcarius lapponicus*
	Long-billed Dowitcher	*Limnodromus scolopaceus*
	Long-tailed Duck	*Clangula hyemalis*
	Pacific Golden-Plover	*Pluvialis fulva*

	Pectoral Sandpiper	*Calidris melanotos*
	Purple Sandpiper	*Calidris maritima*
	Red Knot	*Calidris canutus*
	Red-breasted Merganser	*Mergus serrator*
	Red-throated Loon	*Gavia stellata*
	Rough-legged Hawk	*Buteo lagopus*
	Ruddy Turnstone	*Arenaria interpres*
	Sanderling	*Calidris alba*
	Semipalmated Plover	*Charadrius semipalmatus*
	Semipalmated Sandpiper	*Calidris pusilla*
	Snow Bunting	*Plectrophenax nivalis*
	Steller's Eider	*Polysticta stelleri*
	Stilt Sandpiper	*Calidris himantopus*
	Tundra Swan	*Cygnus columbianus*
	Western Sandpiper	*Calidris mauri*
	Whimbrel	*Numenius phaeopus*
	White-rumped Sandpiper	*Calidris fuscicollis*
	Yellow-billed Loon	*Gavia adamsii*
Total	**36**	
Aridlands	Allen's Hummingbird	*Selasphorus sasin*
	Anna's Hummingbird	*Calypte anna*
	Ash-throated Flycatcher	*Myiarchus cinerascens*
	Bell's Vireo	*Vireo bellii*
	Bewick's Wren	*Thryomanes bewickii*
	Black-throated Sparrow	*Amphispiza bilineata*
	Brewer's Sparrow	*Spizella breweri*
	Bronzed Cowbird	*Molothrus aeneus*

	Brown-crested Flycatcher	*Myiarchus tyrannulus*
	Cave Swallow	*Petrochelidon fulva*
	Chihuahuan Raven	*Corvus cryptoleucus*
	Common Ground Dove	*Columbina passerina*
	Common Poorwill	*Phalaenoptilus nuttallii*
	Costa's Hummingbird	*Calypte costae*
	Couch's Kingbird	*Tyrannus couchii*
	Green-tailed Towhee	*Pipilo chlorurus*
	Hooded Oriole	*Icterus cucullatus*
	Lesser Nighthawk	*Chordeiles acutipennis*
	Phainopepla	*Phainopepla nitens*
	Prairie Falcon	*Falco mexicanus*
	Pyrrhuloxia	*Cardinalis sinuatus*
	Rock Wren	*Salpinctes obsoletus*
	Sage Thrasher	*Oreoscoptes montanus*
	Varied Bunting	*Passerina versicolor*
	Vermilion Flycatcher	*Pyrocephalus rubinus*
	White-throated Swift	*Aeronautes saxatalis*
	White-winged Dove	*Zenaida asiatica*
Total	**27**	
Boreal Forest	Alder Flycatcher	*Empidonax alnorum*
	Blue-headed Vireo	*Vireo solitarius*
	Bohemian Waxwing	*Bombycilla garrulus*
	Connecticut Warbler	*Oporornis agilis*
	Gray-cheeked Thrush	*Catharus minimus*
	Lincoln's Sparrow	*Melospiza lincolnii*
	Philadelphia Vireo	*Vireo philadelphicus*

	Pine Grosbeak	*Pinicola enucleator*
	Ruby-crowned Kinglet	*Regulus calendula*
	Rusty Blackbird	*Euphagus carolinus*
	White-throated Sparrow	*Zonotrichia albicollis*
	Yellow-bellied Flycatcher	*Empidonax flaviventris*
Total	**12**	
Coasts	American Oystercatcher	*Haematopus palliatus*
	Black Guillemot	*Cepphus grylle*
	Black Skimmer	*Rynchops niger*
	Black Turnstone	*Arenaria melanocephala*
	Black-legged Kittiwake	*Rissa tridactyla*
	Brandt's Cormorant	*Phalacrocorax penicillatus*
	Brown Pelican	*Pelecanus occidentalis*
	Common Eider	*Somateria mollissima*
	Common Murre	*Uria aalge*
	Glaucous Gull	*Larus hyperboreus*
	Glaucous-winged Gull	*Larus glaucescens*
	Northern Gannet	*Morus bassanus*
	Pelagic Cormorant	*Phalacrocorax pelagicus*
	Pigeon Guillemot	*Cepphus columba*
	Reddish Egret	*Egretta rufescens*
	Thick-billed Murre	*Uria lomvia*
	Western Gull	*Larus occidentalis*
	Wilson's Plover	*Charadrius wilsonia*
	Yellow-crowned Night-Heron	*Nyctanassa violacea*
Total	**19**	

Eastern Forest	Acadian Flycatcher	*Empidonax virescens*
	American Redstart	*Setophaga ruticilla*
	American Woodcock	*Scolopax minor*
	Baltimore Oriole	*Icterus galbula*
	Black-and-white Warbler	*Mniotilta varia*
	Black-billed Cuckoo	*Coccyzus erythropthalmus*
	Black-whiskered Vireo	*Vireo altiloquus*
	Blue Jay	*Cyanocitta cristata*
	Brown Thrasher	*Toxostoma rufum*
	Chimney Swift	*Chaetura pelagica*
	Eastern Bluebird	*Sialia sialis*
	Eastern Phoebe	*Sayornis phoebe*
	Eastern Towhee	*Pipilo erythrophthalmus*
	Eastern Wood-Pewee	*Contopus virens*
	Field Sparrow	*Spizella pusilla*
	Golden-winged Warbler	*Vermivora chrysoptera*
	Gray Catbird	*Dumetella carolinensis*
	Gray Kingbird	*Tyrannus dominicensis*
	Great Crested Flycatcher	*Myiarchus crinitus*
	Indigo Bunting	*Passerina cyanea*
	Least Flycatcher	*Empidonax minimus*
	Mississippi Kite	*Ictinia mississippiensis*
	Orchard Oriole	*Icterus spurius*
	Ovenbird	*Seiurus aurocapilla*
	Painted Bunting	*Passerina ciris*
	Prothonotary Warbler	*Protonotaria citrea*
	Red-headed Woodpecker	*Melanerpes erythrocephalus*
	Rose-breasted Grosbeak	*Pheucticus ludovicianus*

	Ruby-throated Hummingbird	*Archilochus colubris*
	Scarlet Tanager	*Piranga olivacea*
	Summer Tanager	*Piranga rubra*
	Swainson's Warbler	*Limnothlypis swainsonii*
	Swallow-tailed Kite	*Elanoides forficatus*
	White-eyed Vireo	*Vireo griseus*
	Willow Flycatcher	*Empidonax traillii*
	Wood Thrush	*Hylocichla mustelina*
	Worm-eating Warbler	*Helmitheros vermivorum*
	Yellow-bellied Sapsucker	*Sphyrapicus varius*
	Yellow-billed Cuckoo	*Coccyzus americanus*
	Yellow-breasted Chat	*Icteria virens*
	Yellow-throated Vireo	*Vireo flavifrons*
Total	**41**	
Forest Generalist	American Robin	*Turdus migratorius*
	Blue Grosbeak	*Passerina caerulea*
	Broad-winged Hawk	*Buteo platypterus*
	Brown Creeper	*Certhia americana*
	Cedar Waxwing	*Bombycilla cedrorum*
	Chipping Sparrow	*Spizella passerina*
	Cooper's Hawk	*Accipiter cooperii*
	Dark-eyed Junco	*Junco hyemalis*
	Fox Sparrow	*Passerella iliaca*
	Golden-crowned Kinglet	*Regulus satrapa*
	Hermit Thrush	*Catharus guttatus*
	House Wren	*Troglodytes aedon*
	Long-eared Owl	*Asio otus*

	Northern Flicker	*Colaptes auratus*
	Northern Goshawk	*Accipiter gentilis*
	Olive-sided Flycatcher	*Contopus cooperi*
	Red-breasted Nuthatch	*Sitta canadensis*
	Red-eyed Vireo	*Vireo olivaceus*
	Red-shouldered Hawk	*Buteo lineatus*
	Sharp-shinned Hawk	*Accipiter striatus*
	Swainson's Thrush	*Catharus ustulatus*
	Veery	*Catharus fuscescens*
	Warbling Vireo	*Vireo gilvus*
Total	**23**	
Grassland	Bobolink	*Dolichonyx oryzivorus*
	Burrowing Owl	*Athene cunicularia*
	Clay-colored Sparrow	*Spizella pallida*
	Dickcissel	*Spiza americana*
	Eastern Kingbird	*Tyrannus tyrannus*
	Eastern Meadowlark	*Sturnella magna*
	Ferruginous Hawk	*Buteo regalis*
	Grasshopper Sparrow	*Ammodramus savannarum*
	Horned Lark	*Eremophila alpestris*
	Lark Bunting	*Calamospiza melanocorys*
	Lark Sparrow	*Chondestes grammacus*
	Loggerhead Shrike	*Lanius ludovicianus*
	Long-billed Curlew	*Numenius americanus*
	Savannah Sparrow	*Passerculus sandwichensis*
	Scissor-tailed Flycatcher	*Tyrannus forficatus*
	Sedge Wren	*Cistothorus platensis*

	Swainson's Hawk	*Buteo swainsoni*
	Upland Sandpiper	*Bartramia longicauda*
	Vesper Sparrow	*Pooecetes gramineus*
	Western Kingbird	*Tyrannus verticalis*
	Western Meadowlark	*Sturnella neglecta*
Total	**21**	
Habitat Generalist	American Crow	*Corvus brachyrhynchos*
	American Kestrel	*Falco sparverius*
	Bank Swallow	*Riparia riparia*
	Barn Swallow	*Hirundo rustica*
	Black Vulture	*Coragyps atratus*
	Blue-gray Gnatcatcher	*Polioptila caerulea*
	Brewer's Blackbird	*Euphagus cyanocephalus*
	Brown-headed Cowbird	*Molothrus ater*
	Cliff Swallow	*Petrochelidon pyrrhonota*
	Common Grackle	*Quiscalus quiscula*
	Common Nighthawk	*Chordeiles minor*
	Common Yellowthroat	*Geothlypis trichas*
	Fish Crow	*Corvus ossifragus*
	Golden Eagle	*Aquila chrysaetos*
	Great Horned Owl	*Bubo virginianus*
	Groove-billed Ani	*Crotophaga sulcirostris*
	Killdeer	*Charadrius vociferus*
	Merlin	*Falco columbarius*
	Mourning Dove	*Zenaida macroura*

	Northern Rough-winged Swallow	*Stelgidopteryx serripennis*
	Peregrine Falcon	*Falco peregrinus*
	Purple Martin	*Progne subis*
	Red-tailed Hawk	*Buteo jamaicensis*
	Say's Phoebe	*Sayornis saya*
	Short-eared Owl	*Asio flammeus*
	Song Sparrow	*Melospiza melodia*
	Tree Swallow	*Tachycineta bicolor*
	Turkey Vulture	*Cathartes aura*
	White-crowned Sparrow	*Zonotrichia leucophrys*
	White-tailed Kite	*Elanus leucurus*
Total	**30**	
Western Forest	Band-tailed Pigeon	*Patagioenas fasciata*
	Black Swift	*Cypseloides niger*
	Black-chinned Hummingbird	*Archilochus alexandri*
	Black-headed Grosbeak	*Pheucticus melanocephalus*
	Bridled Titmouse	*Baeolophus wollweberi*
	Broad-tailed Hummingbird	*Selasphorus platycercus*
	Bullock's Oriole	*Icterus bullockii*
	Cassin's Kingbird	*Tyrannus vociferans*
	Cassin's Vireo	*Vireo cassinii*
	Cordilleran Flycatcher	*Empidonax occidentalis*
	Dusky Flycatcher	*Empidonax oberholseri*
	Dusky Grouse	*Dendragapus obscurus*
	Gray Flycatcher	*Empidonax wrightii*
	Gray Vireo	*Vireo vicinior*

	Hammond's Flycatcher	*Empidonax hammondii*
	Hepatic Tanager	*Piranga flava*
	Lazuli Bunting	*Passerina amoena*
	Lewis's Woodpecker	*Melanerpes lewis*
	Mountain Bluebird	*Sialia currucoides*
	Mountain Quail	*Oreortyx pictus*
	Northern Pygmy-Owl	*Glaucidium gnoma*
	Pacific-slope Flycatcher	*Empidonax difficilis*
	Plumbeous Vireo	*Vireo plumbeus*
	Red-breasted Sapsucker	*Sphyrapicus ruber*
	Red-faced Warbler	*Cardellina rubrifrons*
	Red-naped Sapsucker	*Sphyrapicus nuchalis*
	Rufous Hummingbird	*Selasphorus rufus*
	Scott's Oriole	*Icterus parisorum*
	Spotted Towhee	*Pipilo maculatus*
	Townsend's Solitaire	*Myadestes townsendi*
	Vaux's Swift	*Chaetura vauxi*
	Violet-green Swallow	*Tachycineta thalassina*
	Western Bluebird	*Sialia mexicana*
	Western Screech-Owl	*Megascops kennicottii*
	Western Tanager	*Piranga ludoviciana*
	Western Wood-Pewee	*Contopus sordidulus*
	Williamson's Sapsucker	*Sphyrapicus thyroideus*
Total	**37**	
Wetland	American Avocet	*Recurvirostra americana*
	American Bittern	*Botaurus lentiginosus*
	American Black Duck	*Anas rubripes*

American Coot	*Fulica americana*
American White Pelican	*Pelecanus erythrorhynchos*
Bald Eagle	*Haliaeetus leucocephalus*
Barrow's Goldeneye	*Bucephala islandica*
Belted Kingfisher	*Megaceryle alcyon*
Black Phoebe	*Sayornis nigricans*
Black Tern	*Chlidonias niger*
Black-crowned Night-Heron	*Nycticorax nycticorax*
Bufflehead	*Bucephala albeola*
California Gull	*Larus californicus*
Canada Goose	*Branta canadensis*
Canvasback	*Aythya valisineria*
Cattle Egret	*Bubulcus ibis*
Clark's Grebe	*Aechmophorus clarkii*
Common Goldeneye	*Bucephala clangula*
Common Loon	*Gavia immer*
Common Merganser	*Mergus merganser*
Common Tern	*Sterna hirundo*
Double-crested Cormorant	*Phalacrocorax auritus*
Eared Grebe	*Podiceps nigricollis*
Forster's Tern	*Sterna forsteri*
Fulvous Whistling-Duck	*Dendrocygna bicolor*
Glossy Ibis	*Plegadis falcinellus*
Great Black-backed Gull	*Larus marinus*
Great Blue Heron	*Ardea herodias*
Greater Yellowlegs	*Tringa melanoleuca*
Green-winged Teal	*Anas crecca*
Harlequin Duck	*Histrionicus histrionicus*

Herring Gull	*Larus argentatus*
Hooded Merganser	*Lophodytes cucullatus*
Horned Grebe	*Podiceps auritus*
Hudsonian Godwit	*Limosa haemastica*
King Rail	*Rallus elegans*
Least Bittern	*Ixobrychus exilis*
Least Sandpiper	*Calidris minutilla*
Lesser Scaup	*Aythya affinis*
Lesser Yellowlegs	*Tringa flavipes*
Limpkin	*Aramus guarauna*
Little Blue Heron	*Egretta caerulea*
Mallard	*Anas platyrhynchos*
Marbled Godwit	*Limosa fedoa*
Marsh Wren	*Cistothorus palustris*
Mew Gull	*Larus canus*
Mottled Duck	*Anas fulvigula*
Northern Pintail	*Anas acuta*
Osprey	*Pandion haliaetus*
Pied-billed Grebe	*Podilymbus podiceps*
Redhead	*Aythya americana*
Red-necked Grebe	*Podiceps grisegena*
Ring-billed Gull	*Larus delawarensis*
Ring-necked Duck	*Aythya collaris*
Roseate Spoonbill	*Platalea ajaja*
Ruddy Duck	*Oxyura jamaicensis*
Short-billed Dowitcher	*Limnodromus griseus*
Snowy Egret	*Egretta thula*
Solitary Sandpiper	*Tringa solitaria*

	Sora	*Porzana carolina*
	Spotted Sandpiper	*Actitis macularius*
	Surf Scoter	*Melanitta perspicillata*
	Swamp Sparrow	*Melospiza georgiana*
	Tricolored Heron	*Egretta tricolor*
	Trumpeter Swan	*Cygnus buccinator*
	Virginia Rail	*Rallus limicola*
	Western Grebe	*Aechmophorus occidentalis*
	White-faced Ibis	*Plegadis chihi*
	Wood Duck	*Aix sponsa*
	Wood Stork	*Mycteria americana*
	Yellow-headed Blackbird	*Xanthocephalus xanthocephalus*
Total	**71**	

Table S3.2. Generalized linear model estimates, standard errors (SE), z-values, and 95% confidence intervals for the distance-based Moran's Eigenvector Maps (MEMs) and Latitude and Longitude for different migratory bird breeding biome groups that were added to the model as covariates to account for spatial autocorrelation and spatial variation in species richness.

Response	Predictors	Estimates	SE	z value	95% CI
Eastern forest	MEM10	0.10	0.02	4.73	0.06, 0.14
	MEM23	0.11	0.02	5.65	0.07, 0.15
	MEM1	0.06	0.02	3.54	0.03, 0.10

	MEM15	-0.06	0.02	-3.37	-0.09, -0.02
	MEM24	0.06	0.02	3.69	0.03, 0.10
	MEM22	0.11	0.02	4.63	0.07, 0.16
	MEM31	-0.07	0.02	-3.34	-0.12, -0.03
	MEM13	-0.03	0.02	-1.64	-0.06, 0.01
	Longitude	0.59	0.03	22.83	0.54, 0.64
	Latitude	0.11	0.03	4.24	0.06, 0.16
Forest generalist	Longitude	0.08	0.02	5.18	0.05, 0.11
	Latitude	0.29	0.02	18.44	0.26, 0.32
	MEM2	-0.08	0.01	-5.64	-0.11, -0.05
Western forest	Longitude	-0.58	0.08	-6.91	-0.75, -0.41
	Latitude	-0.54	0.14	-3.74	-0.84, -0.24
	MEM2	-0.24	0.08	-2.86	-0.42, -0.07
	MEM3	0.64	0.09	6.91	0.45, 0.83
Boreal forest	Longitude	0.23	0.07	3.40	0.10, 0.36
	Latitude	0.37	0.06	6.13	0.25, 0.49
Habitat generalist	MEM2	-0.06	0.01	-4.37	-0.09, -0.04
	Longitude	0.06	0.01	3.99	0.03, 0.08
	Latitude	0.09	0.02	6.01	0.06, 0.12

Tundra	Longitude	-0.04	0.12	-0.30	-0.26, 0.18
	Latitude	0.31	0.08	3.92	0.17, 0.46
	MEM3	0.50	0.09	5.53	0.33, 0.67
	MEM1	0.01	0.11	0.05	-0.21, 0.22
Aridlands	MEM2	0.06	0.04	1.50	-0.02, 0.13
	Longitude	-0.92	0.15	-6.12	-1.21, -0.62
	Latitude	-0.77	0.07	-10.87	-0.91, -0.63
	MEM3	0.05	0.03	-3.10	-0.00, 0.11
	MEM1	-0.15	0.05	-1.60	-0.25, -0.06
	MEM4	-0.19	0.12	1.61	-0.42, 0.04
Coasts	MEM2	0.45	0.08	5.71	0.30, 0.61
	Longitude	-0.45	0.07	-6.20	-0.59, -0.31
	Latitude	-0.22	0.07	-3.14	-0.35, -0.08
	MEM5	0.32	0.06	4.97	0.20, 0.45
Grassland	MEM2	-0.25	0.02	-10.06	-0.30, -0.20
	Longitude	0.35	0.11	3.25	0.15, 0.56
	Latitude	0.25	0.03	9.39	0.20, 0.30
	MEM1	0.35	0.10	3.41	0.16, 0.56
	MEM4	-0.12	0.03	-4.83	-0.17, -0.07

Wetland	MEM2	0.12	0.02	4.65	0.07, 0.16
	Longitude	0.14	0.06	2.21	0.01, 0.26
	Latitude	0.12	0.03	4.55	0.07, 0.16
	MEM1	0.20	0.06	3.36	0.08, 0.32
	MEM9	0.12	0.02	5.12	0.07, 0.17

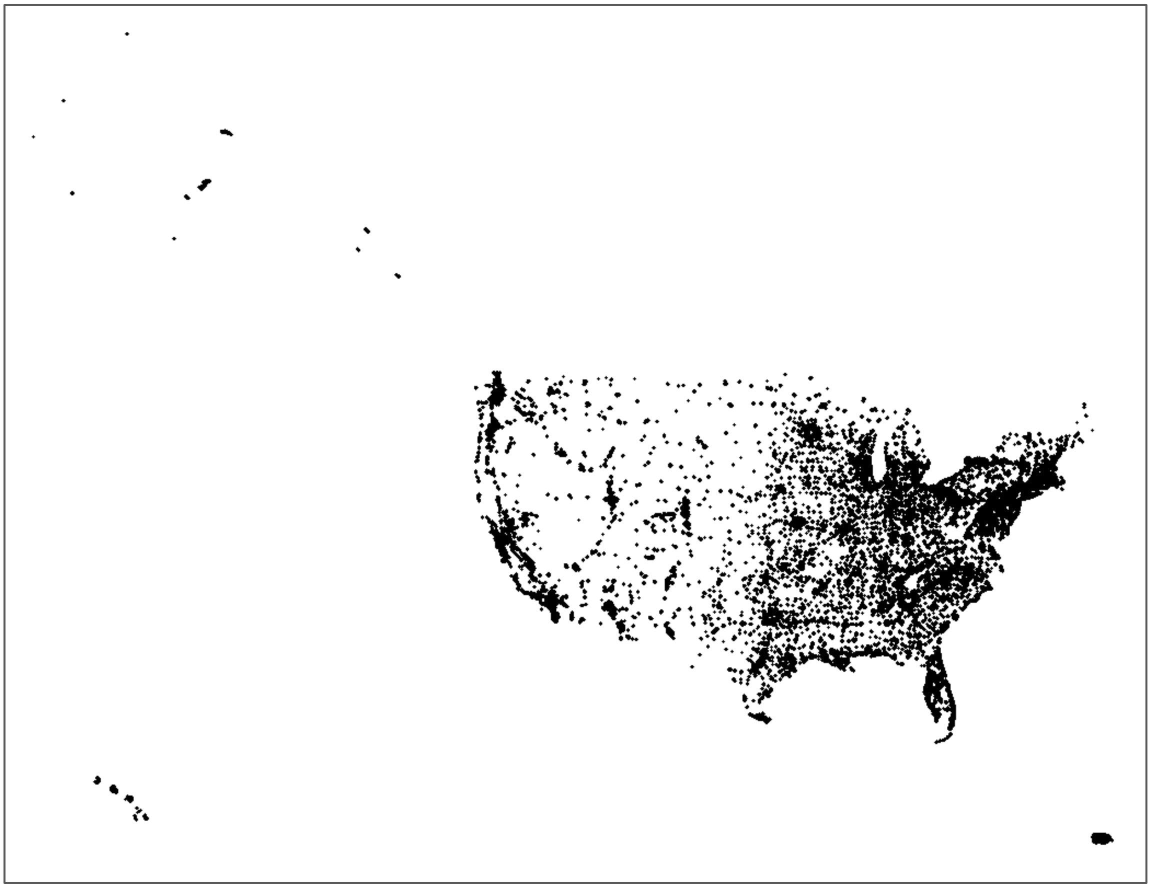
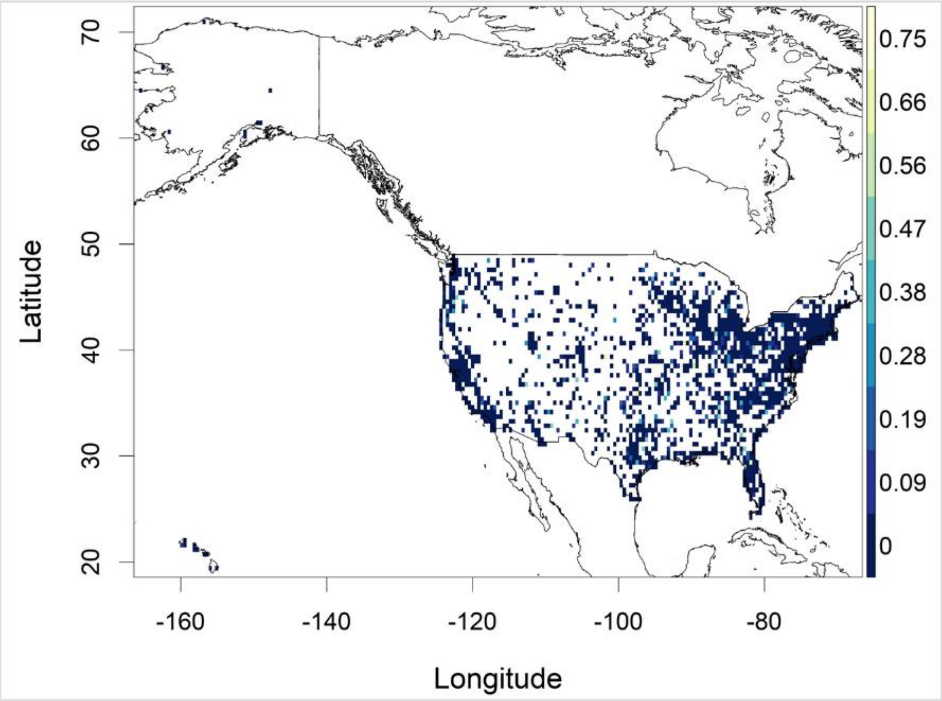


Figure S3.1. Map showing urban areas across the United States that were used to georeference eBird records to urban areas. These U.S. Census Bureau's urban area shapefiles define an urban area as a densely developed territory with a population size of at least 2,500.


70
60
50
40
30
20
Latitude
-160
-140
-120
-100
-80
Longitude
0.75
0.66
0.56
0.47
0.38
0.28
0.19
0.09
0

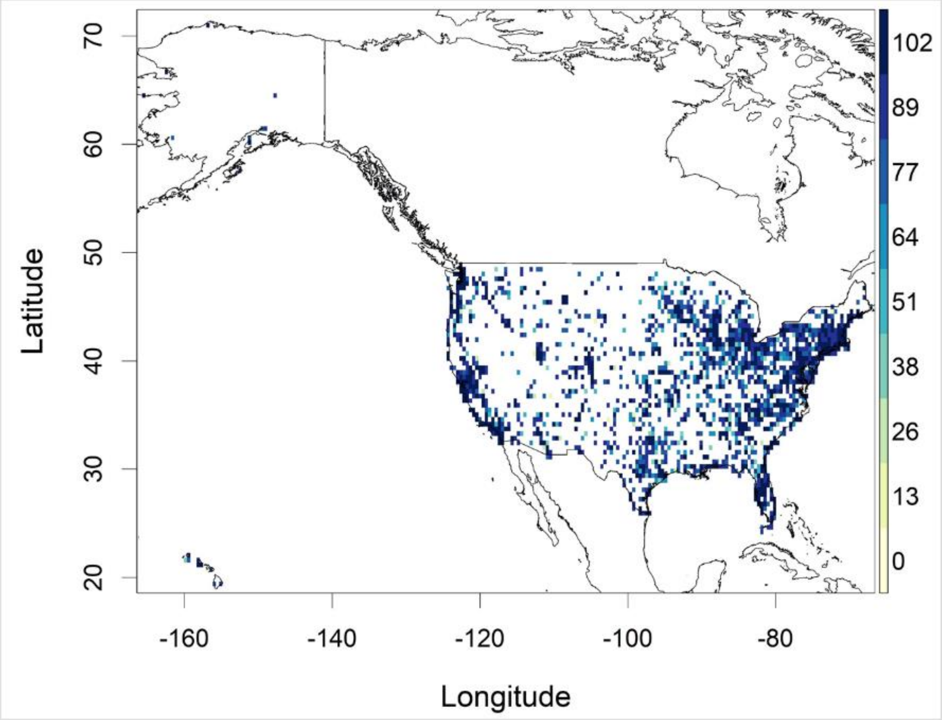
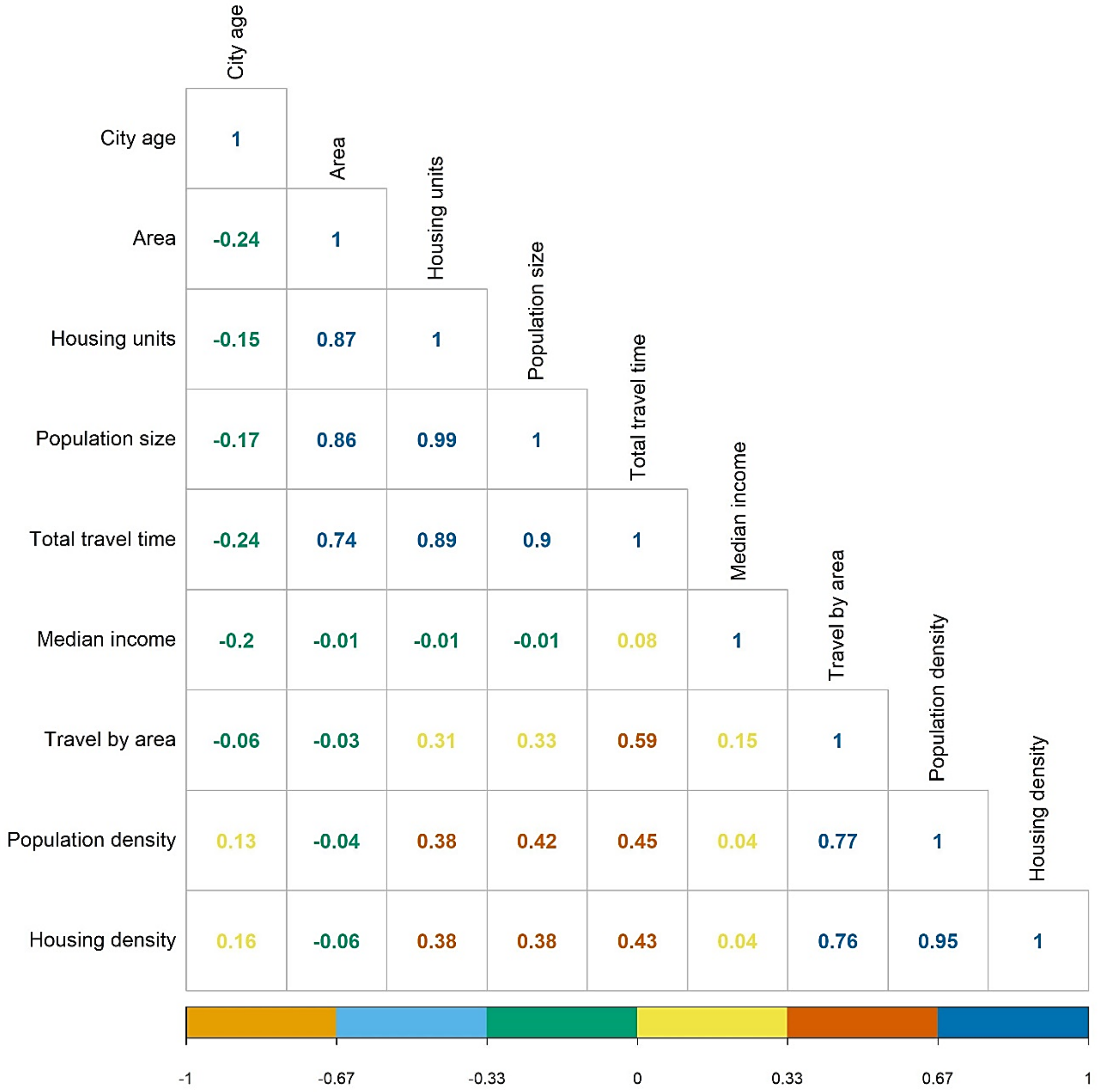

70
60
50
40
30
20
Latitude
-160
-140
-120
-100
-80
Longitude
102
89
77
64
51
38
26
13
0

Figure S3.2. Map showing the final slope of the species accumulation curves—the accumulated increase in the number of species with the addition of database records (above), and the completeness percentages (below) of eBird survey effort across the United States for each geographic unit.



	City age	Area	Housing units	Population size	Total travel time	Median income	Travel by area	Population density	Housing density
City age	1								
Area	-0.24	1							
Housing units	-0.15	0.87	1						
Population size	-0.17	0.86	0.99	1					
Total travel time	-0.24	0.74	0.89	0.9	1				
Median income	-0.2	-0.01	-0.01	-0.01	0.08	1			
Travel by area	-0.06	-0.03	0.31	0.33	0.59	0.15	1		
Population density	0.13	-0.04	0.38	0.42	0.45	0.04	0.77	1	
Housing density	0.16	-0.06	0.38	0.38	0.43	0.04	0.76	0.95	1

Figure S3.3. Spearman correlation coefficients between city features obtained from the U.S. Census Bureau that were considered as possible predictor variables for our analyses.

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Supplementary Information 5

No evidence of *Toxoplasma gondii* infection in urban and rural squirrels

Contents:

Table S5.1 Primers and probe sequences used in the PCR to detect *Toxoplasma gondii* and cellular r18S DNA.

	Target	Name	Sequence 5_→ 3_	5_ Modification	3_ Modification
Primers	*T. gondii*	T2	CGGAGAGGGAGAAGATGTT		
	T. gondii	T3	GCCATCACCACGAGGAAA		
	Cell r18S	F	GATTAAGTCCCTGCCCTTT		
	Cell r18S	R	GATAGTCAAGTTCGACCGTCTT		
Probes	*T. gondii*		CTTGGCTGCTTTTCCTGGAGGG	FAM	BHQ1
	Cell r18S		CACACCGCCCGTCGCTACTACC	Cy5	BHQ2

Table S5.2 Results of the enzyme-linked immunosorbent assay (ELISA) test carried out on the 15 serum samples from grey squirrels (*Sciurus carolinensis*) to detect IgG serum antibodies to *Toxoplasma gondii.* Sample to Positive Ratio (S/P) percentage (S/P%) was calculated for each sample. Samples with S/P% less or equal to 40% were considered negative; samples with S/P% between 40 and 50% doubtful or inconclusive; and samples with an S/P% higher than 50% positive.

Number	ID	S/P %	Result
Sq01	268-19	0.7	N
Sq02	327-19	-0.1	N
Sq03	326-19	-1	N
Sq04	329-19	-0.6	N
Sq05	342-19	0.4	N
Sq06*	343-19	0.5	N
Sq07	344-19	-1	N
Sq08	347-19A	-0.4	N
Sq09	347-19B	-0.6	N
Sq10	351-19	-0.6	N
Sq11	355-19	-1.1	N
Sq12	359-19	-1.1	N
Sq13	389-19	0.8	N
Sq14*	391-19	-0.7	N
Sq15	392-19	-0.5	N

* Not enough sample