From Alleys to Alleles: Diet and Genetic Makeup of Washington Coyotes

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Abstract

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Abstract

Our increasingly urbanized world offers species substantial benefits and drawbacks. Those species able to establish populations in urban areas will find ample food and water resources, but also higher conspecific densities, increased interactions with humans, and higher risk of vehicular mortality. Cities are a new phenomenon in evolutionary time, and those species able to cope with the novelty often display specific qualities like dietary generalism and rapid reproduction rates. However, our understanding of how urban areas fundamentally change the eco-evolutionary dynamics of these species is limited. Here I present conceptual details and empirical data collected with non-invasive methods that demonstrate how these eco-evolutionary dynamics are altered for an adaptable mesocarnivore, the coyote (*Canis latrans*). The coyote can serve as a model species for understanding eco-evolutionary dynamics across North America as they persist in nearly every ecosystem type and every major metropolitan region. In this dissertation, I begin with a conceptual piece elucidating how urbanization and increased interactions with people may fundamentally alter the evolutionary constraints on wildlife coloration via 11 genetic and non-genetic mechanisms. Chapter 2 builds upon the literature on urban gene flow barriers. This chapter highlights how linear barriers can lead to fine-scale genetic structuring. In Chapter 3, I build upon this city-wide

gene flow analysis and model drivers of genetic connectivity from Northeastern Washington to the Kitsap Peninsula, demonstrating different gene flow drivers for different ecosystem types. Geneflow appeared largely driven by impervious surface, location on an island versus the mainland, and water. Geographic distance was a much stronger predictor of genetic distance in urban areas, indicating that dispersal distances are limited and coyotes are likely displaying natal-biased dispersal patterns. In addition, comparison of our urban and wildland areas to three physical islands suggests that gene flow in our urban areas is more similar to that of islands with a bridge to the mainland than that of wildland areas. For Chapter 4, I deploy state-of-the-art metabarcoding methodology to understand coyote diet with the city of Seattle. I predict that coyote diet should mirror human diet where they are eating large amounts of anthropogenic resources and that access to natural prey depends on access to green space. Since green space is largely distributed in Seattle by wealth and access to healthy foods is inequitably distributed, I predicted that coyote diet would largely be driven by human social variables. I found that drivers of different dietary categories varied and that both social and environmental variables were important for understanding urban coyote diets. Lastly, in Chapter 5, I compare diet diversity and niche partitioning among wildland, island, and urban coyotes. I predicted that urban areas would have the highest degrees of individual specialization with an increased diversity of resources to specialize in. Instead, I found that wildland areas have the highest degrees of specialization, likely indicating that urban areas see decreased inter and intraspecific competition as a result of anthropogenic food supplementation and increased resource availability despite increased conspecific densities. Taken together, this body of research demonstrates the eco-evolutionary dynamics that are changed as a result of urbanization, using the coyote as a model species.

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1. Chapter 1 – Introduction

1.1 Background

While many species have struggled to persist during the Anthropocene, with historic declines and extinctions worldwide (Pimm et al. 2014), the coyote (*Canis latrans*) shines as species that has truly thrived (Hody & Kays 2018). Coyotes have nearly doubled their historic range and have successfully established populations in nearly every ecosystem type across North America ranging from deserts to boreal forests, and even metropolitan areas (Hody & Kays 2018). In cities, coyotes face a variety of benefits (e.g., food resources) and challenges (e.g., road mortality, human-wildlife conflict) presented by being in close proximity to high densities of people (Poessel et al. 2012, Murray et al. 2015). And while we know that coyote diet differs between urban and non-urban areas (Murray et al. 2015, Larson et al. 2020, Sugden et al. 2020, Sugden et al. 2021) and that urban areas present many potential genetic barriers (Riley et al. 2006, Bird et al. 2023, Kreling et al. 2024), we have little idea of how the basic eco-evolutionary dynamics are altered for coyotes in urban regions and how this contributes to their success in these areas.

Coyotes have likely been present in Seattle, Washington since the 1920s. After the extirpation of gray wolves (*Canis lupus*) and favorable habitat modification, coyotes were able to establish populations in the growing city (Hody.& Kays 2018). While some have welcomed their appearance, others express fear for their pets and children (Quinn 1995). Much of human-coyote conflict stems from their diet and thus understanding their dietary preferences and patterns is important for mitigating potential conflicts (Murray et al. 2015). Prior work on coyote diet in this area determined that Seattle coyotes had substantially different diets from their non-urban counterparts with urban coyotes predominantly eating house cats (*Felis catus*) and squirrels (*Scirus sp., Tamiascurius sp.*) while less urban coyotes predominantly consumed voles, but these results were from morphological identification and are subject to heavy bias (Quinn et al. 1997, Loureiro de Sousa et al. 2019, Massey et al. 2021). The adaptability of coyotes may in part be due to their generalist diet (Sorace & Gustin 2009, Xavier Palacio 2019). However, some work has suggested that while at the population-level coyotes have a generalist diet,

individuals may specialize in specific resources to minimize intraspecific competition (Sacks et al. 2008, Larson et al. 2020, Sugden et al. 2021). Individual specialization often leads to evolutionary differences over time (Bolnick et al. 2003) and may have implications for human-wildlife conflict (Swan et al. 2017). For instance, depredation of domestic animals may be carried out by just a few individuals specializing in domestic animals rather than occurring at the population level (Swan et al. 2017).

While diet may be a significant driver of coyote's adaptability, the plasticity in their diet may be underlied by genetic means. Thus, gene flow may have important underlying implications for urban coyotes. Seattle is also an ideal location for testing island biogeography principles for urban wildlife with physical oceanic islands just offshore (MacArthur & Wilson 1967). In terms of genetics, islands tend to see little immigration or emigration, have reduced genetic diversity, and increased rates of inbreeding. Like physical islands, urban locations are often surrounded by large bodies of water (Fujita & Mori 1996, Dunn et al. 2022) and have myriad potential anthropogenic barriers such as highways that could inhibit movement in or out of the city (Miles et al. 2019, Dunn et al. 2022). No work to our knowledge has previously been conducted on terrestrial wildlife gene flow in this region as a whole.

My dissertation aims to untangle the effects of urbanization on eco-evolutionary dynamics in relation to genetics and diet. In chapter 2, I begin with a conceptual piece disentangling how urbanization may affect animal coloration. In chapters 3 and 4, I identify barriers to gene flow in the city of Seattle and then expand geographically to understand facilitators and inhibitors of gene flow across the state of Washington and to understand if urban areas are acting as genetic islands. In chapters 5 and 6, I aim to elucidate coyote dietary trends across Seattle to understand drivers of food consumption and compare urban coyote diet and individual specialization to offshore islands and wildland areas.

1.2 Goals

In chapter 2, I present a conceptual paper on how urbanization may influence the coloration of wildlife via reduction in evolutionary constraints. The goal of this paper is to elucidate a variety of mechanisms in which coloration could be affected by urbanization and serve as a jumping off point for future research. In

chapters 3 and 4, I explore how the built and natural environment shape gene flow for coyotes first in the city of Seattle, then at a larger scale, across much of Washington state. While coyotes are in no need of conservation action, understanding the effects of anthropogenic infrastructure on gene flow for common and mobile animals can help us understand the potential barriers and facilitators of movement for other less common species. These chapters also lays the basis for understanding any potential selective evolution happening, by addressing the neutral evolutionary forces of gene flow and genetic drift. Additionally, the 4th chapter asks if island biogeography can effectively explain gene flow in urban areas given similar conceptual trends between urban and island landscapes. In chapters 5 and 6, I examine how urbanization and anthropogenic influences shape diet and resource partitioning for coyotes. First, I examine this within the city of Seattle, asking if coyote diet reflects the diet of humans within their home ranges and whether environmental or human social variables better explain diet items detected in scat. Next, I compare resource partitioning among urban, island, and wildland coyotes to understand how individuals specialization may be influenced by land cover type, diversity of prey options, and what this implies for inter- and intra-specific competition in each of these areas.

1.3 Implications

This dissertation aims to produce results that further our understanding of eco-evolutionary dynamics and ecological theory in relation to urbanization. By the end of this dissertation, we will have addressed how urbanization changes diet diversity, resource partitioning, and gene flow across three unique Washington landscapes. Dietary results can additionally be used by management officials to predict potential hotspots of conflict based on dietary makeup of individuals (e.g., domestic animal depredation). Gene flow and barrier analyses can be used to facilitate genetic connectivity for wildlife and help understand potential source-sink dynamics as well as provide the basis for future selective evolution research.

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2. Chapter 2 – So overt it's covert: wildlife coloration in the city

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2.1 Abstract

With novel human-wildlife interaction, predation regimes, and environmental conditions, in addition to often fragmented and smaller populations, urban areas present wildlife with altered natural selection parameters and genetic drift potential compared to non-urban regions. Plumage and pelage coloration in birds and mammals has evolved as a balance between avoiding detection by predator or prey, sexual selection, and thermoregulation. However, with altered mutation rates, reduced predation risk, increased temperatures, strong genetic drift, and increased interaction with people, the evolutionary contexts in which these colorations arose are radically different than what is present in urban areas. Regionally alternative color morphs, leucistic or melanistic individuals that aren't typical of most avian or mammalian populations, may become more frequent as a result of adaptive and/or neutral evolution. Thus, I conceptualize that in urban areas conspicuous color morphologies may persist, leading to an increase in frequency of regionally non-typical pelage coloration. Here I discuss the potential for conspicuous color morphs to arise and persist in urban mammalian and avian populations, as well as the mechanisms for such persistence, as a result of altered environmental conditions and natural selection pressures.

Key Words: coloration, evolution, urban ecology

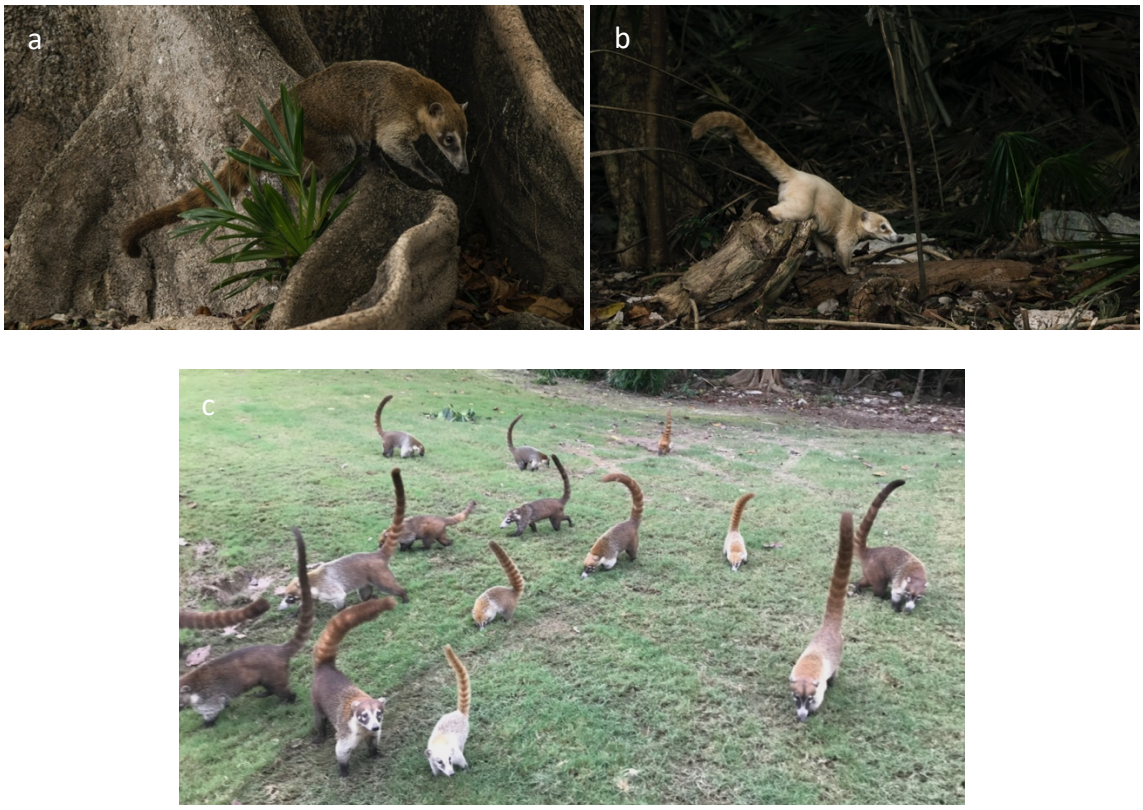

Figure 2.1: Three images white-nosed coatis (*Nusua narica*). (a) An adult coati with normal pelage coloration and pattern blends into its surroundings. (b) A conspicuous adult coati with leucism does not blend into its surroundings. This adult leucitic coati was one of few individuals to display leucism in this population. (c) Multiple coatis foraging with normal coloration and two juvenile coatis exhibiting leucism. Photographs were taken on a golf course outside a resort, a place with few or no predators left for coatis, in Playa del Carmen, Quintana Roo, Mexico in 2018 (Photo Credit: Samantha Kreling).

2.2 Introduction

Human-induced rapid environmental change is known to be a driver of evolutionary change in organisms during the Anthropocene (Sih et al. 2011, Hendry 2016, Alberti et al. 2017). As one of the most extreme examples of human-modified environments, urban areas serve as potential hotspots for rapid evolution (Donihue and Lambert 2015, Schell 2018, Rivkin et al. 2019, Diamond and Martin 2021, Lambert et al. 2021). Highly urbanized regions are characterized by increased human presence and impervious surfaces, as well as lack of green space, high exposure to pollutants, high levels of artificial light and ambient

noise, and increased temperatures (Grimm et al. 2008, MacDonnell et al. 2009, Imhoff et al. 2010, Niemelä 2011, Szulkin et al. 2020). Additionally, urban areas typically have elevated levels of vehicle traffic, human-wildlife conflict, and physical landscape structures that may alter gene flow (e.g., habitat fragmentation, linear barriers) or mortality rates (e.g. vehicle collisions, conflict removals) within and among urban wildlife populations (Johnson and Munshi-South 2017, Winchell et al. 2016, Miles et al. 2019, Schell et al. 2021 Cosentino and Gibbs 2022). All these factors have been found to cause non-adaptive evolutionary phenotypic or genotypic responses in urban wildlife populations and in select cases scientists have even conclusively discovered adaptive evolution in response to the urban environment (Oke et al. 1973, Noël et al. 2006, Giraudeau et al. 2014, Serieys et al. 2015, Campbell-Staton et al. 2020, Adducci II et al. 2020, Lambert et al. 2021, Cronin et al. 2022).

Yet despite recent advancements in the fields of urban evolutionary ecology, pelage coloration has been largely ignored (but see Leveau 2021). Coloration in birds and mammals serves many purposes, and several hypotheses have been proposed to explain the variation in plumage and pelage pattern and color (Caro 2005). Substantial evidence supports avoidance of detection, sexual selection and secondary fitness signaling, and thermoregulation as drivers of coloration (Roulin 2004, Caro 2005, Protas and Patel 2008, Stuart-Fox et al. 2017, Pembury Smith and Ruxton 2020). However, interactions between these drivers are not well-understood. With endothermic species, for example, how pelage and plumage coloration is affected by temperature is difficult to predict due to the numerous concurrent selection pressures and thermoregulatory mechanisms within individuals. For example, desert dwelling species might take on light colorations to reflect sunlight and blend into their surroundings; yet many desert species are black in coloration (Buxton 1923, Caro 2008, Caro and Mallarino 2020). That being said, over large geographic scales, typical trends of coloration and pattern have evolved convergently in many different lineages globally, suggesting similar selection pressures throughout many different regions and time periods (Baker and Parker 1979, Caro 2005, Kronforst et al. 2012, Pembury Smith and Ruxton 2020). Despite pelage and plumage coloration's immense importance to a wide diversity of behavioral and physiological purposes, like camouflage, territoriality, thermoregulation, and sexual selection

signaling, a recent review of the impact of urbanization on coloration across taxa found only 62 studies, 30 of which were spread across only 3 species (Rock Dove also known as the domestic pigeon (*Columba livia*), great tit (*Parus major*), peppered moth (*Biston betularia*); Leveau 2021).

This paper builds on the literature on intraspecific pelage coloration evolution in both urban and non-urban systems to develop new hypotheses for how pelage and plumage coloration may change in urban areas. In this paragraph, I will lay out several hypotheses that will be addressed throughout the manuscript (Table 2.1; visual summarization in Figure 2.2). I will explore the theoretical release from evolutionary constraints of novel pelage coloration phenotypes as an example of the potential for neutral and/or adaptive traits to persist in human-dominated landscapes. As a result of increased exposure to toxins, pollutants, and chemical mutagens, in conjunction with strong genetic drift and higher densities of wildlife than in non-urban areas, I predict an increase in heritable rare pelage coloration. Additionally, I hypothesize that through the human shield effect and subsequent decreased predation pressure, changes in pelage coloration that would be negatively adaptive in non-urban areas may be neutral or beneficial in urban areas, allowing for alternative colorations to persist or even proliferate. Finally, there is potential for alternative color morphs to be selected for if they offer better thermoregulatory power to combat increased heat load in urban areas, improve detoxification ability to mitigate increased toxin loads, or provide better visibility by humans to avoid direct human-mediated mortality (e.g., black squirrels hit by cars less frequently than gray squirrels (Cosentino and Gibbs 2022).

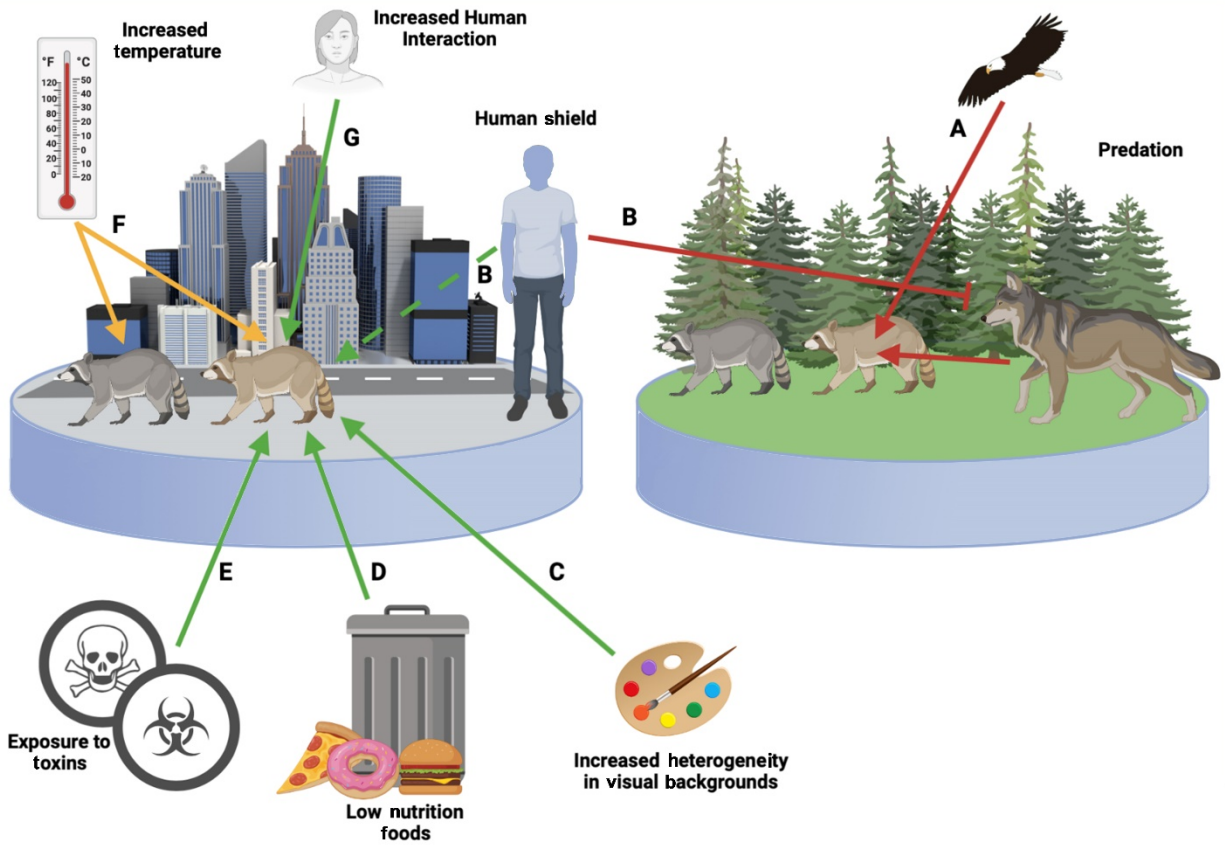

Figure 2.2: Conceptual figure to illustrate potential drivers and mechanisms influencing the theoretical release of novel coloration phenotypes in urban compared to rural areas. A) Predators remove conspicuous individuals prior to breeding. B) In urban areas, human presence directly or indirectly acts as a shield for prey and smaller carnivore species from large carnivores, thus reducing predation selection pressure. Additionally, food supplementation for urban carnivores with anthropogenic resources may reduce predation risk. Combined removal of predation selection pressure releases novel phenotypes to persist in urban areas. C) Where predation risk still exists, individuals with novel colorations have a higher probability of finding backgrounds that match their coloration due to a proliferation and wider variety of colors and textures across urban landscapes. D). Low nutrition foods may provide a non-heritable cause for increase in novel coloration in birds and mammals. In particular, tyrosine deficiencies may present similar, but non-heritable, patterns to leucism. E) Increased exposure to toxins and pollutants may increase mutation rates, giving rise to a potential increase in frequency of novel pelage phenotypes. F) Increased temperatures may select for different pelage colorations that assist in thermoregulation. However, because of the complexity of thermoregulation in endotherms, the direction for this selection is difficult

to predict. G) Increased human visibility may result in direct reductions in mortality for conspicuous individuals or through increased interest in which conspicuous individuals are favored by human viewers as 'novel' and 'rare' sights.

Table 2.1: List of hypotheses and related mechanisms driving potential increases in conspicuous color morphs within urban environments. H1:H13 are demarcated proceeding their corresponding discussions in the text below.

Hypothesis Title	**Hypothesis**	**Mechanism**
H2: Founder Effect	Populations founded by individuals with alternative coloration with little gene flow may create populations with high frequencies of these traits.	Limited gene flow into populations will amass traits found within the original founding individuals among the population.
H1: Increased Mutation Load	Increased mutation load in urban areas leads to increase in aberrantly colored individuals.	Air pollution and other toxic sources that are found at higher densities in urban areas induce germline heritable mutations.
H3: Low-nutrition Diet	Low-nutrition diets may cause a non-heritable whitening of pelage.	Diets for wildlife in urban areas are typically composed of low-nutrition, high carbohydrate foods. This can lead to a lack of sufficient quantities of amino acids such as tyrosine which are necessary to produce melanin, causing a lightening or whitening of pelage.

H4: Urban Graying	High oxidative stress for wildlife in urban areas leads to non-heritable graying of pelage.	High stress environments such as that in urban areas causes increases in oxidative stress, leading to graying of pelage.
H5: Human Shield	Predation constraints on pelage coloration will be lifted through lowered predation rates, resulting in an increase of conspicuous coloration.	Humans have for the most part eradicated large predators from urban areas. While there are often higher densities of mesopredators in urban than non-urban areas, anthropogenic food supplementation results in lowered predation rates, releasing camouflage constraints in both predators and prey.
H6: Background-matching Heterogeneity	Alternative color morphs that blend into anthropogenic backgrounds may be selected for in high predation environments.	In addition to the backgrounds for wildlife to match in wildland areas, urban areas have a variety of anthropogenically-created backgrounds such as bricks and concrete. In cities with higher predation pressure, localized populations of wildlife with altered coloration that blend into selected backgrounds may be selected for.
H7: Human Visibility	Conspicuous morphs may proliferate in areas of high human density and where visibility of the animal	Increased visibility of conspicuous individuals conveys advantage in the form of reduced mortality.

	increases fitness or survival rate (i.e., non-conscious increased survival via humans).	
H8: Thermoregulation	Color morphs that offer thermoregulatory advantages will proliferate.	Urban areas are significantly warmer than rural areas. If the additional heat load is large enough to produce thermal stress on organisms, then color morphs that offer thermoregulatory benefits will be selected for.
H9: Melanistic Detoxification	Melanistic morphs may have selective advantage through heavy metal detoxification.	Melanin binds certain heavy metal ions rendering them inert and storing them in structures such as fur or feathers, thus detoxifying the body. In urban areas where toxin load is higher and may have significant fitness consequences, individuals that are better at detoxification may have a selective advantage.
H10: Sexual Selection	Urbanization may alter sexual selection preferences related to coloration.	Sexual selection can be a strong selection force. If urbanization alters what individuals select for in a mate based on coloration, then coloration in urban areas may regionally vary from non-urban conspecifics.
H11: Human Interest	Conspicuous morphs may proliferate in areas of high	Preferential treatment to conspicuous individuals through physical protection and

	human density and where humans give preferential treatment to conspicuous individuals (i.e., conscious increased survival via humans).	nutritional rewards may increase survival of conspicuous color morphs.
H12: Hybridization	Hybridization with domesticated animals may produce abnormal pelage coloration in wildlife.	Mating between wild animals and closely related domestic species may result in wildlife of abnormal coloration that may mimic coat colorations produced by leucism or melanism.
H13: Domestication Syndrome	When wild animals are domesticated (intentionally or unintentionally), a variety of traits linked to behavioral states such as docility and boldness may be altered and produce variation within the 'domestication suite,' including pelage coloration.	The 'domestication suite' is a standard set of phenotypic traits that tend to be altered during the process of domestication. One of these traits is pelage coloration. Urban areas often select for bold wildlife individuals, and unintentional rewarding to these individuals may produce similar selection outcomes as intentional domestication.

2.3 Wildlife Coloration in Urban Systems

In this section I will review relevant literature and discuss the potential ways that urban wildlife may be released from the evolutionary constraints that drove the patterns of pelage and plumage coloration that we see in most wildlife (Figure 2.3).

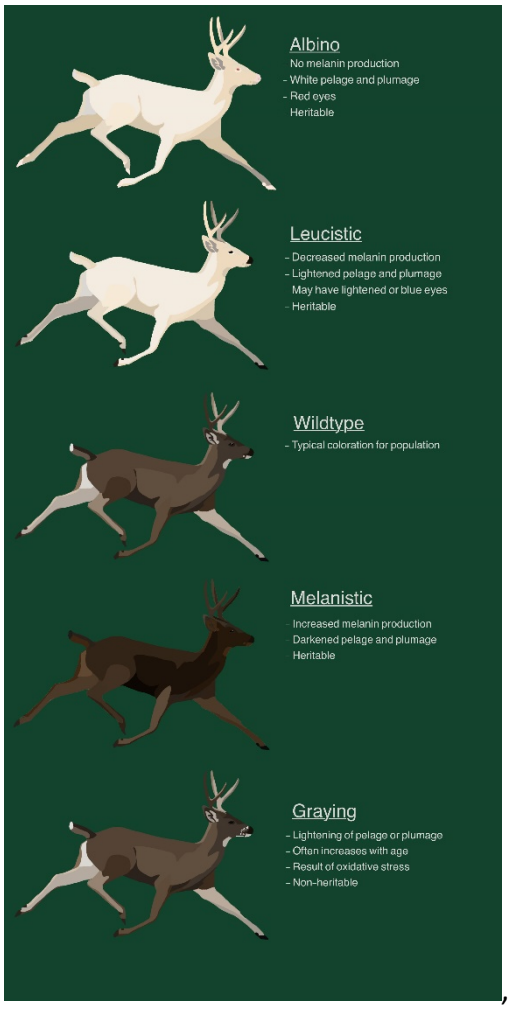

Figure 2.3: Examples of different coloration patterns that can occur because of increased (melanism) or decreased (leucism, albinism) melanin production and oxidative graying compared to wildtype or regionally 'normal' coloration.

2.3.1 Genetic Basis of Mammalian and Bird Coloration

The mechanism for deposition of pigment in bird feathers and mammalian fur is nearly identical (Lubnow 1963, van Grouw 2013). Coloration in both taxa is predominantly due to the pigments produced in cells known as melanocytes (Fox and Ververs 1960). These melanocytes produce two types of melanin: Eumelanin is responsible for black, gray, and dark brown colors, and phaeomelanin is responsible for red and light brown colors (Lubnow 1963, Hoekstra 2006). Multiple genes regulate the production of these pigments (Lamoreux et al. 2010). Abnormalities in coloration that affect melanism can occur from single point mutations, often in the melanocortin 1 receptor (MC1R) or agouti (AGOUTI) genes (Lamoreux et al. 2010), and there are often multiple mutagenic pathways that may be responsible for the same or similar color aberrations (van Grouw and de Jong 2009, van Grouw 2017).

2.3.2 Genetic Drift

With all the hypotheses discussed in this manuscript, genetic drift may have large impacts on the feasibility of alternative coloration traits persisting in urban areas. The high levels of fragmentation, often small founding populations, and potential limitations to gene flow associated with urban areas means there is strong potential for genetic drift (Miles et al. 2019), or the random change in the frequency of alleles within a population (Fisher 1922, Wright 1945). The combination of increased mutation load and drift (e.g., founder effects, bottlenecks), or selection may lead to regionally elevated frequencies of different wildlife traits (Miles et al. 2019). Founder effects, a specific type of genetic drift, may have particularly strong effects on wildlife populations where there may be limited population establishment events dependent on species attributes and corridor availability which may be limited in urban areas (Aziz and Rasidi 2014, Gallo et al. 2017, Kimmig et al. 2019, **H1**). This could lead to elevated levels of certain phenotypes if the original founders possessed those traits or recessive alleles that may produce those characteristics in future generations (Boileau et al. 1992, Crispo et al. 2005). Indeed, we've seen strong population founder effects in coat coloration of domestic cats based on settler origin in North Eastern United States' cities and cities across Europe (Todd 1964, 1966; Goncharenko and Zyat'kov 2012). For

scenarios where adaptive selection is occurring, its interactions with strong genetic drift in urban areas may either work to proliferate the mutations faster through the population by randomly dropping the frequency of 'typical coloration' alleles. Alternatively, if there is only weak to moderate strength selection for alternative coloration, drift may outweigh selection timelines and randomly lose these new mutations. Additionally, if the mutations causing abnormal coloration are recessive in nature, random loss of these alleles in the population before frequencies of the phenotypic trait are high enough for selection to engage may occur (Andrews 2010, Lynch et al. 2016).

2.3.4 Mutation Rate

Urban regions have concentrated historic and ongoing industrialization and development and are often littered with hotspots of chemical pollutant exposure (McDonnell et al. 1997, Apeagyei et al. 2011). Additionally, urban areas have higher densities of humans and increased vehicular traffic, leading to elevated air pollution levels (Lawson et al. 2011, Cakmak et al. 2012, Da Silveira Fleck et al. 2014). Many of the most common types of pollutants in urban areas are known carcinogens and mutagens (Cohen and Pope 1995, Turner et al. 2020) and have been demonstrated to increase the general mutation rate in both humans and animals (Ellegreen et al. 1997, Yauk et al. 2008, Dubrova 2019). Certain studies have also shown some of these mutations to be heritable (Yauk and Quinn 1996, Somers et al. 2002). With an increased mutation rate, novel phenotypes, including abnormal coloration of wildlife pelage, may arise more frequently in urban compared to non-urban areas (**H2**). Additionally, urban areas often host higher abundances of urban adapter species than rural regions (Tucker et al. 2020), increasing the likelihood that a mutation both occurs and is fixed by selection because of higher abundances of individuals (Kimura and Ohta 1971). Importantly, mutation itself as an evolutionary force, is relatively weak. In order to proliferate within a population, mutations would either need to be under strong directional selection or occur at higher frequencies within a population due to genetic drift.

Importantly there are also non-genetic or non-heritable causes of coloration abnormalities. Leucism for example may be caused by dietary deficiencies of tyrosine, an amino acid necessary for the

synthesis of melanin (van Grouw 2013, **H3**). This is plausible as wildlife in urban areas have been found to have less nutritious diets compared to non-urban areas (Isaksson and Andersson 2007, Murray et al. 2015). Similarly, oxidative stress which is found at higher levels in urban animals (Hutton and McGraw 2016), may lead to non-germline mutations in wildlife, causing graying or a lightening of coloration (Møller and Mousseau 2001, Izquierdo et al. 2018, **H4**). Finally, leucism may have negative effects for birds where melanin deposits within feathers provide mechanical strength to the structure and resistance to feather wear (Lee and Grant 1986, Bonser 1996, Kose and Møller 1999, Butler and Johnson 2004). Regardless, this presumed decrease in fitness may be negligible in the context of urban regions where individuals typically have to travel smaller distances for food and face less predation risk (Berger 2007, Suraci et al. 2019, O'Donnell and delBarco-Trillo 2020, Sadoul et al. 2021).

2.3.5 Natural Selection

2.3.5.1 Camouflage

For both predator and prey, camouflage can be imperative for survival (Pembury Smith and Ruxton 2020). Camouflage as an anti-predator mechanism for prey has been well-documented (Nachman et al. 2003, Caro 2005, Rosenblum et al. 2009, Stevens et al. 2011, Harris et al. 2020). Drivers of camouflage in predators have been less studied than anti-predator responses for prey, but background matching crypsis can be important for successful hunting and overall fitness (Pembury Smith and Ruxton 2020).

Prey and some mesopredator species may be more abundant in urban areas as result of the 'human shield,' the phenomenon where human presence and urbanization act as an inhibitor for apex predator establishment and persistence, allowing prey and smaller predatory species to thrive (Berger 2007, Geffroy et al. 2015, Suraci et al. 2019, Sadoul et al. 2021). While an increase in mesopredators may seem problematic for conspicuous prey, research has shown urban areas have decreased predation rates despite higher predator density due to anthropogenic food supplementation (Fischer et al. 2012, Eötvös et al. 2018). In non-urban settings with strong predation pressure, individuals that do not match their environments are easily spotted and removed from the population quickly (Belk and Smith 1996, Caro

2005). For example, species who experience camouflage mismatch due to climate change have seen significant declines because of increased predation, to the extent that some species like the snowshoe hare may require evolutionary rescue to persist (Mills et al. 2013, Zimova et al. 2016). With less predation risk and thus less need to blend in to avoid predation in urban regions, prey coloration may be released from these constraints (**H5**). While mutations are rare, individuals with alternative coloration may be able to persist and even proliferate without predation removing individuals before they reproduce, especially if there is strong genetic drift (Miles et al. 2019).

While predation pressures are generally reduced in urban areas, locations within cities may have regionally strong predation pressures. With higher levels of landscape heterogeneity than non-urban areas (Irwin and Bockstael 2007, Schell 2020), urban areas have additional types of background patterns and colors that may allow for a greater variety of color morphs to exist while continuing to avoid predation through crypsis (Figure 2.4, **H6**). Urban areas typically have a greater variety of background types to match than non-urban areas. For example, urban areas typically have patches of naturalistic backgrounds that you would find in non-urban areas such as forest fragments and vegetated open spaces. In addition to these naturalistic backgrounds, several human-fabricated backgrounds such as brick, asphalt, concrete, and lawn are abundant in cities and can often cover vast areas contiguously (Leveau 2021). Selection via predation is most likely to occur in small-bodied prey species with high fecundity and that have relatively small home ranges, confining them to a particular environmental background color and pattern (Nachman et al. 2003, Rosenblum et al. 2009). For population-level change to occur, sufficiently high predation risk to select for aberrantly colored individuals would be required. As an example of urban background pattern matching, Kettlewell (1955) documented selection for alternate color morphs in an urban area. Selection acted in favor of the more cryptic black peppered moths that blended into the trees that were covered with soot from nearby industrial factories, while light colored moths were easily identified and predated on.

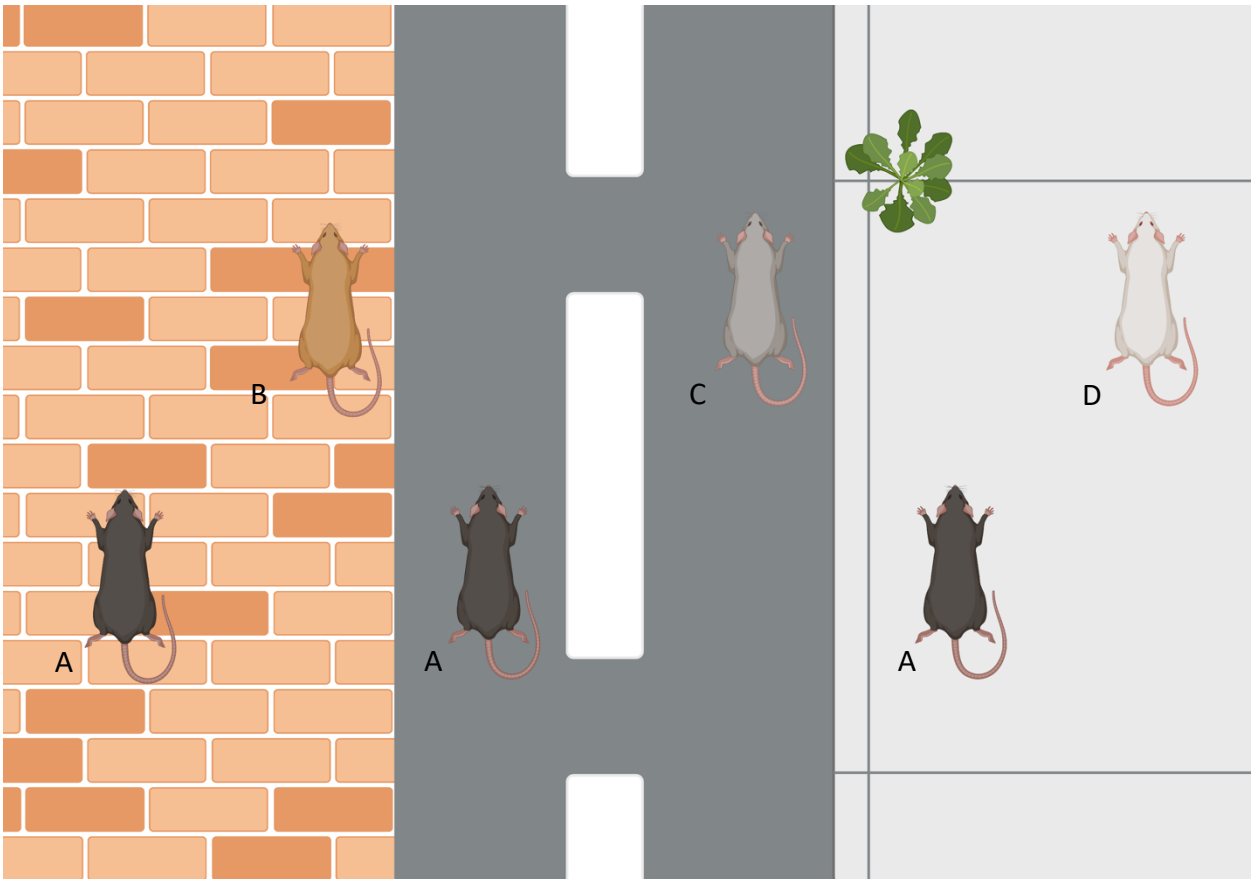

Figure 2.4: In addition to the background patterns and colorations found in natural habitat fragments in urban areas, urban areas offer a proliferation of human-created backgrounds that prey species may evolve to match to. This conceptual figure compares the typical coloration of black rats (rat A) to an alternative color morph (rats B, C, and D) that matches three common background colors and patterns in urban areas including bricks, asphalt, and concrete.

Conspicuous coloration may even offer advantages over cryptic coloration in areas with little predation risk and where primary mortality is predominately from motor vehicle collisions or other visually mediated human-induced mortality sources (**H7**). This appears to be the case in Gibbs et al. (2019) where melanistic morphs of eastern gray squirrels (*Sciurus carolinensis*) dominate in the studied urban area and represented a disproportionately small percentage of roadkill. Recently, a multi-city study has shown repeated proliferation of melanistic morphs of this species over urban-rural clines. Notably the authors of the study also acknowledge that melanism was positively associated with northward latitude,

suggesting the melanistic morph may have better thermotolerance for extreme cold temperatures in winter and that there may therefore be multiple selection pressures at play (Cosentino and Gibbs 2022).

For predator species, access to anthropogenic foods may remove predation-based selection pressures for camouflage. In urban areas, wildlife species tend to supplement their diets with anthropogenic resources that are either intentionally or unintentionally left accessible (Contesse et al. 2004, Williams et al. 2006, Murray et al. 2015). This supplementation of food that does not require active pursuit may thus allow a loosening of the predicted selection pressures on pelage coloration surrounding predator-prey relationships (Skelhorn and Rowe 2016, Pembury Smith and Ruxton 2020). As far as I am aware, no studies have addressed the frequencies of alternative color morphs in relation to predation regimes in urban areas.

2.3.5.2 Thermoregulation

For endotherms such as birds and mammals, the relationship between color and body temperature is complex and highly dependent on several factors such as behavior, structural properties of the feathers or hair, and amount of fatty insulation (Walsberg 1983, Stuart-Fox et al. 2017). Coloration is further complicated by interacting selection pressures such as predation avoidance and links to physiological processes (Cloudsley-Thompson 1999, Stuart-Fox et al. 2017). There are a few, albeit often contradictory, trends in endotherm coloration and thermal temperature (Stuart-Fox et al. 2017). For one, Gloger's rule specifies that in humid areas endothermic animals are often darker than in less humid regions, though this has also been attributed to additional benefits conferred by melanization such as hydroregulation and UV protection (Gloger 1833, Burtt and Ichida 2004, Kamilar and Bradley 2011). Desert and other hot-ecosystem endotherms tend to either mirror the color of the soil and sand, a buff or sandy color, or be black in coloration (Buxton 1923, Caro 2008, Caro and Mallarino 2020). Both coloration patterns may have potential benefits to animals in extreme ambient temperatures. As the amount of insulation in an individual declines, the potential effect of coat coloration on thermoregulation increases (Finch et al. 1980, Dawson et al. 2014).

Urban centers have a steep increase in impervious surface and reduction in tree cover when compared to non-urban areas (Arnfield 2003). This leads to an average increase of 1.1 - 2 °C in ambient temperatures in urban areas during the day (Hibbard et al. 2017, Allen et al. 2018). This increase in heat has already led to selection for thermal tolerant members in populations of urban anoles (Campbell-Staton et al. 2020). While mechanisms that confer thermal tolerance in this population of anoles are not physically visible to the human eye like a change in pelage coloration would be, it suggests that urban heat islands could lead to selection for particular color phenotypes if they confer a thermoregulatory advantage (**H8**). For instance, if all things are held constant between two individuals with differing fur coloration, if one coloration provides better thermoregulatory power, it may be selected for in that environment. Interestingly, different color morphs may convey opposite effects, having positive benefits in freezing temperatures and negative effects in extreme heat conditions or vice versa, further complicating the potential of directional selection (Caro 2008, Hetem et al. 2009). As far as I found, the aforementioned study on melanization in squirrels across the Eastern United States is the only mammalian study to look at a potential correlation between color morphology and ambient temperature in urban areas (Cosentino and Gibbs 2022). A single herpetological study has shown some correlations between color polymorphism and temperature in a semi-urban environment (Evans et al 2020), but this has seemingly not been tested in other vertebrates or explicitly along an urban-rural gradient.

2.3.5.3 Detoxification & Immunity

Individuals in urban areas are likely to have increased exposure to toxins and heavy metals which have significant effects on fitness (Trust et al. 1990, Dauwe et al. 2004, Greenberg and Briemberg 2004, Snoeijs et al. 2004, Hsu et al. 2006, Rainbow 2007, Eeva et al. 2009, Plum et al. 2010). The polymers that constitute melanin have negatively charged carboxyl, hydroxyl, and amine functional groups. These free electrons have been shown to bind to positive metal ions, thus acting as a detoxicant (Larson and Tjälve 1978, Liu et al. 2004, Bridelli and Crippa 2008). Melanistic morphs may therefore be able to detoxify their bodies by storing inert metal ions in melanin-laden structures such as feathers or fur (Chatelain et al.

2014). This mechanism has been suggested as a reason for more melanistic morphs of rock pigeons (*Columba livia*) in urban areas compared to non-urban areas (Obukhova 2007, Chatelain et al. 2014, 2016).

Through this detoxification, melanin may indirectly improve immune functioning of individuals, as heavy metals will often weaken the immune system (McGraw 2003, Hong and Simon 2008, Chatelain et al. 2014, Serieys et al. 2018, Murray et al. 2019). For example, one study exposed rock pigeons with a variety of melaninization to zinc and lead. They found that while birds with more melanin retained a higher concentration of these heavy metals in feather structures, their blood work showed similar metal concentrations to that of lighter colored birds. However, darker juvenile birds had a higher survival rate than their light-colored counter parts (Chatelain et al. 2016). This suggests that melanization does play some role in fitness and survivorship, potentially through detoxification, but the mechanism may be complex and mediated by other traits. Other studies have also shown a correlation between melanin and metal concentration in feather structures in white-tailed eagles and barn owls (Niecke et al. 1999, 2003). Additionally, melanocytes have been speculated to play a direct role in vertebrate immunity and parasite resistance, though the mechanism is unclear (Mackintosh 2011, Gasque and Jaffar-Bandjee 2015, Coté et al. 2018, **H9**). Little experimental work has been done to understand the role of melanin in the immune system in wildlife and most information that exists is entirely correlative.

2.3.6 Sexual Selection

Urbanization may alter wildlife sexual selection preferences regarding coloration **(H10)**. For the purposes of this paper, sexual selection's implications on coloration primarily applies to avian species as most mammalian species are not sexually dimorphic in color (Price 2006, McPherson & Chenoweth 2012, Cooney et al. 2019). While many of the colors that are under sexual selection in birds are carotenoid-based and derived from diet rather than melanin-based (McGraw 2006), there are a few examples of altered sexual selection of melanin-based coloration in urban areas. Great tits (*Parus major*) in Barcelona, Spain were found to have smaller black ties than forest birds. The tie is known to be a signal for sexual

selection, and this was speculated to be a result of altered sexual selection in urban areas. However, this paper also noted that birds with smaller ties were less exploratory and less bold and may have been selected in urban areas for these behavioral traits instead (Senar et al. 2014). White tail feathers in dark-eyed juncos (*Junco hyemalis*) are also a signal for sexual selection. An urban population in San Diego, California had an average 22% decline in white plumage which could be a result of altered sexual selection parameters (Yeh 2004). Notably, the authors mention that this result could also be due to genetic drift or phenotypic plasticity. Both examples show a decrease in coloration and sexual signaling. A recent review looking at which species of birds are likely to establish population in urban regions found that species with less plumage dichromatism, when males are more brightly colored than females, were more likely to inhabit urban areas (Iglesias-Carrasco et al. 2019). While the review did not provide many conclusive reasons as to why species with less plumage dichromatism persist in cities more frequently, they theorized that production of color may be more costly in urban regions. Perhaps displaying fewer or smaller colorations in sexual signals mirrors this trend. While there isn't much concrete evidence for altered sexual selection in avian species based on melanin-based coloration in urban areas, other sexually selected traits such as acoustic signals have been found to differ along an urban-rural gradient (Cronin et al. 2022). In European treefrogs (*Hyla arborea,* Troïanwski et al. 2015), painted gobies (*Pomatoschistus pictus*, de Jong et al. 2018), and common cuttlefish (*Sepia officinalis*, Kunc et al. 2014), altered coloration in noisy areas (e.g. urban) is thought to mitigate the effects of noise pollution on acoustic cues and mate selection; since birds also use calls to attract mates, this suggests that avian species could potentially alter coloration to attract mates in the presence of heavy noise pollution where their calls are less likely to be heard by potential mates.

2.3.7 Human Interest

Prior to the development of modern-day genetic techniques, scientists often categorized uniquely colored wildlife individuals as separate species. These 'species' were often regarded as highly prized and rare (van Grouw 2017). Many cultures around the globe have similarly assigned significant value and

importance to individuals and populations of wildlife with unique and conspicuous coloration (Saunders 1998, Chief Looking Horse 2010, Service et al. 2020). For example, Kitasoo people of British Columbia have traditions and stories reaching back immemorial regarding *Moksgm'ol,* leucistic black bears ('spirit bears') of the Great Bear Rainforest (Service et al. 2020). Additionally, many areas in the US have bans on hunting leucistic or albino white-tailed deer (Wisconsin Department of Natural Resources 1940, Iowa General Assembly 1988, Illinois General Assembly 2021) and other leucistic wildlife (Stencel and Ghent 1987). While melanistic wildlife individuals exist across the country, I could only identify protective laws for leucistic animals. Coloration of animals may also sway our willingness to conserve them from a psychological standpoint, prioritizing the things humans find aesthetically pleasing (Prokop and Randler 2018). Studies using community sourced pictures of aberrantly colored birds showed that people were more likely to send in photos of birds with rare coloration rather than less conspicuous color morphs because of their more unique coloration (Husby 2017, Zbyryt et al. 2021). Thus, this increased visibility and inherent aesthetical fascination with conspicuous color morphs may lead to selective protection or beneficial behavior of humans towards conspicuously colored individuals (**H11**).

2.3.8 Behavior, Hybridization & Domestication Syndromes

Domestic animals exist at high densities alongside people in urban areas. While unusual, hybridization between domestic animals and closely related wildlife species can occur (Adams et al. 2003, Chapman and Jones 2011, Leonard et al. 2014, Galov et al. 2015, Stronen et al. 2022). Hybridization in general, whether it be between two domestic species or a domestic and non-domestic species, can result in a variety of different pelage and plumage colorations (Hauffe et al. 2004, Schmutz et al. 2007, Zhang et al. 2014, Aguillon et al. 2020). With urban wildlife in close proximity to abundant domestic animals, there is the potential for hybridization to occur and introduce new coat or plumage phenotypes into the population (Adams et al. 2003). The high frequency of black-coated wolves in North America is a result of hybridization with domestic dogs and subsequent maintenance via heterozygote advantage and disease resistance (Wayne and vonHoldt 2012, Cubaynes et al. 2022). In European wolves, dark coloration is

very rare, but typically associated with urbanization and presence of feral dogs (Randi and Lucchini 2002). Recently in Queens, New York, three coyotes were found to be recent (F1 and F2) hybrids with domestic dogs. Some of the offspring had extremely abnormal coat coloration as a result (Caragiulo et al. 2022). If these urban populations are small enough and with few enough migrants from other wild populations, these alternative colorations may be able to persist at a higher frequency than in non-urban populations (**H12**).

Additionally, urban wildlife populations are preferentially established by individuals with bolder personalities (Caspi et al. 2022). In some species, melanin-production is pleiotropically linked to behavior and is often associated with bolder and more exploratory behavior (Ducrest et al. 2008, Mateos-Gonzalez & Senar 2012). Bolder individuals that are more likely to approach humans and adapt to the novelty of urban environments may be selected for once they are established as well (Brooks et al. 2020). Selection for these individuals that tolerate humans and may even lose their fear of humans, mimics domestication studies such as the infamous Russian silver fox farm experiments. These experiments showed that selection for less fearful and bolder animals eventually lead to domestication and as by-products of domestication, a variety of phenotypic traits, the 'domestication syndrome', became common that were not frequent or present in the wild individuals (Trut 1999; Hare et al. 2005, Hare and Tomasello 2005, Wilkins et al. 2014). One of these byproducts was changes in coat coloration, such as piebald coloration, white feet, chest spots, and tail tips (Trut 1999, Wilkins et al. 2014). Over time, unintentional selection for individuals that are bolder in urban areas may develop alternative coat coloration as a byproduct of unintentional domestication similar to the 'self-domestication' hypothesis for wolves (Hare and Tomasello 2005, Hare 2017, **H13**). Brooks et al. (2020) addressed this question in coyotes (*Canis latrans*) and similar to earlier work found that urban coyotes are bolder than their rural conspecifics (Breck et al. 2019) but failed to confirm higher frequencies of domestication syndrome coat phenotypes in urban coyotes.

2.4 Discussion, Implications, and Future Work

In this section I review the potential implications of having aberrantly colored wildlife in cities and address how scientists can begin to assess trends in coloration across urban-rural gradients.

2.4.1 Why aren't we seeing high rates of aberrantly colored wildlife in all urban areas?

Ultimately, the selection pressures that influence pelage coloration in wildlife are complex and interacting. Many of the stated hypotheses are dependent on strong genetic drift and limited gene flow from wildland populations to allow for the proliferation of wildlife with abnormal coloration. Additionally, mutations in general are relatively infrequent, and typically have adverse effects. The chances of MC1R or AGOUTI mutations causing abnormal coloration, are thus rare in general. We may not be seeing aberrantly colored wildlife in all urban regions because these mutations may simply have rarely occurred in urban areas and not had the chance to persist or were not coupled with strong enough genetic drift or selection in their respective environments to allow for proliferation. Similarly, there are varying selection pressures among and within different cities. If predation pressure is still high in some cities or parts of cities, conspicuous individuals are unlikely to persist there, as they would likely be removed from the population before reproduction. Additionally, alternative color morphs may have unknown underlying physiological differences that affect fitness negatively. Even if these regionally abnormally colored individuals exist and are in the correct genetic landscape to allow persistence, if there are unknown negative consequences that affect fitness or survival, they will likely not thrive in the population. On the other hand, if abnormal coloration conveys fitness or survival advantages on one axis of selection, with a multitude of selection pressures influencing wildlife coloration, advantages conveyed by alternative pelage coloration must strongly outweigh any potential opposing selection pressures. Finally, on evolutionary time scales, cities are a relatively new landscape and studying wildlife in urban areas is an even more recent development. Scientists likely have little idea of what the frequency of different color morphs in urban and non-urban areas are. This subject has rarely been studied and the hypotheses suggested in this manuscript are all in need of further research.

2.4.2 Implications

Physical appearances in wildlife serve distinct and important purposes. From sexual selection and secondary fitness signaling to anti-detection mechanisms, pelage pattern and coloration help determine the survival and reproductive success of individuals. Given time and the correct genetic landscape, urban areas may allow or even select for alternative color morphs. However, proliferation of conspicuous color morphs due to one mechanism may have dramatic implications for the other purposes of pelage coloration.

Unintended consequences of human-mediated selection may have vast and wide-ranging effects on wildlife populations. For example, laws that protect leucistic and albino wildlife may encourage proliferation of these specific color morphs. There are multiple states in the United States with protections on wildlife with abnormal coloration (Wisconsin DNR 1940, Iowa General Assembly 1988). Even at the municipal level, there are towns that provide protections to these alternatively colored individuals, such as for white morphs of Eastern gray squirrels in Olney, Illinois (City of Olney Municipal Code). Towns like Olney (e.g., Marionville, MS, USA; Marysville, KS, USA; Kenton, TN, USA; Kent, OH, USA; Exeter, Ontario, CA) even profit from these uniquely colored individuals through tourism, incentivizing their protection. However, protection of these individuals may have unintended population consequences. Leucism and albinism have been linked to potentially negative health and fitness outcomes in wildlife, such as weakened feathers in birds or may be the result of inbreeding as seen in domestically raised white tigers (Lee and Grant 1986, Bonser 1996, Kose and Møller 1999, Butler and Johnson 2004, Xu et al. 2013). Thus, artificially selecting for these color morphs could significantly decrease the average fitness of these populations, making them more vulnerable to human and climate-related disturbances.

Additionally, with the many behavioral and physiological roles that wildlife pelage and plumage coloration plays, selection for aberrantly colored individuals on one axis may have negative consequences on another. For example, if aberrantly colored individuals are selected for in cities based on a thermoregulatory advantage, how will this affect secondary fitness signaling and mate selection?

Additionally, if aberrantly colored individuals are selected for based on withstanding the greater temperatures found in urban heat islands, they may be more susceptible to climate volatility and sudden cold snaps that may present themselves more frequently as climate change progresses and daily weather patterns becomes less predictable (Sheshardri et al. 2021).

Finally, there has been a global increase in human-wildlife conflict in recent decades, which is predicted to only get worse with climate change (Abrahms 2021, Schell et al. 2021). In urban areas, people are often at closer proximity to wildlife than non-urban areas. If people begin favoring wildlife individuals with alternative coloration, through protective laws or supplemental feeding, the proximity to these individuals may further increase, leading to higher likelihoods of conflict arising (Thirgood et al. 2005). Additionally, if alternative coloration is caused non-genetically, as through urban graying or low nutrition foods, these individuals may be more susceptible to accumulating pathogens or diseases as a result of lowered immune responses (Murray et al. 2019). Coupled with close proximity to humans, this could potentially increase the risk of disease spillover (Murray et al. 2019, Messmer 2020).

2.4.3 Future Studies

While Leveau (2021) found more than 60 studies that to some extent address frequencies of different color morphs between urban and non-urban regions, the vast majority of these studies were concentrated on just three species. Addressing pelage and plumage coloration frequency across different study systems and taxa will be imperative for understanding the likelihoods of proliferation for different phenotypes. Additionally, few of the hypotheses I suggest above have been studied at all. I suggest that researchers prioritize understanding how environmental change due to urbanization can alter strength and direction of adaptive and non-adaptive evolutionary forces across phenotypic traits. Urban evolutionary ecology is a nascent field and urban adaptive and non-adaptive evolution is thus poorly understood. I suggest building on literature regarding urban evolution in general and applying these findings to coloration morphology specifically. While beginning to change, to date, much of the research on urban wildlife evolution has been conceptual in nature or correlative rather than mechanistic (Diamond and Martin 2021, Lambert et

al. 2021). Much more research on the mechanisms behind adaptation or regional genetic changes in urban wildlife is needed. Additionally, those studies that do look at urban evolution have primarily focused on specific locales and lack replication across urban areas (Lambert et al. 2021).

Scientists should leverage existing museum collections, camera trap networks (Urban Wildlife Information Network), community science data (Zbyryt et al. 2021, Cosentino and Gibbs 2022), and census data (e.g., the Squirrel Census) to begin to monitor and understand phenotypic frequencies of different color morphs across urban-rural gradients. Additionally, conducting laboratory studies may help understand when atypical color morphs may be advantageous. Borrowing methods from studies such as Campbell-Staton et al. (2020), scientists can conduct thermal tolerance studies to understand how pelage and plumage color morphology affects heat load in different thermal environments. Similarly, laboratory experiments can help us further understand how exposure to different chemicals and mutagens may influence mutation load and subsequent heritability of those mutations, and if there are any biases in the genetic regions they are likely to mutate within. Exposing laboratory animals to polluting compounds common in urban areas (e.g., lead, various hydrocarbons, fine particulate matter) and tracking potential genetic changes will help us understand the likelihood of different mutations, such as those that produce coloration changes, occurring. Finally, while difficult to enact, transplant experiments with atypically colored individuals between urban and non-urban conditions, could help to understand how predation pressures and general environmental conditions influence survival and reproduction rates of these individuals.

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3. Chapter 3 - City divided: Unveiling family ties and genetic structuring of coyotes in Seattle

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3.1 Abstract

Linear barriers pose significant challenges for wildlife gene flow, impacting species persistence, adaptation, and evolution. While numerous studies have examined the effects of linear barriers (e.g., fences, roadways) on partitioning urban and non-urban areas, understanding their influence on gene flow within cities remains limited. Here, we investigated the impact of linear barriers on coyote (*Canis latrans*) population structure in Seattle, Washington, where major barriers (i.e., interstate highways and bodies of water) divide the city into distinct quadrants. Just under 1,000 scats were collected to obtain genetic data between January 2021-December 2022, allowing us to identify 73 individual coyotes. Notably, private allele analysis underscored limited interbreeding among quadrants. When comparing one quadrant to each other, there were up to 16 private alleles within a single quadrant, representing nearly 22% of the population allelic diversity. Our analysis revealed weak isolation by distance, and despite being a highly mobile species, genetic structuring was apparent between quadrants even with extremely short geographic distance between individual coyotes, implying that Interstate 5 and the Ship Canal act as major barriers. This study uses coyotes as a model species for understanding urban gene flow and its consequences in cities, a crucial component for bolstering conservation of rarer species and developing wildlife friendly cities.

Key Words: Coyotes, landscape genetics, linear barriers, population structuring, private alleles, urban

3.2 Introduction

Genetic diversity has many implications for species persistence, evolution, and adaptation (DeWoody et al. 2021). In an age of rampant landscape and climate change, maintaining high genetic

diversity among species and populations will be increasingly important (Exposito-Alonso et al. 2022). High genetic diversity can confer increased fitness, higher disease resistance, increased adaptive capacity, and prevent inbreeding depression, making high-diversity populations and species more resilient to extinction (O'Brien & Evermann 1988, Keller & Waller 2002, Beever et al. 2016). Genetic diversity within populations depends on the ability of individuals to move across the landscape, and how easily genes flow between and within populations (Storfer et al. 2007). Frequent migrants can maintain high population genetic diversity and higher population-wide adaptive capacity, but this connectivity can also homogenize populations and inhibit localized adaptation (Hoeksema & Forde 2008, Sundqvist et al. 2016). Many of the features of urban landscapes such as linear barriers (e.g., fences, roadways) can affect gene flow of carnivore species in rural and wildland areas (Riley et al. 2006; Frantz et al. 2010; Sawaya et al. 2014, 2019; Litvaitis et al. 2015) and limit gene flow between conspecifics in urban and non-urban regions (Evans et al. 2018, Adducci et al. 2020, Smith et al. 2020, Huffmeyer et al. 2022). Few studies, however, have investigated the implications of fine-scale genetic structuring for wildlife within cities (Serieys et al. 2015, Kimmig et al. 2019, Fusco et al. 2021). While urban areas present limitations to population persistence and gene flow, many species have found ways to thrive in these areas, including various carnivore species that generally have large space requirements and historically low tolerance for anthropogenic disturbances (Woodroffe 2006, Ripple et al. 2014, Torres-Romero & Giordano 2022).

While many species can thrive in urban environments, city life also has its challenges. High pollution and toxin burdens (McDonnell et al. 1997, Apeagyei et al. 2011, Lawson et al. 2011, Cakmak et al. 2012, Da Silveira Fleck et al. 2014), low quality food (Murray et al. 2019), increased interactions with humans and novel stimuli (Thirgood et al. 2005, Kreling et al. 2023), and barriers to gene flow (Miles et al. 2019) are just some of the factors complicating life in the city for urban wildlife. The combination of gene flow limitations and novel pressures of city life may have a particularly strong impact on population persistence and even evolutionary adaptation (Crispo et al. 2011, Kimmig et al. 2019, Miles et al. 2019, Lambert et al. 2021). Urban environments are highly heterogeneous and fragmented, and lack of habitat corridors may impede dispersal and mobility for certain species (Gehrt et al. 2011). Common linear

barriers like freeways can make movement between different areas of a city risky or impossible for some species (Holderegger & Di Giulio 2010, Serieys et al. 2015, Kreling et al. 2019, Miles et al. 2019). In addition, species' relative tolerance of disturbance and human activity may impose additional limits on wildlife movement (Gaynor et al. 2018, Schell et al. 2020). These factors may impede the movement of individuals, and thus reduce gene flow.

Two main theories in landscape genetics may help predict gene flow through urban environments: isolation by distance and isolation by resistance. Isolation by distance leverages Tobler's first law of geography (Tobler 1970), where individuals that are closer together in geographic space are generally more similar to each other than to those further away (Wright 1943). While isolation by distance considers physical distances, it fails to consider the specific landscape structure and potential physical barriers between two individuals that may affect genetic distance. Isolation by resistance, on the other hand, takes into account that different components of the landscape may provide resistance or facilitation of gene flow (McRae 2006). Two additional models of gene flow have been specifically proposed for urban areas – the urban facilitation model and the urban fragmentation model (Miles et al. 2019). Under the urban facilitation model, urban areas may facilitate gene flow through human-mediated physical movement or indirect facilitation of movement. For example, black widow spiders (*Latrodectus hesperus*) show signatures of urban facilitation with higher genetic diversity and connectivity than rural populations throughout western US cities (Miles et al. 2018). Under the urban fragmentation model, lack of connectivity via linear infrastructure may lead to population structure influenced by resistance more than distance (Gortat et al. 2013, Selonen et al. 2018). For example, mountain lions (*Puma concolor*) in the Santa Monica Mountains show reduced genetic diversity and high inbreeding rates due to population isolation by highways (Huffmeyer et al. 2022).

Here we use non-invasive genetic techniques to understand how linear barriers such as roads and waterways affect population structuring of coyotes within the rapidly growing urban landscape of Seattle, Washington USA. Coyotes (*Canis latrans*) are a highly mobile mesocarnivore species that thrives in urban regions of North America. Because of their adaptability and long dispersal ranges, researchers

typically find little structuring in coyote populations compared to other less mobile species (Heppenheimer et al. 2018, Henger et al. 2020), though in some instances structuring has been found (Adducci et al. 2020, Sacks et al. 2004, 2008). However, Sacks et al. (2004) found structuring due to ecotypes and Adducci et al. found structuring when comparing urban to non-urban coyotes and few studies have truly investigated coyote population genetics at the within-city level. Some additional studies suggest that urban areas may facilitate coyote movement due to their skill in navigating barriers such as roads and human-created paths (Heppenheimer et al. 2018, Henger et al. 2020), while Riley et al. (2006) found that Interstate 101 acted as a strong barrier for coyotes. However, urban areas have seemingly retained high population genetic diversity and low inbreeding rates (Roy et al. 1994, Williams et al. 2003, Henger et al. 2020, Heppenheimer et al. 2018). Several urban coyote studies have also recognized some degree of differentiation between urban and non-urban coyotes, suggesting some limitations to gene flow in and out of urban areas (Damm et al. 2015, DeCandia et al. 2019, Adducci et al. 2020). However, the degree to which linear barriers may limit gene flow of carnivores within cities rather than across a city or along urbanization gradients, remains less understood and coyotes can act as a model species for understanding how gene flow may be effected by urbanization for highly mobile species.

Like many urban areas, Seattle contains multiple high-traffic highways and industrial areas that may act as barriers to gene flow, as well as a heterogeneous mixture of landscape covers and housing densities (Figure 3.1). Additionally, Seattle is surrounded by large bodies of water, and contains multiple rivers or channels that may further inhibit coyote movement. Using non-invasive scat collection and microsatellite genotyping, we investigated if two linear features of the landscape acted as barriers to gene flow, influencing population structure of coyotes in the Seattle area. In addition, we expected coyote genetic relationships to reflect geographic distances, because of the high density of coyotes that could inhibit longer-range movements, making nearer individuals much more likely to be related to each other than those far away in the absence of physical barriers. Understanding how landscape barriers facilitate or constrain gene flow of wildlife in urban areas can assist city planners in facilitating co-existence with urban wildlife and create more wildlife-friendly cities that aid in dispersal and movement (Kay et al.

2022, Lambert & Schell 2023). Additionally, examining underlying neutral evolutionary forces, such as gene flow and genetic drift, is an important first step for understanding urban evolution (Miles et al. 2019, Diamond & Martin 2021).

3.3 Methods

3.3.1 Study Region

We conducted non-invasive scat collection within the city limits of Seattle, Washington (latitude: *c.* 47.500° to 47.734°; longitude: *c*. -122.436° to -122.236°; Figure 3.1). Seattle is one of the fastest growing cities in the United States (US Census Bureau 2023), with a population of nearly 750,000 and a larger metropolitan area of just over 4 million (US Census Bureau 2022). The city is a matrix of dense housing, industrial centers, and natural land cover types. Seattle is bordered by the Puget Sound to the west and Lake Washington to the east (Figure 3.1). The city is split nearly in half by the Lake Washington Ship Canal (hereafter 'Ship Canal'), a 12.9 km waterway that connects the Puget Sound to Lake Washington. Additionally, Seattle has several major multi-lane highways that cross the city including Interstate-5 (I-5), Interstate-90 (I-90), and State Route 99. I-5 is a highly trafficked interstate with a 60-mph speed limit and divides the city in half vertically. South of the Ship Canal, the highway is eight lanes. North of the Ship Canal, there are eight lanes and three additional auxiliary lanes. This highway experiences considerable traffic volumes ranging from an average of 117,000-247,000 cars per day depending on the highway segment (WSDOT 2022). I-90 cuts eastward through the city between the industrial district and the I-90 bridge, but most of the highway is overpassed or tunneled, making it unlikely to be a barrier to wildlife movement. State Route 99 runs south-north just west of I-5. The majority of State Route 99 is four lanes, but traffic along each section of the route varies considerably. As it passes through the southern industrial portions of Seattle, the road ranges from speed limits of 40-50mph with several stop lights along the roadway. Through downtown, State Route 99 is underground, and between downtown and Green Lake, the speed limit remains a constant 40 mph. North of Green Lake, the road has several stoplights and high

traffic variability. While this road is busy during the daytime, rush hour traffic in the morning and evening slows traffic speeds considerably and night use is limited. The West Duwamish River separates the large neighborhood of West Seattle from the rest of the city. To the north and south of Seattle city limits are smaller, lower density cities and suburbs.

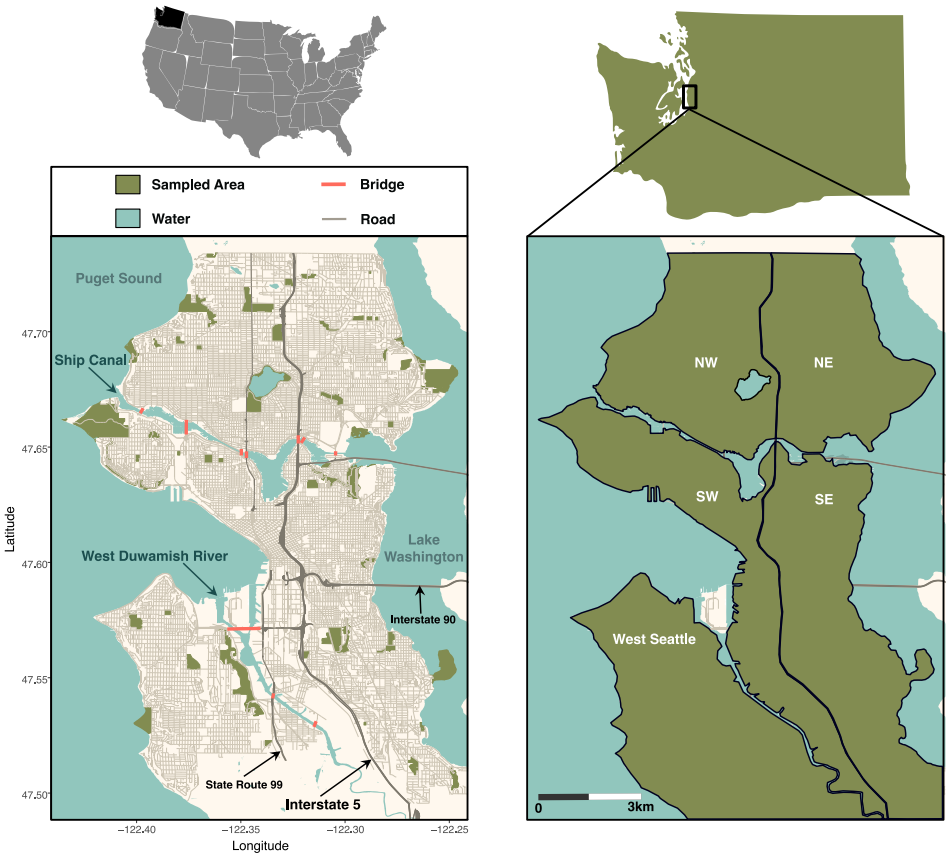


Figure 3.1: On the left: A map of Seattle with bridges, roads, highways, and sampled areas shown. Parks that were not sampled are not shown on the map, but can be visualized in Appendix A Figure S1. Important landmarks and potential barriers are labeled. On the right: Map of Seattle with hypothesized areas between potential linear barriers.

3.3.2 Scat Collection

Scats were collected from municipal parks within Seattle city limits between January 2021 – December 2022. Between May – September 2021, 34 parks were routinely sampled approximately every two weeks and an additional 16 parks were opportunistically sampled. Additional scats were opportunistically collected across a subset of the 50 parks that had regular coyote activity during 2021, before and after the main collection period as well as in 2022. A small portion of these scats were collected by volunteers. These scats, along with those researcher-collected scats, were visually inspected and only went on to DNA extraction if they morphologically appeared to be from coyotes rather than domestic dogs. All scats were collected into a sterile Ziploc bag and then double-bagged in a sterile Whirlpak. After collection, scats were stored in a -80°C freezer for at least two weeks to ensure no risk of viable *Echinococcus spp.* eggs (Hildreth et al. 2004). Scats either stayed in this freezer or were moved to a -20°C freezer for storage until DNA extraction.

3.3.3 DNA Extraction & Microsatellite Genotyping

All lab work aside from capillary electrophoresis was conducted at the University of Washington School of Environmental and Forest Sciences Genetics Lab. To obtain DNA material, scats were thawed and the inside and outside of each was rubbed with a flocked swab that was wetted with phosphate buffered saline solution. A modified protocol for the QIAmp DNA Investigator Kit (Qiagen; Hilden, Germany) was used to extract DNA from the samples.

To obtain individual coyote genotypes, 10 microsatellite loci and two sex ID loci were amplified from the samples using multiplexed PCR panels. Three PCR replicates were amplified per sample for both panels to screen for genotyping errors (*sensu* Prugh et al. 2005). The first panel included the following nuclear microsatellite loci: FH2001, FH2010, FH2054, FH2088, FH2328. The second panel consisted of the loci: CXX2235, FH2096, FH2137, FH2140, FH2159 and 2 additional microsatellite loci on the X and Y chromosomes to determine sex (DBX6, DBY7; Prugh et al. 2005, Seddon 2005). Cycling conditions were the same for both panels were as follows: initial denature step of 95°C for 15 min,

followed by 10 touchdown cycles of (94°C for 30s, 68°C - 1°C per cycle for 30s, 72°C for 45s), followed by 30 cycles of (94°C for 30s, 58°C for 30s, 72°C for 45s), followed by a final extension of 60°C for 15 min. After PCRs were completed, plates were frozen until they were shipped to Yale's Keck DNA Sequencing Core for fragment analysis on an Applied Biosystems 3130 Series Genetic Analyzer using capillary electrophoresis. Allele sizes were quantified using GeneMapper (Currie-Fraser & Shah 2010) and consensus genotypes from the PCR replicates were formed following the procedures described by Prugh et al. (2005) before being imported into GeneAlEx in Excel (Peakall & Smouse 2006). All consensus genotypes were examined to rule out domestic dog origin based on unique alleles found in reference dog samples and STRUCTURE clustering.

Any sample with 12 or more confirmed alleles was considered a successful genotype. Of the successful sample genotypes, unique IDs were assigned to genotypes observed in at least two scat samples, where at least one sample contained 20 or more confirmed alleles and the other sample had at least 12 matching alleles. This conservative higher threshold is to ensure that we did not accidentally assign a genotype to the wrong individual and to confirm that the scats were not of domestic dog origin. PIDsib is the probability that two siblings randomly drawn from a population will have the same genotype (Waits et al. 2001), was determined via GeneAlEx using a reference coyote population of 73 individuals. The sex ID markers were removed from this analysis, as these were fixed markers on the X chromosome and binary on the Y chromosome, and denoted sex rather than allelic diversity. For confident assignment of individuals, PIDsib values less than 0.01 are ideal (Waits et al. 2001). The values calculated from the 73 Seattle coyotes was 0.016 across 5 loci or 10 confirmed alleles, 0.006 across 6 loci, or 12 confirmed alleles, giving us our cutoff values of 12 confirmed alleles for a genotype to be considered successful. For all proceeding allelic analyses, the sex ID markers were removed.

3.3.4 Analysis Groups

Based on major linear infrastructure within the city that we suspected may reduce connectivity (I-5 and the Ship Canal), we assigned each coyote to 1 of 4 corresponding sections of Seattle based on the average

geographic location of all scat samples collected from each individual (Figure 3.1). The four sections were: north of the Ship Canal and west of I-5 (NW), north of the Ship Canal and east of I-5 (NE), south of the Ship Canal and west of I-5 (SW), and south of the Ship Canal and east of I-5 (SE). Hereafter, these groups are referred to as the 'quadrant groups.' Importantly, we failed to collect any confirmed coyote scats with successful genotypes in the West Seattle area, which precluded analyses in this region.

3.3.5 STRUCTURE & DAPC Analysis

STRUCTURE (v2.3.4) is a program that allows for analysis of multilocus genotypes to understand population structuring (Pritchard et al. 2000). Discriminant analysis of principal components (DAPC) is a multivariate analysis that identifies clusters within data (Jombart et al. 2010). Both clustering methods are commonly employed together to better understand population structuring (Miller et al. 2020). We ran STRUCTURE across all samples to compare the algorithm's clustering to the quadrant groups. We then ran DAPC analyses on the quadrant groups and STRUCTURE-defined groups. For DAPC and STRUCTURE analysis, best practices involve removing highly related individuals to prevent spurious clustering (Anderson & Dunham 2008). However, at the fine scale within cities, population structuring is likely coming from individual family groups and their dispersed offspring, so removing related individuals may obscure population structure (Barthel et al. 2020). To account for this, we ran two separate analyses. First, we identified first-order related pairs (full sibling or parent-offspring pairs; $r \geq 0.5$) using the Ritland & Lynch (1999) relatedness estimator in GeneAlEx, and we removed one individual per pair and used only the remaining individuals in analyses. Second, we retained all related individuals in the sample pool and ran the same analyses. Differences in results between the two sets of analyses were minimal. Thus, the first analysis was moved to the Appendix.

We used the command-line version of STRUCTURE to identify different groups within our data. STRUCTURE generates maximum-likelihood model-based estimations for ancestry and genetic structuring (Pritchard et al. 2000). We ran parallel admixture STRUCTURE analyses for K=1-10 groups using the GNU 'parallel' function (Tange 2023). For each analysis we used a burn-in value of 100,000

with 100,000 replicates. These analyses were blind to scat collection location information and were not informed by the quadrant groups. We identified the most likely K value by finding the greatest mean log likelihood value among the tested K values. We then ran a single STRUCTURE analysis on the data using this value and extracted the proportions of each individual assigned to each group. We then used this information to construct admixture plots in R.

To perform DAPC analyses in R, we first converted the input file to a *genind* object using the 'df2genind' function from the *adegenet* package (Jombart 2008, Jombart & Ahmed 2011). Importantly, the DAPC analysis was privy to quadrant groups and STRUCTURE-defined groups. This informs the clustering algorithm and may give separate results than *de novo* clustering with DAPC, but are generally correlated with F_{ST} values (Miller et al. 2020). The number of principal components (PC) was calculated using the 'xvalDapc' function, and the DAPC was conducted using the 'dapc' function from the *adegenet* package. DAPCs were visualized using the *ggplot2* package (Wickham 2016). We tested K=1-40 (number of clusters) to determine the optimal K value. For all DAPC analyses, the number of PC retained was determined by the lowest Mean Squared Error achieved. All eigenvalues for all DAPC analyses were retained as there were a small number of PC retained in all analyses.

3.3.6 Mantel Tests

Genetic mantel tests are non-parametric tests that assess isolation by distance via a correlation between Euclidean geographic distance and a metric of genetic distance (Mantel 1967). If barriers strongly inhibit gene flow, we expect that correlations between geographic and genetic distances would be weak because adjacent coyotes separated by a barrier should be genetically distant. For each individual coyote, we calculated the centroid location of all scats collected from this individual and then made pairwise calculations of Euclidean distance to each other coyote. For genetic distance, we calculated pairwise Nei's bias-corrected genetic distance (Nei 1978) using the 'nei.dist' function from the *poppr* package in R (v2.9.4; Kamvar et al. 2014). This function modified Nei's genetic distance so that it could be applied to genetic distances between individuals rather than just between populations. Nei's genetic distance is

particularly useful when the underlying genetic distance and structure is suspected to be from genetic drift (Nei 1978). The mantel test used Pearson's product-moment correlation and 999 permutations and was conducted in R using the 'mantel' function from the *vegan* package in R (v2.6-4; Oksanen et al. 2022).

3.3.7 Allelic Measures

To explicitly test the effect of the two barriers (the Ship Canal and I-5) on genetic differentiation and allelic measures, we calculated allelic metrics for the quadrant groups. To account for the positive relationship between sample size and richness, we calculated rarified allelic richness per locus and group using the 'allellic.richness' function from the *hierfstat* package which accounts for uneven sample sizes between groups (v0.5-11; Goudet & Jombart 2022). As is standard, we also calculated Weir & Cockerham's (1984) F_{ST} using the 'pairwise.WCfst' function from the *adegenet* package. Allelic metrics of genetic distance, such as F_{ST}, G_{ST}, or D, are generally based on heterozygosity, which is not a direct measure of genetic differentiation or diversity, and these metrics are continually debated in the population genetics literature (Whitlock 2011, Wang 2012, Verity & Nichols 2014, Jost et al. 2017). Thus, we also calculated private alleles between one group and the whole population, as well as between pairwise groups. Private alleles are unique to a single group (Szpiech & Rosenberg 2011) and can be used as indicators of gene flow and genetic structuring (Slatkin 1985, Barton & Slatkin 1986). To account for sample size differences, we calculated the number of rarified private alleles across all quadrants and between all quadrants using HP-Rare (Kalinowski 2005). Each pairwise comparison was resampled at the minimum sampling level between the quadrants. Finally, we calculated inbreeding coefficient using the 'inbreeding' function from the *adegenet* package (Jombart 2008) and average the sampled F values across each individual. We then ran a linear regression testing the correlation between inbreeding estimates and assigned quadrant group.

3.4 Results

3.4.1 Scat Collection & Genotyping

We collected 931 scats from January 2021 to December 2022. Of these, 191 failed to sufficiently amplify to form confirmed consensus genotypes, and 23 were suspected to be of domestic dog origin and were removed from analyses. Seventy-three individuals (36 females and 37 males) were identified from the remaining 717 scat samples. The number of scat samples per individual ranged from 2 to 44, with an average of 9.81 scats per individual (*SD* = 9.72, Median = 5). No coyotes were detected in more than one quadrant.

3.4.2 STRUCTURE

STRUCTURE indicated six groups (*n* = 73 coyotes, Table 3.1). STRUCTURE groups largely aligned with quadrant groups defined by the I-5 and Ship Canal linear barriers (Table 3.2). STRUCTURE groups 2, 3, 4, and 5 had 100% of the individuals belonging to a single quadrant group. Only STRUCTURE groups 1 and 6 had some mixture of individuals from different quadrant groups. Because STRUCTURE is not designed for use at such fine geographic scale, we used the relative agreement among STRUCTURE-defined groups as support for proceeding with quadrant-based analyses.

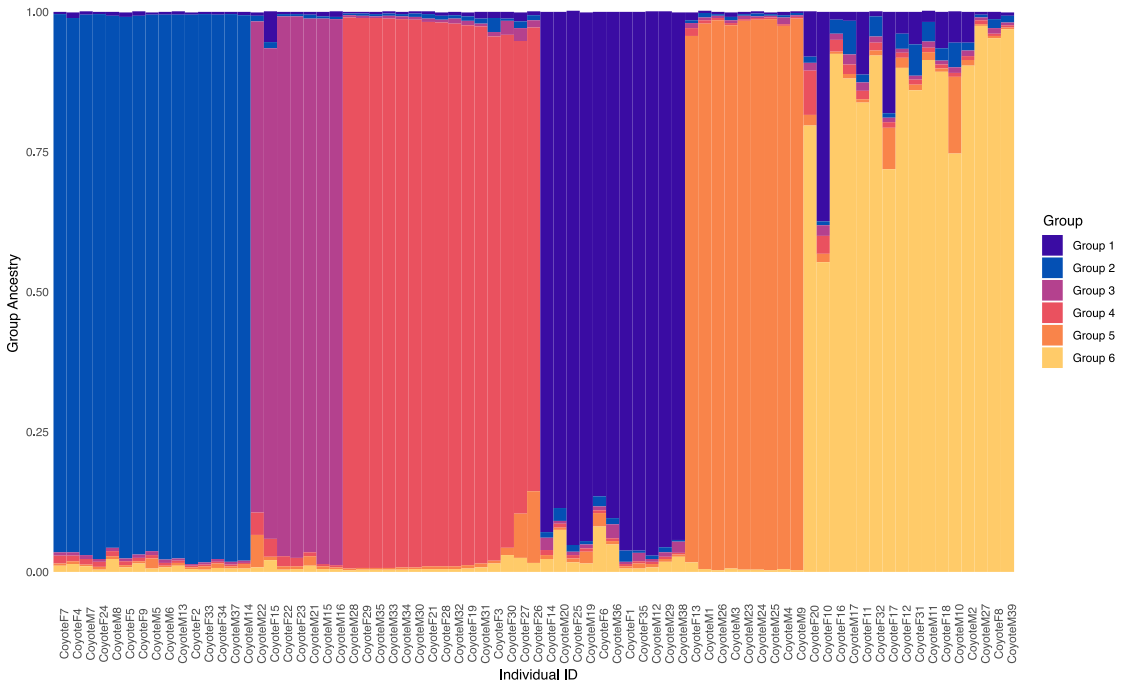
Table 3.1: Mean log likelihood values for varying K values in STRUCTURE. Bolded values are the highest mean ln likelihood and the number of groups tested in subsequent analyses.

K	**Mean Ln Likelihood (variance) with related individuals**
1	-2214.5 (29.0)
2	-2043.4 (85.3)
3	-1932.4 (86.9)
4	-1815.5 (121.5)
5	-1747.1 (219.2)
6	**-1691.1 (209.2)**
7	-1700.6 (236.0)
8	-1686.8 (393.5)
9	-1706.2 (406.9)
10	-1698.5 (413.5)

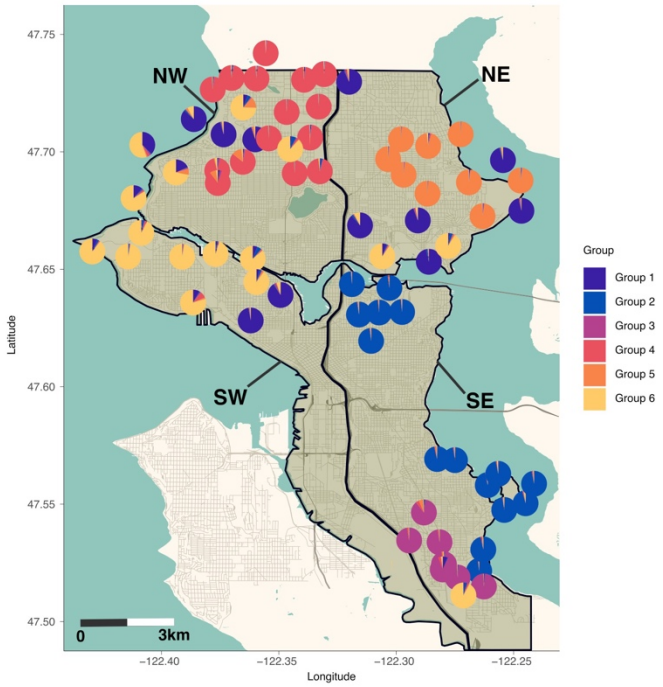
Table 3.2: Number of individuals from each quadrant group in each STRUCTURE-defined group. STRUCTURE-defined groups that consist of only 1 quadrant are bolded.

STRUCTURE Group	**NE**	**NW**	**SE**	**SW**
1	6	3	0	2
2	**0**	**0**	**15**	**0**
3	**0**	**0**	**7**	**0**
4	**0**	**15**	**0**	**0**
5	**9**	**0**	**0**	**0**
6	2	5	1	8

STRUCTURE admixture values (Figure 3.2A) and maps (Figure 3.2B) are to facilitate interpretation.



A)



B)

Figure 3.2: A) Plot of STRUCTURE-generated admixture values for each coyote from the sample pool. B) Admixture pie charts for each individual coyote detected. Points are jittered for visibility and are near where each coyote was most frequently sampled.

3.4.3 DAPC

For quadrant groups, 20 principal components (PC) and 3 DA eigenvalues were retained. The NE, NW, and SW groups showed differentiation from the SE group along the first principal component axis (horizontal; Figure 3.3A). The NE and SE groups showed differentiation from the NW and SW groups along the second principal component axis (vertical). For the STRUCTURE-defined groups, 20 PC and 5 DA eigenvalues were retained. For this analysis, groups 2 and 5 appear differentiated from each other and all other groups along the first principal component axis (Figure 3.3B). Additionally, groups 1 and 6 and groups 3 and 4 appear slightly differentiated across the first principal component axis. Groups 1, 2, 5, and 6 appear differentiated from groups 3 and 4 along the second principal component axis.

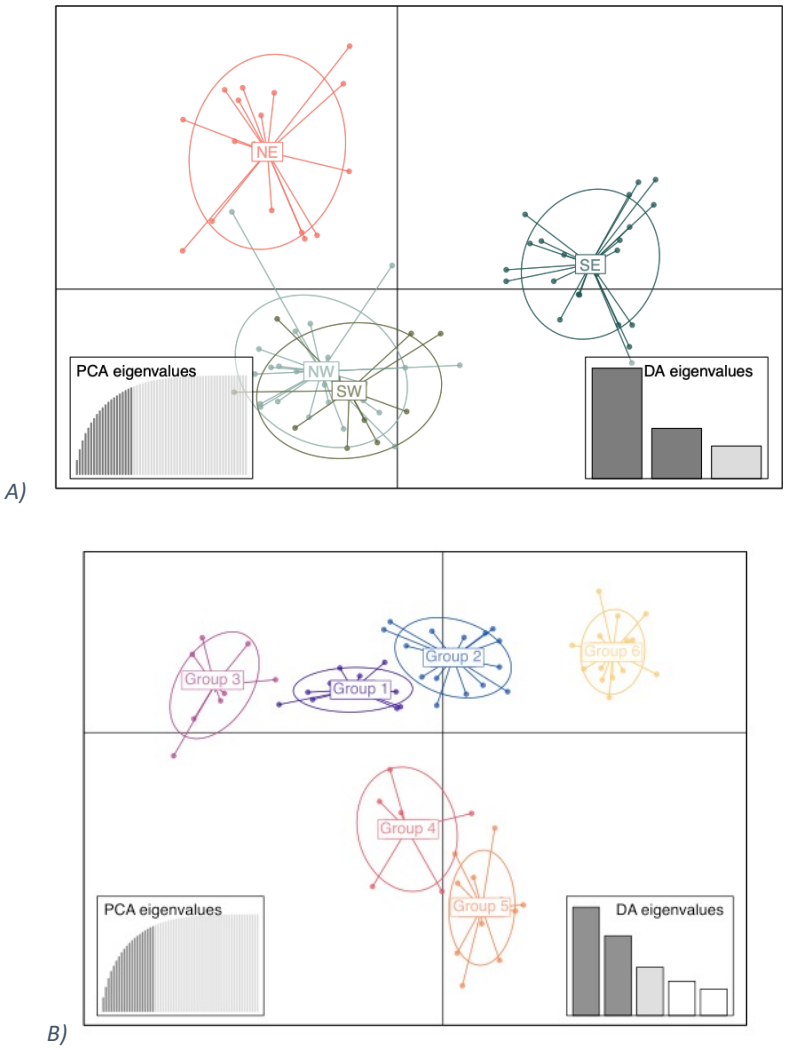


Figure 3.3: *DAPC results for quadrant groups for datasets A) for quadrant groups and B) DAPC results for STRUCTURE-defined groups.*

3.4.4 Mantel Test

We found very weak evidence of isolation by distance (Figure 3.4); although the mantel test correlating Nei's genetic distance to geographic distance was significant ($p = 0.006$, $r = 0.108$), geographic distance explained only 1.2% ($R^2 = 0.012$) of the variation. This weak correlation disappeared when related individuals were removed (Appendix A, Figure 3.2).

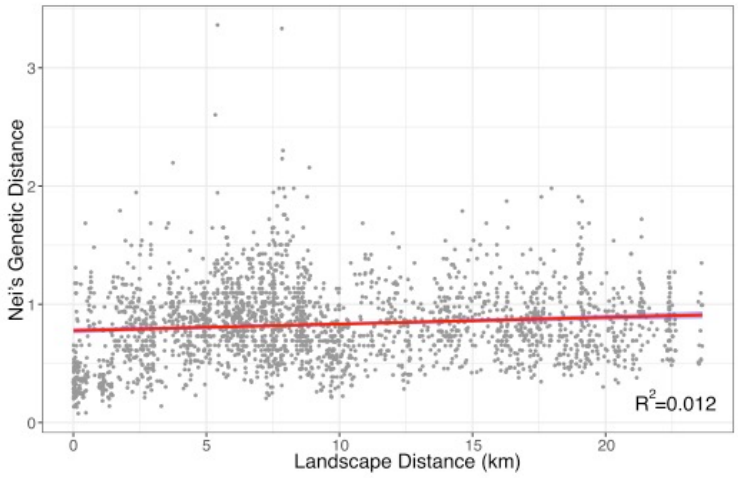


Figure 3.4: Mantel tests between Nei's genetic distance and landscape geographic distance in kilometers. Trend lines are reported in red with light gray confidence intervals. Note that these intervals are so small that they are barely visible above the trend line.

3.4.5 Allelic Richness, Private Alleles, F_{ST}

In total, across all loci and groups, 72 different alleles were present among the quadrant groups. Mean allelic richness per locus across the genotype for each quadrant group was 4.54, 4.37, 4.05, and 4.22 for the NE, NW, SW, and SE groups, respectively. Allelic richness per locus ranged from 1.87 to 7.46 across groups and loci (S2).

For the quadrant groups, all analyses showed some number of private alleles for each group. The NE group had just under 4 private alleles (n = 17 individuals, S3). The NW group had 3.5 private alleles (n = 14 individuals). The SE group had a total of 5.8 private alleles (n = 23). The SW group had 5.8 private alleles (n = 10).

The number of private alleles between individual quadrant groups ranged from 7.4 to 15.7 (Table 3). With 72 total alleles across the groups, having just under 16 private alleles between groups represents nearly 22% of the total population allelic diversity. At the low end of the range, 7 private alleles represent

just under 10% of the population-level allelic diversity. When the sum of the private alleles between each pair of quadrants is calculated, values range from 18.5 to 29.6 ($\bar{X}$ = 24.1).

All dyads showed moderate degrees of differentiation (>0.05), with F_{ST} values ranging from 0.069 – 0.091 (Table 4). The average inbreeding estimate across all individuals in Seattle was 0.163 (*SD* = 0.057). Within each group average inbreeding estimates were similar: 0.161, 0.164, 0.146, 0.169 for NW, NE, SW, and SE groups respectively (*SD* = 0.067, 0.060, 0.041, 0.056). Individual average inbreeding estimates ranged from 0.095 to 0.400. The linear regression analysis revealed weak, but insignificant correlation between group and average inbreeding estimate (p = 0.688, R^2 = 0.02).

Table 3.3: Rarified private alleles between all quadrants. The 'Individuals' column gives the number of coyotes included in each quadrant. The following columns are genotyped loci, and each cell value is the number of private alleles at each locus for each quadrant in comparison to the other listed quadrant. Below the sum of the number of private alleles in each quadrant is the sum of private alleles between the two quadrants.

	Individuals	**FH2001**	**FH2010**	**FH2054**	**FH2088**	**FH2328**	**CXX2235**	**FH2096**	**FH2137**	**FH2140**	**FH2159**	**Sum**
SE	23	0.986	0.739	0.000	1.000	2.736	0.936	0.000	0.936	0.997	1.986	10.316
NE	17	1.081	0.000	0.261	1.000	3.003	2.029	1.003	3.328	2.128	1.003	14.836
												25.152
SE	23	3.044	0.957	0.957	0.000	0.957	1.000	0.000	2.042	2.000	2.000	12.957
NW	23	1.001	0.000	0.000	1.000	1.956	1.957	1.000	1.043	3.959	2.000	11.916
												24.873
SE	23	2.443	0.391	1.391	0.000	1.358	1.419	0.875	2.573	2.509	2.740	15.699
SW	10	1.373	0.000	0.003	1.040	2.053	1.211	1.995	1.851	2.387	2.029	13.942
												29.641
NW	23	0.000	0.000	0.000	1.000	2.000	1.739	0.000	0.000	1.739	0.936	7.414
NE	17	2.261	0.000	1.015	0.003	1.325	2.081	0.000	3.325	1.044	0.004	11.058
												18.472
NW	23	0.000	0.000	0.784	0.000	1.773	2.014	1.000	1.486	4.157	1.632	12.846
SW	10	0.696	0.000	0.000	0.133	1.603	1.336	1.017	1.288	2.444	1.119	9.636
												22.482
NE	17	1.572	0.000	1.523	0.000	2.832	1.535	0.998	2.827	2.529	0.529	14.345
SW	10	0.084	0.000	0.094	1.002	2.995	0.516	1.010	0.170	2.194	1.377	9.442
												23.787

Table 3.4: F_{ST} values for quadrant groups.

	NE	**NW**	**SE**	**SW**
NE	--	--	--	--
NW	0.080	NA	--	--
SE	0.091	0.084	--	--
SW	0.089	0.081	0.069	--

3.5 Discussion

Linear barriers can represent a significant challenge for wildlife dispersal and gene flow in urban and non-urban settings alike. Coyotes represent an ideal model species for understanding how barriers may affect even the most mobile of urban wildlife species. While previous studies have investigated how linear barriers such as highways affect coyotes in non-urban areas, few have examined how such barriers may affect gene flow within cities. Seattle, with its high number of linear barriers and apparent geographic quadrants, offers a unique case study to understand how multiple barriers may limit the movement of genes within an urban matrix. While STRUCTURE defined 6 separate genetic groups, most of these groups aligned with the quadrants delineated by I-5 and the Ship Canal. Two of these groups appear to be mixtures of many family groups or groups that didn't fit nicely into one of the more defined family groups, and not all individuals within Group 1 and Group 6 have genotypes that are very similar. On the other hand, Groups 2 through 5 represent well-defined family groups of parents and offspring, or other near relatives. Despite coyotes being exceptionally mobile and plastic, we found that both linear barriers acted as surprisingly strong barriers to gene flow. Our findings suggest that urban wildlife populations may be characterized by genetic structuring at far finer scales than has been previously tested, with implications for urban evolution and population health.

To understand if coyotes are isolated by these barriers and test the urban facilitation model, we first assessed if isolation by distance was strong across the population. We found that there was a very

weak correlation between genetic distance and geographic distance, suggesting that the structure observed in this population may be a result of significant fragmentation via barriers and/or resistance in the landscape. In this study, each individual coyote is no more than 27 km apart, a distance easily crossed by young dispersing coyotes, or traveled by transient adults. Many locations for many individual coyotes are less than 1 km apart yet they are genetically distant. Numerous other studies have found that geographic distance is very poor at explaining genetic distance in urban regions (Vargová et al. 2023, Taichi et al. 2024). For example, mantel tests for coyotes in Los Angeles, California found similar isolation by distance values, $R^2 = 0.016$ compared to our $R^2 = 0.012$ (Adducci et al. 2020). In urban areas, prevalence of linear barriers may render isolation by distance unimportant, allowing for geographically close individuals to be genetically distant.

While many studies use FST, GST, and D as metrics of gene flow and genetic distance, all these metrics are based on expected levels of heterozygosity and have a variety of known limitations (Szpiech & Rosenberg 2011). On the other hand, few studies have used private alleles to assess gene flow. While this metric is difficult to compare across studies, it provides a more reliable and less-biased estimate of gene flow within studies and may be especially insightful for fine-scale studies (Slatkin 1985, Barton & Slatkin 1986, Brunton et al. 2022). In our study, each quadrant held unique alleles in pairwise comparisons as well as when compared to all quadrants at once. If genes were mixing among quadrants regularly or pups were dispersing into other quadrants frequently, we would expect to see no or limited numbers of private alleles in pairwise comparisons between quadrants and among the entire population (Slatkin 1985, Barton & Slatkin 1986). On average, there were 24 private alleles across our 10 loci genotype panel between pairwise quadrants. Other studies have found similar numbers of private alleles among fewer samples and larger geographic ranges where a higher number of private alleles would be expected (Szpiech & Rosenberg 2011). For example, leopards (*Panthera pardus*) across the cape of South Africa consisted of three separate populations, with the central population holding 27 private alleles, the largest number found in the study. However, that analysis was conducted with only 40 tissue samples, used more microsatellite loci ($n = 13$), and the study area spanned over 500 km in width (McManus et al.

2015). For urban white-footed mice (*Peromyscus leucopus*) in New York City, the maximum number of private alleles found when comparing one site to all sampling sites within the city was 10 across 18 loci (Munshi-South & Kharchenko 2010). The maximum number of rarified private alleles we found when comparing one quadrant to the other 3 quadrants was 5.8. While Munshi-South & Kharchenko (2010) found values representing nearly double the number of private alleles found by us, mice reproduce much faster than coyotes and have shorter dispersal distances. Their study also covered a larger geographic area and used more loci in the genotyping panel. Considering this, having half as many private alleles in coyotes, whose recorded urban dispersal distances range from 1.7 to 60 km (Zepeda et al. 2021) demonstrates significant lack of interbreeding between quadrants. Munshi-South & Kharchenko (2010) also found similar F_{ST} values as we found among the quadrant groups.

Coyotes are a highly mobile and reproductively prolific species. In urban areas, most green spaces should therefore be colonized quickly once coyotes arrive. However, despite repeated sampling, we did not genetically identify any coyotes to the west of the West Duwamish River. Additional sampling to the south of this area, combined with anecdotal evidence from residents, suggests that there are very few coyotes in this region. Based on personal conversations and newspaper articles, residents saw many coyotes 4-5 years prior to the start of this study but report a large mange outbreak shortly before the disappearance of coyotes. Through personal communications, we know that these individuals were not removed by federal agencies but could have been removed by local government or even community members taking matters into their own hands (Hunold & Lloro 2022). Mange can be sign of immunosuppression which can develop because of chronic poisoning, something that happens occasionally throughout Washington (personal communications with USDA Wildlife Services). Since we did not detect any coyotes in West Seattle, there appears to be little if any movement of coyotes from the southeast side to the southwest side of Seattle. I-5 is not the only barrier in this location, as Seattle's main industrial area runs directly to the west of the southern portion of I-5 and directly east of the West Duwamish River, which may further provide barriers to coyote movement. After sampling for this study

was completed, it has since seemed that coyotes are beginning to re-inhabit West Seattle as of late 2023 (https://carnivorespotter.org/).

In the northern portion of Seattle, the lack of interbreeding between quadrants is rather surprising given there are numerous overpasses, underpasses, and culverts along I-5. Additionally, coyotes should be able to easily swim across the short distance of the Ship Canal (Robinson & Cummings 1947, Way 2002**)**. While research has often addressed singular urban barriers, investigating multiple potential barriers and the interactions among barriers may present a more insightful understanding of urban gene flow and the subsequent implications for urban wildlife. Additionally, addressing gene flow at smaller spatial scales may help to identify specific barriers that may not be as obvious across larger spatial scales or may not seem like they would function as a barrier.

Lack of gene flow among quadrants may be a result of coyotes attempting and failing to cross these barriers or deciding not to attempt a crossing due to perceived risk. While multiple bridges cross the Ship Canal and numerous underpasses and overpasses exist across I-5, our findings indicate there is little breeding among individuals across these barriers. This lack of functional connectivity could indicate that even with features that enhance structural connectivity of a landscape, the perceived and actual risk of crossing these barriers may prevent individuals from doing so (Clevenger & Waltho 2005). While our results show some level of differentiation between all quadrants, an alternative explanation for the lack of interbreeding among quadrants may be lack of empty territories. However, in urban areas, vehicle collisions and management decisions cause significant mortality, up to 40% (Grinder & Krausman 2001, Gehrt et al. 2011), likely leaving 'widowed' parents frequently in search of new mates or even freeing up entire territories. Additionally, abundant anthropogenic food resources may support coyotes even when optimal habitat is not available (Gese et al. 2012, Parsons et al. 2022). Furthermore, there were nearly 20 sizeable green spaces where we collected few (~0-2) coyote scats despite repeated sampling. While lack of scat does not necessarily mean no coyote use, it may indicate that there are still habitat patches vacant for dispersing pups to move into. Thus, the lack of gene flow may reflect the resistance of the landscape as opposed to resistance due to territoriality. Alternatively, these green spaces could be within the

territories of existing families that don't use them much, but still patrol and exclude other individuals from these areas or may not be defendable, and thus territory doesn't need to be marked with scat. Open green spaces may also be a result of local lethal removals, though based on personal communications only one family group removal occurred from federal agencies during time period, and most local trappers do not trap coyotes within the city (personal communications).

While urban areas act as refugia from larger predators for some species (Shannon et al. 2014) and come with an abundance of resources, they also present a variety of potential costs. For smaller, less mobile species, the linear barriers present in urban areas may limit gene flow substantially. Even for larger, more mobile species, the threat of traffic, varying social tolerances for wildlife, and fragmented natural spaces may present significant barriers to gene flow (Serieys et al. 2015, Kimmig et al. 2019, Schell et al. 2020). While limited gene flow often does not present immediate threats to populations, sustained gene flow limitation may lead to excessive inbreeding. In turn, this may lead to an increase in the number of deleterious alleles found within a population and reduce the population's adaptive capacity, making that population more susceptible to local extinction (Saccheri et al. 1998, Coltman et al. 1999, Keller et al. 2002, Spielman et al. 2004, Keyghobadi 2007). Limited gene flow over sustained periods may lead to local differentiation among populations, potentially affecting neutral and adaptive selection, with important implications for urban wildlife evolution (Andrews 2010). Using neutral markers, multiple studies have found some level of differentiation between urban and rural populations of many different species (Ruthkowski et al. 2006, Chiappero et al. 2011, Gortat et al. 2013, Adducci et al. 2020, Ziege et al. 2020), though others have found no differentiation (Huang et al. 2008, Zeisset & Beebee 2010, Honnen & Monaghan 2017, Indykiewicz et al. 2018, Selonen et al. 2018). These conflicting results among studies highlight the fact that gene flow varies by system and species, even within the same metropolitan regions and at extremely fine geographic scales. Additionally, understanding these neutral evolutionary forces such as gene flow and genetic drift, is an imperative first step to understanding how animals adapt to urban environments.

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4. Chapter 4 - Assessing coyote gene flow across Washington's diverse landscapes through the lens of island biogeography

4.1 Abstract

Island biogeography theory provides a framework for understanding genetic drift, inbreeding, gene flow, speciation, and divergence in wildlife populations. This theory, however, has been under-utilized by urban evolutionary ecologists and may produce insights into the impacts of urbanization on wildlife. We hypothesize that cities may function as semi-isolated "islands" with restricted gene flow to surrounding areas. To test this hypothesis, we investigated genetic variation and landscape drivers of gene flow among 728 coyotes (*Canis latrans*) in urban, wildland, and island regions of Washington State. Urban coyotes exhibited similar allelic richness and F_{ST} values as the two islands with bridges to the mainland, while the island without a bridge had higher F_{ST} and lower richness. Higher correlations among genetic and geographic distances on islands—and to a lesser extent, in urban areas— than the wildland study areas, suggests semi-insular gene flow and short dispersal distances. Pairwise genetic relatedness among coyotes increased with greater impervious surface cover and decreased with higher elevation and water bodies. Combined, these results support natal-biased dispersal, whereby dispersing individuals tend to settle in habitats similar to those they were born in. These findings support the hypothesis that urban environments function as "genetic islands," with restricted dispersal leading to increased genetic differentiation, and highlight the importance of incorporating both built and natural features of the landscape into gene flow models.

Key Words: *Canis latrans*, landscape genetics, microsatellites, urban ecology

4.2 Introduction

Island biogeography is a foundational theoretical framework to understand how habitat area and isolation affect biodiversity through colonization and extinction rates (MacArthur & Wilson 1967). This framework has been applied extensively to fragmented terrestrial ecosystems (Higgs 1981, McCoy 1983, Prugh et al. 2008) and has recently been highlighted as an under-utilized and poignant framework for

understanding the movement and gene flow of urban species (Dunn et al. 2022). Individuals living in small, isolated habitat patches or on actual islands often have limited gene flow with other populations and few immigrants or emigrants (MacArthur & Wilson 1967). Over time, this isolation can lead to reduction in genetic diversity, speciation, or even local extinction (Sobel et al. 2010). Like oceanic islands, cities may see limited movement of individuals into or out of the area. Cities are often surrounded by dense infrastructure and linear features such as waterways, highways, and railroads that can act as barriers for many species (Riley et al. 2006, Miles et al. 2019, Kreling et al. 2024). However, the extent to which urban areas mirror island biogeographic patterns has rarely been tested.

Islands often have unique conditions that have different selection pressures than the mainland, which can facilitate evolution (MacArthur & Wilson 1967). Urban regions similarly have distinct characteristics, such as high amounts of impervious surface, abundant human foods, and increased interactions with people (Diamond & Martin 2021). Understanding how gene flow is affected by landscape type and urbanization not only has important implications for species persistence and genetic diversity, but also for local adaptation (Meek et al. 2023). However, while urban areas conceptually mirror islands in many ways, metropolitan regions are often densely populated by the species that are able to persist there (Fischer et al. 2012). With higher access to caloric resources than their non-urban counterparts, urban animals are often able to produce larger litters (Santini et al. 2018). With this productivity, however, comes competition for space. Thus, movement out of metropolitan areas may be elevated as young individuals disperse and try to find territories of their own. In this case, urban areas may act more as source regions than islands (Kanda et al. 2009, Milsap 2017).

The coyote (*Canis latrans*) is a highly mobile, adaptable canid species that inhabits every terrestrial habitat in North America, including major metropolitan regions (Hody & Kays 2018). Coyote populations are typically genetically diverse, but this species can show signs of founder effects, and occasionally, decreased genetic diversity in urban regions where populations have been highly fragmented (DeCandia et al. 2019). Additionally, some studies have found genotypic differences between urban and wildland coyotes, suggesting variability in patterns of gene flow (Adducci et al. 2020).

Although previous studies have documented barriers to coyote gene flow in urban landscapes (Riley et al. 2006, Kreling et al. 2024), the mechanisms that facilitate or inhibit genetic connectivity in and around urban areas for coyotes remain poorly understood. Understanding the factors that shape gene flow in urban environments for coyotes can provide insight regarding the relevance of island biogeography theory to cities, which may inform conservation strategies for urban species and facilitate human-carnivore coexistence (Miles et al. 2019).

We hypothesize that metropolitan areas function as urban islands, exhibiting restricted gene flow with surrounding regions and levels of genetic isolation that are similar to those found on actual offshore islands. Here, we take advantage of the unique landscape of western Washington, where offshore islands are located adjacent to a major metropolitan area, to test this urban island hypothesis. Specifically, we use microsatellite genotypes from non-invasively collected scat samples and salvaged carcasses across urban, island, and wildland regions of Washington to understand drivers of gene flow, and to specifically assess whether the Seattle-Tacoma Metropolitan Area is acting as a genetic island for coyotes. As with many cities, Seattle is bordered by large bodies of water. Additionally, the region has oceanic islands just off the coast of Seattle, facilitating comparison of urban regions to actual islands. We used two wildland study areas as comparisons to understand if genetic patterns in urban areas are more similar to islands or wildland areas. If urban areas are acting as genetic islands, we expect to see similar allelic richness as on islands and decreased richness compared to wildland areas. We also expect moderate to high levels of genetic differentiation (as measured by F_{ST} values) between islands and wildland areas and between urban and wildland areas, but low levels of differentiation between the two wildland areas. We expect that genetic distance will be less correlated with geographic distance in urban areas as a result of increased anthropogenic barriers. Taken together, these analyses of genetic patterns across more than 700 coyotes inhabiting islands, cities, and wildland regions yield new insights regarding impacts of urbanization on gene flow.

4.3 Methods

4.3.1 Study Area

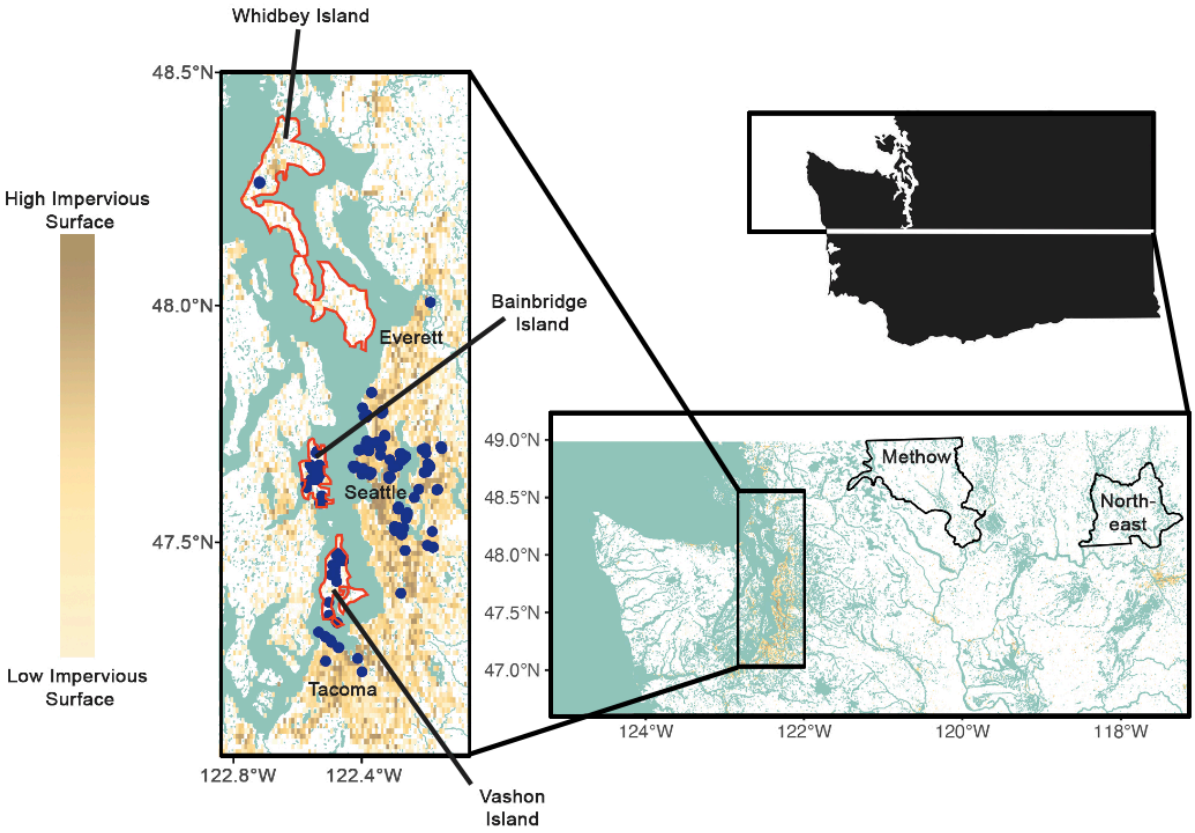

Figure 4.5: Map of study areas across the state of Washington. On the top right, a silhouette of the state of Washington with a bounding box to show the inset location. On the bottom right, is an inset of the northern half of Washington state with the wildland study sites labeled 'Methow' and "Northeast" is shown. On the left, is an inset of the urban and island coyotes. Major cities are labeled and each island (Bainbridge, Vashon, and Whidbey) are outlined in red and labeled. Blue dots represent the average location for each individual coyote genotyped within this region. Both inset maps are plotted with relative levels of impervious surface. For clarity, Canadian islands are not depicted within this map.

We categorize our study areas into three groups – urban, island, and wildland Washington (Figure 4.1). Urban coyote samples were obtained from scat samples and salvaged carcasses obtained from a region ~2,200 km^2 in size bounded longitudinally by Tacoma and Everett, and latitudinally by Tacoma and

Redmond. The majority of the urban samples came from cities such as Seattle (47.611°, -122.335°) and Tacoma (47.250°, -122.436°). The Seattle-Tacoma Metropolitan region is one of the fastest growing regions in the United States and the 15th largest Metropolitan area in the United States, with over 4 million inhabitants. This metro area is a matrix of dense housing, industrial centers, and natural land cover types and experiences a temperate climate, with warm dry summers and cool, exceedingly wet winters (Kottek et al. 2006). Natural forest fragments are dominated by Douglas fir (*Pseudotsuga menziesii*), red cedar (*Thuja plicata*), bigleaf maple (*Acer macrophyllum*), and western hemlock (*Tsuga heterophylla*). The region is bordered by the Puget Sound to the west and the Cascade Mountain Range to the east. The region has several major highways that crisscross the area including Interstate-5, I-90, State Route 99, and I-405. Between and surrounding Seattle and Tacoma are smaller, less densely populated cities.

Our island study areas consist of Vashon Island (95.6 km^2; 47.424°, -122.479°), Whidbey (436.9 km^2; 48.162°, -122.602°), and Bainbridge Island (71.95 km^2; 48.176°, -122.612°). All islands are located within the Puget Sound. At its closest point, Vashon Island is 2.7 km away from Tacoma and 1.6 km from the Kitsap Peninsula. Bainbridge Island is 260 m from its closet point on the Kitsap Peninsula and is connected at this point via the Agate Passage Bridge. Whidbey Island is connected to the mainland via the Deception Pass Bridge at the northern end of the island. Land cover on the islands consists of low density housing, agricultural lands, and fragmented forest spaces dominated by the same tree species as on the mainland. All islands experience cool Mediterranean climates and elevations below 150 m.

Our wildland study areas are located in the Methow Valley (5,300 km^2; 48.554°, -120.131°) and northeastern Washington (4,535 km^2; 48.547°, −117.896) and were the study areas for the Washington Predator Prey Project (https://predatorpreyproject.weebly.com/). The Methow study area consists of elevations from 230-2830m and is dominated by coniferous forests and shrub-steppe. Elevations in the northeastern study area range from 370-2080m and the site is dominated by montane conifer forest and agriculture-filled valleys. Both areas contain few anthropogenic structures and experience cold, wet winters, and warm summers (Ganz et al. 2024).

4.3.2 Sample Collection

Scats were collected from 2017-2023 semi-systematically, where regular transects were surveyed 1-2 times per month and additional scats were opportunistically collected. Seattle coyote scats were primarily collected in summer (June – September) 2021, with some opportunistic collection year-round and a less intensive field season in the summer of 2022. Scats in the cities surrounding Seattle were collected primarily during summers 2022 and 2023. Vashon Island scats were collected once per month during summer 2021, with additional scats collected by volunteer residents from 2017-2021 year-round. Bainbridge Island scats were collected by a technician who lived on the island in summer 2021. Wildland scats were collected during summer and winter 2018-2020 as part of the Washington Predator Prey Project.

Each scat was collected into a Ziploc bag and then placed in a Whirlpak. After collection, scats were immediately frozen upon return from a field site. Scats spent a minimum of two weeks in a -80°C freezer as a safety precaution to kill any potential *Echinococcus* eggs (Hildreth et al. 2004). After two weeks, scats either continued to be stored at -80°C or were transferred to a -20°C freezer prior to DNA extraction.

Additional tissue samples were obtained from opportunistically salvaged carcasses sourced from coyote-vehicle collisions, PAWS Rehabilitation Center, or the USDA Wildlife Services (i.e., “conflict removals”) across the Greater Seattle Metropolitan Area during 2021-2024.

4.3.3 DNA Extraction

DNA extractions and polymerase chain reactions (PCRs) were conducted at the University of Washington School of Environmental and Forest Sciences Genetics Lab. The outside of each scat was swabbed with a flocked swab wetted with phosphate buffered saline solution. Then DNA was extracted using a modified protocol for the QIAmp DNA Investigator Kit (Qiagen; Hilden, Germany). For carcass samples, DNA was extracted either from ear or tongue tissue using the Qiagen DNA Blood and Tissue Kit, following the standard protocol.

4.3.4 Microsatellite Genotyping

We amplified DNA at 12 microsatellite loci and each sample had three replicate PCRs to screen for genotyping errors (*sensu* Prugh et al. 2005). The microsatellite loci were split between two panels. The first panel included the following nuclear microsatellite loci: FH2001, FH2010, FH2054, FH2088, FH2328. The second panel consisted of the loci: CXX2235, FH2096, FH2137, FH2140, FH2159 and 2 additional microsatellite loci on the X and Y chromosomes to determine sex (DBX6, DBY7; Prugh et al. 2005, Seddon 2005). Cycling conditions were the same for both panels and performed as touchdown-PCR: initial denature step of 95°C for 15 min, followed by 10 touchdown cycles of (94°C for 30s, 68°C - 1°C per cycle for 30s, 72°C for 45s), followed by 30 cycles of (94°C for 30s, 58°C for 30s, 72°C for 45s), followed by a final extension of 60°C for 15 min. PCR products were immediately frozen after completion and then sent to Yale's Keck DNA Sequencing Core for fragment analysis on an Applied Biosystems 3130 Series Genetic Analyzer using capillary electrophoresis. We quantified allele sizes with GeneMapper (Curie-Fraser & Shah 2010) and formed consensus genotypes from the replicates following procedures described by Prugh et al. (2005). Genotypes were then imported into GeneAlEx in Excel (Peakall & Smouse 2012). All consensus genotypes were examined to rule out domestic dog origin based on alleles found in reference dog samples.

PIDsib is the probability that two siblings randomly drawn from a population will have the same genotype (Waits et al. 2001). For confident assignment of individuals, PIDsib values less than 0.01 are ideal (Waits et al. 2001). For all samples except those from Vashon Island (see below), individual IDs were assigned to genotypes observed where at least one sample contained 20 or more confirmed alleles (except for Vashon Island). Samples did not need to match another genotype to be given a unique ID. Carcass-based samples were always complete (i.e., 24 alleles) and accepted without a match. Because of high degrees of inbreeding, for Vashon Island samples, we required all 24 alleles to have successfully amplified and matched another sample without any allelic mismatches. Even then, based on PIDsib values, there was a 2% chance assigning a genotype to one individual when it represented two

individuals. For all allelic analyses, the sex ID markers were removed because these were fixed markers on the X chromosome, and binary on the Y chromosome denoting sex rather than allelic diversity.

4.3.5 Allelic Measures

To detect underlying patterns of genetic diversity, we first calculated allelic richness across all samples and within each sampling group. To account for the positive relationship between sample size and richness, we calculated rarified allelic richness per locus and group using the 'allellic.richness' function from the *hierfstat* package in Program R, which accounts for uneven sample sizes among groups (v0.5-11; Goudet & Jombart 2022). As is standard, we also calculated Weir & Cockerham's (1984) Fixation Index (F_{ST}) using the 'pairwise.WCfst' function from the *adegenet* R package (Jombart 2008). F_{ST} measures population differentiation and provides a framework for estimating population structure.

4.3.6 Genetic & Geographic Distance

For genetic distance modeling, we reduced our dataset to coyotes that had a pairwise relatedness value below 0.5 to remove first-ordered parent-offspring and full-sibling pairs (Lynch and Ritland 1999). Relatedness was calculated in GeneAlEx (Peakall & Smouse 2006) and one coyote from each first-order pair was manually removed from the dataset. This removal ensured that we assessed gene flow without including groups of pups that had not yet dispersed from their natal territories and had not yet contributed to gene flow within the system. While this may also eliminate some dispersed individuals, inspection of our dataset indicated that we captured very few dispersal events, most of which came from salvaged carcasses. We also limited the dataset for this analysis to coyotes that had complete genotypes, as Nei's genetic distance estimator cannot accommodate missing data. We calculated pairwise Nei's bias-corrected genetic distance (Nei 1978) using the 'nei.dist' function from the *poppr* package in R (v2.9.4; Kamvar et al. 2014). This function modified Nei's genetic distance so that it can be applied to genetic distances between individuals rather than just between populations. Nei's genetic distance is especially useful in fragmented landscapes where gene flow may be affected by genetic drift (Nei 1978).

For each individual coyote from which we collected multiple scats, we calculated the centroid of all scats collected from the individual. Whidbey Island coyotes were typically conflict removals from naval base airports of undisclosed locations. For these individuals, we assigned a location equidistant between the two naval bases from which they were possibly collected. We used location of death for all other salvaged carcasses. We then made pairwise calculations of Haversian geodesic distance (hereafter 'geographic distance') from each coyote to all other coyotes in the dataset using the 'distance' function from the *terra* package in R (Hijmans 2023). This measurement calculates a straight-line distance between each coyote with a correction to account for the curvature of the Earth.

4.3.7 Mantel Test

We used a Mantel test to assess isolation by distance. This tests the correlation between geographic distance and Nei's genetic distance for all pairs of coyotes (Mantel 1967). We ran one Mantel test for all coyotes as well as separate tests for coyotes in each landscape type (urban, island, and wildland). We expected weaker isolation-by-distance patterns to be found among urban coyotes than among wildland coyotes as urban areas tend to have more fragmentation and linear barriers. The Mantel test used Pearson's product-moment correlation with 999 permutations and was conducted using the 'mantel' function from the *vegan* package in R (v2.6-4; Oksanen et al. 2022).

4.3.8 Spatial Covariates

We extracted covariates from a variety of spatial data layers that we hypothesized could affect gene flow for inclusion as predictors in generalized linear models (Table 4.1). First, we created straight lines between every possible pair of coyotes. We then created a 500m buffer along this line (1 km width) from which we extracted covariates. Some studies have used values extracted from a straight line between nodes, but we included a buffer to better represent the landscape characteristics between each pair (Pless et al. 2021).

To understand how natural features of the landscape affect gene flow, we included tree canopy cover, water, and elevation as covariates. The proportion of tree canopy cover in each buffer was

extracted from the National Land Cover Database 'Tree Canopy' layer (NLCD 2021). We then calculated the proportion of each buffer covered by large water bodies (e.g., lakes, rivers, sounds) extracted from the 'Visible Surface Water' dataset from the Washington Department of Fish Wildlife (WDFW 2021). The Cascade Mountain Range occurs between the wildland and urban/island regions, and we extracted the average elevation across each buffer from a digital elevation model (DEM) obtained via the Washington State Geospatial Data Archive to account for this geographic barrier.

To assess how anthropogenic features of the landscape affect gene flow, we included impervious surface cover and highways as covariates. We extracted the average amount of impervious surface within each buffer using the National Land Cover Database's (NLCD) 'Urban Imperviousness' dataset (NLCD 2019a). We used the TIGER US Census Bureau's 'Primary and Secondary Roads State-based' dataset and extracted only the primary highways within Washington (US Census Bureau 2021). We added a 15m buffer to each highway to account for the wider width of highways compared to normal roads and calculated the proportion of each buffer comprised of highways.

In addition to extracting our spatial covariates, we created an "island status" categorical variable to better quantify how being on an island affects genetic distance. The 'island status' variable categorized a pairing of coyotes based on whether each coyote in a dyad was located on the same island, on different islands, on an island and the mainland (e.g., one on Bainbridge Island, one in Seattle), or both on the mainland. This led to 10 separate categories: Bainbridge-Bainbridge, Vashon-Vashon, Whidbey-Whidbey, Bainbridge-Vashon, Bainbridge-Whidbey, Vashon-Whidbey, Bainbridge-mainland, Vashon-mainland, Whidbey-mainland, mainland-mainland.

Table 4.1: Table of variables used within our models and inclusion justification.

Variable	**Inclusion Justification**
Geographic Distance	Objects closer together in space are often more similar. With coyotes, we'd expect that genetic distance would be somewhat correlated with geographic distance.
Highways	Highways can represent a major mortality source for coyotes (Margenau et al. 2023). Additionally, they may represent gene flow barriers in which coyotes either die crossing a highway or do not attempt crossing the highway (Kreling et al. 2024).
Impervious Surface	Areas with high impervious surface may facilitate coyote movement by creating linear paths for them to travel and may represent areas with increased food resources due to human trash, feeding, and compost. Alternatively, areas with high impervious surface may have little natural space to serve as cover for coyotes and have high densities of roads which may act as a barrier to coyote movement and a source of mortality depending on traffic volume and speed.
Island Status	Island status denotes which island each coyote is on. In theory, coyotes on islands should be more distantly related to each other and to the mainland than coyotes on the same island or on the mainland as a result of water barriers. Individual islands may also have different gene flow rates from the mainland as a result of having bridges or variable distance to the mainland.
Tree Canopy	Areas with high tree canopy cover may facilitate coyote movement by providing habitat and potentially acting as corridors between areas of high impervious surface.

Water	While coyotes can swim, we expect that wide waterways and the Puget Sound will act as strong barriers to gene flow as supported by earlier work (Kreling et al. 2024).

4.3.9 Generalized Linear Modeling

To understand drivers of genetic distance across diverse landscapes, we ran generalized linear models using the natural and anthropogenic spatial covariates we hypothesized could affect gene flow. These models were run with the 'glm' function from the *stats* package in R. We modeled our response variable, Nei's genetic distance, as a function of spatial covariates (described below) with a Gaussian distribution. We did not include a random effect individual ID, because it has been suggested that including more direct measures of spatial autocorrelation is better for modeling dyadic data (Neumayer & Plümper 2010). Thus, geographic distance was always included as a covariate to account for spatial autocorrelation (Saas & Gosselin 2014).

All variables were normalized using the 'scale' function in base R, and we calculated correlations among covariates using the 'corrplot' function from the *corrplot* package (Wei & Simko 2021). Covariates with $r > 0.7$ were not included within the same model to prevent model overfitting (Dormann et al. 2013; Appendix B Figure S1). Impervious surface and elevation as well as impervious surface and highways were highly correlated. We ran separate full models with impervious surface and with elevation, but the two variables were not combined in one model. None of the three variables were ever used in the same model. We used AIC values to evaluate a model set that included a hypothesis-driven full model, a null model, and reduced combinations of covariates by removing non-significant varaibles (6 total models). To visualize the results, we created coefficient plots of covariate β coefficients using the 'plot_model' function from the *sjPlot* package in R (Lüdecke 2024). We evaluated model performance using R^2 values from the 'r2' function in the *performance* package in R (Lüdecke et al. 2021).

4.3.10 DAPC Analyses

To detect underlying spatial structuring within our urban and island coyote samples, we conducted a Discriminant Analysis of Principle Components (DAPC) with highly related individuals removed from the dataset. We ran the DAPC blind, without inputting *a priori* groupings or collection locations. This allows the algorithm to detect structure without being informed, and potentially biased, by these delineations. We used the 'find.clusters' function from *adegenet* package (Jombart 2008) to determine the optimal *k* numbers of groupings in the data. Using the output charts from this function, we selected the lowest number of principle components (PCs) to retain while obtaining maximal cumilitave variance, and the smallest *k* value with low Bayesian information criterion (BIC). We then used these PC and *k* values to inform the 'dapc' function from the *adegenet* package (Jombart 2008). We extracted the probability of assignment to each group for each individual coyote. We separated any individuals that were less than 50% probability of assignment to a single group into a separate 'admixed' category; otherwise, all individuals were assigned to the group with the highest probability.

4.3.11 Multinomial Logistic Regression

To understand drivers of the underlying structure detected with the DAPC analyses, we conducted multinomial logistic regressions for both the rural DAPC group assignments and island/urban group assignments. We used the 'multinom' function from the *nnet* package in R (Venables & Ripley 2002). We compared group assignment to scaled impervious surface levels.

4.4 Results

4.4.1 Sample Collection & Genotyping

We collected 5,331 scat samples and 78 carcass tissue samples, resulting in a total of 728 individual coyotes identified across all areas (Table 4.2). We collected 1,622 scats and 35 carcasses from the urban region and identified 223 unique individuals. Across the three islands, we collected 622 scats and 43 carcasses, identifying 112 unique individuals (38 on Bainbridge Island, 29 on Vashon Island, 45 on

Whidbey Island). In the two wildland study areas, we collected 3,087 scats and no carcasses, resulting in the identification of 409 unique individuals.

Table 4.2: Sample collection summary with number of total scats and carcasses collected for each study area. Urban sampling areas were further broken down by county to facilitate understanding of the geographic coverage of samples, but these county-level divisions were not used in any analyses. Size of each area is reported as the total amount of land (as opposed to land and water) within the geographic boundaries of each area. The right most column lists the total number of unique individuals identified by across the scat and caraccas samples.

Study Area	**Collection Area**	**Land Area Size (km^2)**	**Total Scats Collected**	**Total Carcasses Collected**	**Total Individuals Identified**
Urban	Greater Seatte Area	1,175	1,622	35	207
Wildland	Methow	5,300	2,029	0	254
	Northeast	4,535	1,058	0	155
	Total	9,835	3,087	0	409
Island	Bainbridge	72	407	0	38
	Vashon	96	206	0	29
	Whidbey	437	9	43	45
	Total	605	622	43	112
	Grand Total	**11,615**	**5,331**	**78**	**728**

4.4.2 Allelic Richness & F_{ST}

Rarified allelic richness (to the lowest sample size; n=29) also varied considerably across study sites (Table 4.3). As predicted, wildland coyotes (Methow and Northeast) had the highest allelic richness ($\bar{x}$=100, 108 respectively), followed by urban coyotes ($\bar{x}$=81), and finally island coyotes ($\bar{x}$=50). Among islands, allelic richness varied substantially, with the highest richness on Whidbey Island ($\bar{x}$=72) and lowest on Vashon Island ($\bar{x}$=28).

Table 4.3: The table below represents rarified allelic richness values across study sites. Values were rarified to the lowest number of individuals sampled within a location (n=29). Bainbridge, Vashon, and Whidbey are island locations and Northeast and Methow are wildland locations.

Study Area	Total Alleles	FH2001	FH2010	FH2054	FH2088	FH2328	CXX2235	FH2096	FH2137	FH2140	FH2159
Bainbridge	49.2	4.8	2.0	3.0	4.0	4.8	4.7	2.0	7.7	8.5	7.7
Vashon	27.9	4.0	3.0	1.0	3.0	3.0	2.0	2.0	3.0	2.0	4.9
Whidbey	72.4	6.3	2.7	6.7	5.8	8.3	8.8	4.3	7.5	10.1	11.9
Urban	81.2	7. 4	3.1	4.6	4.8	11.8	8.8	4.5	11.1	13.2	11.9
Northeast	107.7	5.9	5.6	6.6	9.1	17.9	7.8	5.2	13.2	20.0	16.4
Methow	100.5	7.1	4.9	6.0	6.6	16.1	8.4	5.1	12.2	18.9	15.2

Pairwise F_{ST} values among study sites varied considerably but generally aligned with the prediction that coyotes in wildland regions would have low genetic differentiation and coyotes on islands would have substantial differentiation (Figure 4.3). There was little genetic differentiation ($F_{ST} < 0.05$) between the Methow and Northeast (i.e., wildland) coyotes. Vashon Island coyotes had very high levels of genetic differentiation ($F_{ST} > 0.25$) from Whidbey Island and high differentiation ($F_{ST} = 0.15 - 0.25$) from all other study sites. Whidbey Island and Bainbridge Island coyotes also were also highly differentiated ($F_{ST} > 0.25$), and all other pairings (including urban coyotes) had moderate degrees of genetic differentiation ($F_{ST} = 0.05 - 0.15$).

Pairwise F_{ST} Values	Bainbridge	Vashon	Whidbey	Western Washington	Methow	Northeast
Bainbridge	1.000					
Vashon	0.253	1.000				
Whidbey	0.125	0.267	1.000			
Western Washington	0.061	0.193	0.066	1.000		
Methow	0.092	0.210	0.067	0.043	1.000	
Northeast	0.093	0.206	0.070	0.061	0.017	1.000

Figure 4.6: Pairwise Weir-Cockerham F_{ST} Values. Values are color-coded by low differentiation (<0.05), moderate differentiation (0.05-0.15), high differentiation (0.15-0.25), and very high differentiation (>0.25). Lighter colors represent lower degrees of genetic differentiation.

4.4.3 Isolation by Distance

Mantel tests revealed low correlation between geographic distance and genetic distance across all coyotes ($r = 0.216$, $p = 0.001$). Contrary to expectations that genetic correlation with geographic distance would be weaker in urban areas, wildland coyotes had the weakest isolation by distance ($r = 0.059$, $p = 0.001$), urban coyotes had a stronger, though still weak relationship ($r = 0.179$, $p = 0.001$), and island coyotes exhibited the strongest relationship ($r = 0.494$, $p = 0.001$).

4.4.4 Drivers of Gene Flow

When modeling genetic distance across all coyote dyads with complete genotypes (n = 335 individuals, 55,571 dyads), the top model included geographic distance, impervious surface, amount of water, and island status (Table 4.4, Figure 4.3). Impervious surface, water, and Vashon-Vashon island status were associated with decreased genetic distances. Geographic distance and all other island status categories were associated with increased genetic distances.

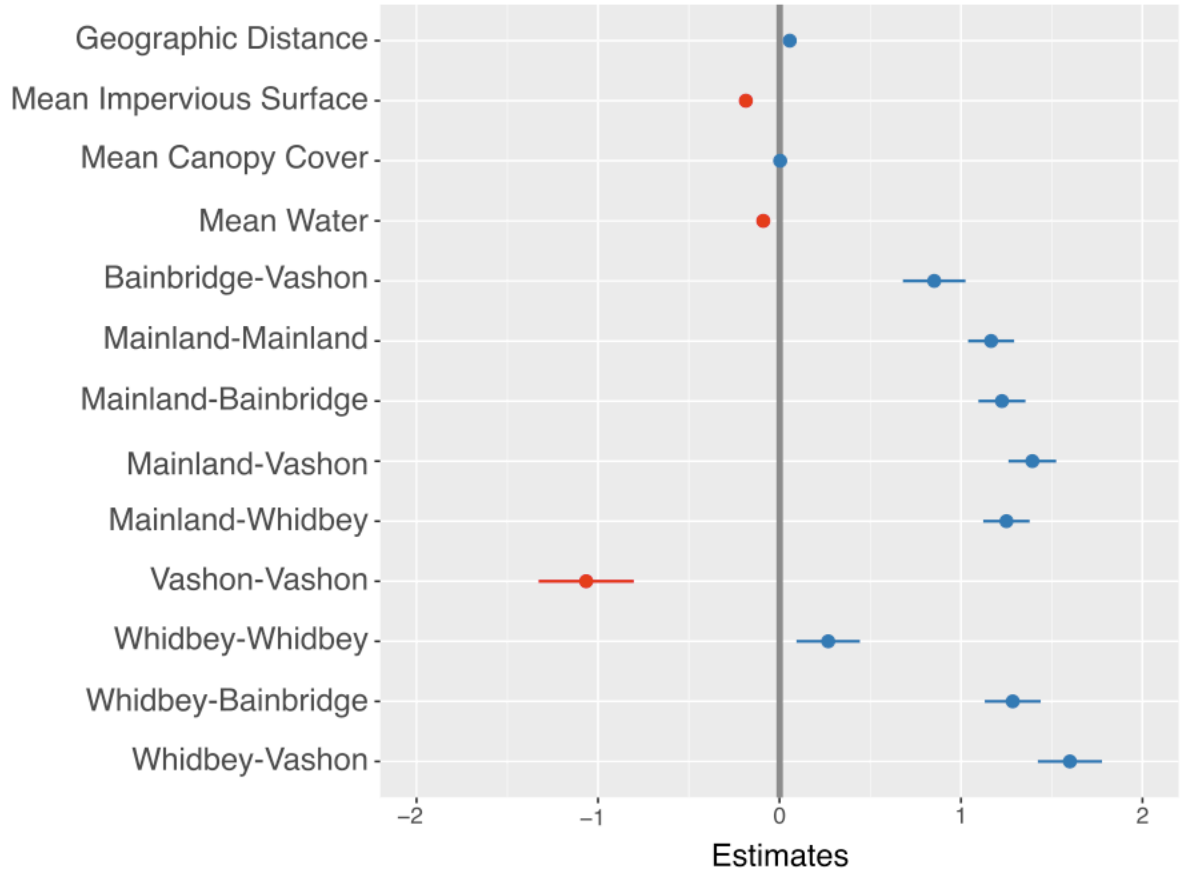

Figure 4.3: Covariate plot for top regression model of Nei's genetic distance against covariates. Variables that are associated with increased genetic distances among individuals (blue) are right of the dark vertical line. Variables that are associated with decreased genetic distances among individuals (red) are left of the dark vertical line. Each dot represents a mean and is accompanied by confidence intervals.

Table 4.4: Model results for all dyads of coyotes across Washington. Beta coefficients are listed for the models next to each variable in the model. Ranges are reported for the categorical variable, 'Island Status'. Degrees of freedom was 55,570. Statistical significance codes are listed after beta coefficients. Model AIC and R^2 values are listed to the right. **p<0.01, ***p<0.001

Model	**AIC**	**ΔAIC**	**R^2**
Nei's Genetic Distance ~ Geographic Distance(β=0.057)*** + Impervious Surface(β=-0.186)*** + Water(β=-0.091)*** + Island Status(β=-1.072 to 1.592)**to***	**152698**		0.087
Nei's Genetic Distance ~ Geographic Distance(β=0.057)*** + Impervious Surface(β=-0.185)*** + Tree Canopy(β=0.004) + Water(β=-0.089)*** + Island Status(β=-1.065 to 1.600)**to***	152700	2	0.087
Nei's Genetic Distance ~ Geographic Distance(β=0.086)*** + Elevation(β=0.210)*** + Tree Canopy(β=-0.020)*** + Highway(β=-0.038)*** + Island Status(β=-1.044 to 1.243)**to***	152785	87	0.086
Nei's Genetic Distance ~ Geographic Distance (β=0.106)*** + Tree Canopy(β=0.053)*** + Highway(β=-0.142)*** + Island Status(β=-0.897 to 1.539)***	153674	976	0.071
Nei's Genetic Distance ~ Geographic Distance(β=0.189)***	157566	4,868	0.036
Null	159605	6,907	0.000

4.4.5 DAPC Analyses & Population Structuring

For the DAPC analysis with urban and island coyotes, we retained 35 principal components and detected $k = 6$ groups (Figure 4.4). All individuals were assigned to a group with greater than 50% probability or more. The number of individuals assigned to each group ranged from 12 to 59 ($\bar{x} = 31$, $SD = 16.89$). Group 3 was composed entirely of individuals from Vashon Island, while the rest of the groups were composed of coyotes from the mainland, Bainbridge Island, and Whidbey Island. Multinomial logistic regression revealed positive associations between amount of impervious surface and assignment to groups 2 and 5 compared to group 1, with a one unit increase of impervious surface corresponding to 0.42 and 0.64 increase in the log odds of being in assigned to groups 2 and 5 respectively vs group 1. Groups 3 and 6 were negatively associated with impervious surface cover, with a one unit increase in impervious surface corresponded to a 3.54 and 0.82 decrease in the log odds of being assigned to groups 3 and 6 respectively vs group 1. There was very little association between impervious surface and assignment to

groups 1 and 4, with a -0.01 and 0.07 change in the log odds of being assigned to group 1 or 4, respectively, with a one unit increase in impervious surface.

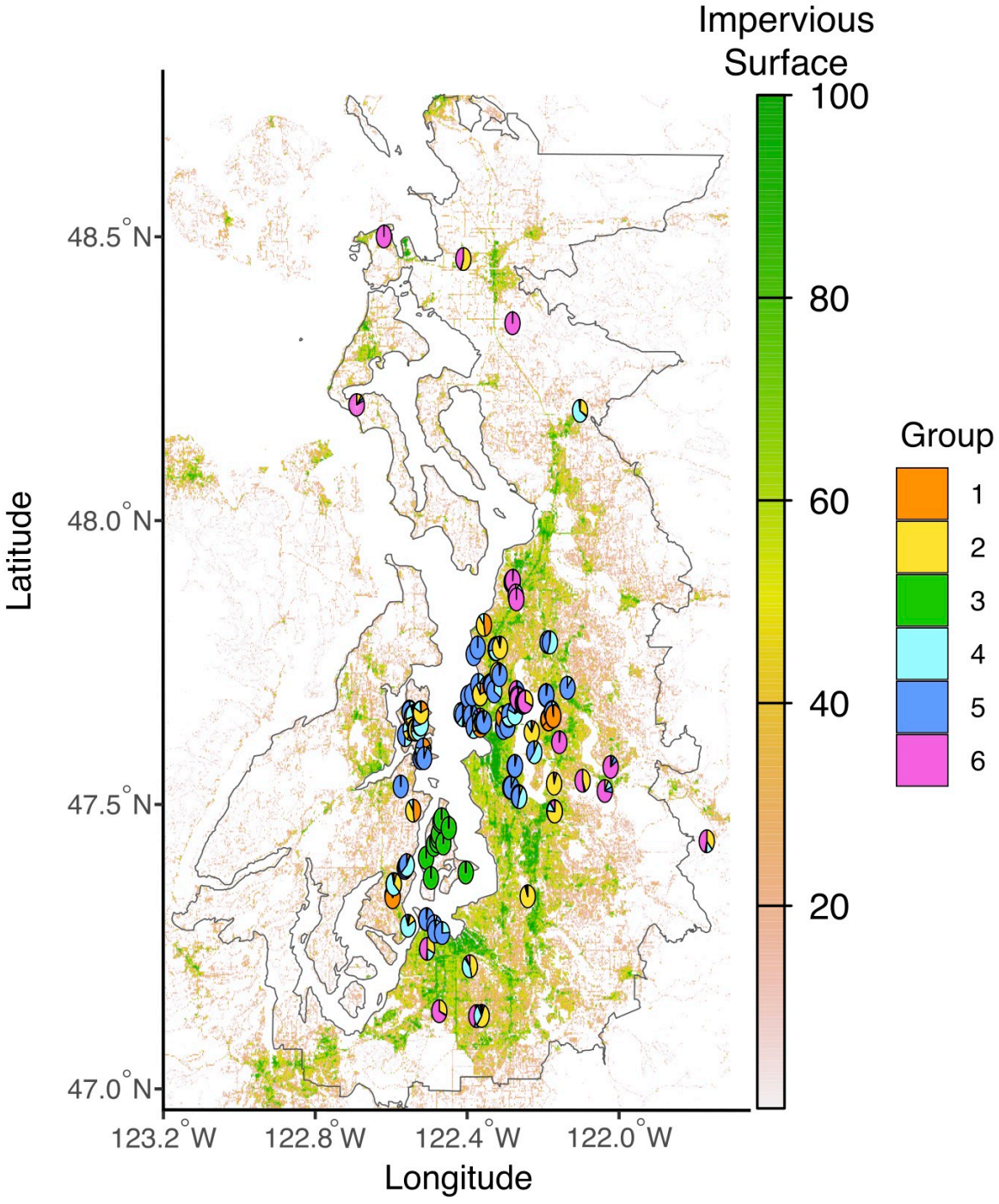

Figure 4.4: Mapped results of DAPC analysis. Each circle represents an individual coyote and its probability of being assigned to each of the 6 identified groups. Mapped beneath the circles is the percent of impervious surface.

4.5 Discussion

In many ways, urban regions conceptually mirror oceanic islands (Dunn et al. 2022) – they offer unique selection pressures and barriers to movement in and out of the region (Miles et al. 2019, Diamond and Martin 2021). Theory would suggest that urban areas with strong levels of fragmentation and potential genetic drift may have decreased genetic diversity, and genetic distance may be poorly correlated with geographic distance (Miles et al. 2019, Dunn et al. 2022). Additionally, genetic distance among individuals may be more likely to be driven by anthropogenic barriers than natural ones (Riley et al. 2006, Adducci II et al. 2020). However, the extent to which urban areas may function as genetic islands has rarely been tested (Kimmig et al. 2020, Dunn et al. 2022). While many studies test for carnivore population isolation and determine subpopulations, few studies explicitly test for drivers of gene flow. Using a large dataset of coyote genotypes from across Washington, we assessed drivers of genetic distance in relation to urbanization and compared these drivers and allelic metrics among urban coyotes, wildland coyotes, and coyotes on physical islands. Our urban areas acted similarly to islands with bridges connected to the mainland, exhibiting decreased allelic richness, increased isolation by distance, and similar levels of genetic differentiation to the wildland coyotes. These findings support the use of island biogeography theory in understanding urban gene flow and animal movement.

Many studies have found that geographic distance is a poor predictor of genetic distance, especially in urban regions characterized by anthropogenic barriers that severely impede animal movements (Semple Delaney et al. 2010, Taylor et al. 2011, Munshi-South 2012). However, while we generally found low explanation of variance among our urban and wildland coyotes, we found that geographic distance explained roughly 3% of the variation in genetic distance for the urban coyotes compared to less than 0.5% in wildland coyotes. For island coyotes, isolation by distance was much stronger, at 25% likely reflecting large genetic distances among rather than within islands. Carnivores in urban areas often have home ranges that are a fraction of the size of their wildland counterparts and disperse shorter distances from their natal territories (Zepeda et al. 2021, Gelmi-Candusso et al. 2024a). In wildland areas, individuals can roam without anthropogenic barriers and lower densities of

conspecifics, allowing dispersals to be longer and less geographically constrained. Increased individual movement can lead to increasingly panmictic populations (Reudink et al. 2011, David 2018). In this way, urban dispersal may be more similar to that of an island, where access to territory and physical landscape barriers constrain dispersal distances. In regression models, geographic distance was a significant predictor of genetic distance, but its effect was relatively weak when other environmental variables were included. Similarly, testing just geographic distance in models was only slightly more predictive than the null model, indicating that features of the landscape are much more important for predicting genetic distance than geographic distance.

Island status was also an important variable in our models and the strength and directionality of genetic distances varied among islands. F_{ST} values indicate that Vashon Island is significantly differentiated from all other sampling groups. High degrees of inbreeding have led to coyotes that are highly related on the island, resulting in multiple fixed markers and low allelic richness. Rarified allelic richness was much higher on both Bainbridge and Whidbey islands, likely due to a physical connection to the mainland via a bridge on each island. However, F_{ST} values still indicate low to moderate levels of genetic differentiation between the two islands and the mainland. Like coyotes on actual islands, urban coyotes had lower allelic diversity than coyotes in both of the wildland study sites. These individuals displayed similar decreased allelic richness patterns to other carnivore species on islands, such as Channel Island Gray Foxes (*Uroxyon littoralis*; Funk et al. 2016) and pygmy raccoons (*Procyon pygmaeus*) and pygmy coatis (*Nasua nelsoni*; Flores-Manzanero et al. 2022). Allelic richness in urban coyotes was closer to that of Whidbey Island than to the wildland study sites, but not as low as that on the other two islands. These findings suggest that urban regions may be semi-permeable islands for mobile species like coyotes, where gene flow is relatively insular, but like islands with a bridge, some gene flow with non-urban regions occurs.

This insular geneflow in urban areas is further supported by the negative effect of impervious surface on genetic distance revealed by the general linear models and supported by the influence of impervious surface on DAPC genetic groupings. While this could suggest that impervious surface is

facilitating movement of coyotes, this finding more likely reflects lack of movement between urban and non-urban regions. Coyotes born in urban areas are likely dispersing within urban regions due to natal-biased dispersal, whereby dispersing individuals tend to settle in areas that are similar to their natal territory (Sacks et al. 2004). Similarly, coyotes outside of urban regions may not be dispersing into urban regions because of natal-biased dispersal as well as lack of open territory space in the higher-density urban regions (Fischer et al. 2012). These results are supported by the DAPC analyses showing distinctive genetic groups correlated with both urban (groups 2 and 5) and rural (groups 3 and 6) land cover, with little admixture within groups. Additionally, the DAPC analyses showed similar patterns in our urban regions and surrounding rural areas as our bridged islands did, in contrast to individuals on Vashon Island which grouped separately from all other individuals. We suggest that future studies explicitly monitor movement and demographic rates to understand rates of gene flow between urban and non-urban regions (Miles et al. 2019).

Few studies have looked at both natural and anthropogenic drivers of gene flow for carnivores across broad scales (Mcrae et al. 2005, McManus et al. 2014, Creel et al. 2019). As seen with other carnivore species, tree canopy cover appears to facilitate gene flow, reducing genetic distance between individuals (Swanepoel et al. 2012, Warren et al. 2016, Evans et al. 2018). Contrary to other studies indicating that roads and highways act as major barriers to carnivore movement and geneflow (Ceia-Hasse et al. 2017, Carvalho et al. 2018, Kimmig et al. 2019), our models did not indicate that highways were acting as significant barriers. However, our prior analysis restricted to Seattle showed that Interstate-5 had significant effects on genetic structuring within the city (Kreling et al. 2024). This discrepancy likely reflects a difference in scale. At fine scales, highways in these landscapes may act as barriers, but they may have little effect on the overall genetic structuring across a broader region (Anderson et al. 2010, Murphy et al. 2018, Sexton et al. 2024). This scale-dependence mirrors other work indicating that genetic structuring can be scale-dependent. For instance, McRae et al. (2005) found that mountain lions (*Puma concolor*) exhibited genetic structuring across regions, but also different structuring within each region as a result of different habitat types.

Our generalized linear modeling approach to identify landscape features affecting gene flow does not account for the non-linear routes that coyotes would likely use to move across the landscape. Resistance or least-cost path models are often used in landscape genetic analyses (e.g., Spear et al. 2010, Zeller et al. 2012, Day et al. 2024), but this approach has several drawbacks including substaintally increased computational demands that was less feasible in our study due to the large number of dyads. For example, Pless et al. (2021) examined factors affecting mosquito geneflow in the southeastern US, and they found that a model with linear paths did not perform as well as an optimized least cost paths model. However, this study had 703 dyads compared to our >50,000, was conducted on a species with very minimal movement capability, and had geographic features within straight lines, such as the Gulf of Mexico, that made linear paths especially unrealistic. Even still, model performance only increased from root mean squared error (RMSE) of 0.0385 for the linear model to 0.0347 for the optimized least cost paths model. Other studies have also shown that mantel-type analyses perform as, or nearly as well as, more complicated resistance models (Shirk et al. 2017), and animal behavior and movement studies have shown that animals often deviate from least cost paths (Sawyer et al. 2011, Shirabe 2018, Wilson et al. 2021). Furthermore, resistance frameworks generally require setting resistance values through subjective expert opinion or hypothesis-driven model selection (Sawyer et al. 2011). However, coyotes often display highly variable behavior across individuals and populations (Murray & Cassady St. Clair 2015, Chamberlain et al. 2021, Thompson et al. 2021). Linear features of the landscape that are often considered highly resistant for most species, such as highways, may actually facilitate movement for certain coyotes as they are known to travel using linear infrastructure (Gelmi-Candusso et al. 2024b). Other individual coyotes may find highways to be a physical or psychological barrier. A species as adaptable and variable as the coyote would require evaluating a wide range resistance values for landscape covariates, and such a model would require substantial computational resources and increase the chances of spurious results (Anderson et al. 2001). While our modeling framework would be inappropriate for identifying the actual movement paths of coyotes across Washington, our large sample size incorporates substantial variability in the proportions of different landscape features separating dyads, and thus we feel our linear analysis

provides useful insights despite its limitations. In addition, our DAPC analysis generally aligned with the linear models in highlighting the importance of impervious surface as an important factor affecting coyote gene flow.

While coyotes are widespread and still expanding their range (Hody & Kays 2018), understanding drivers of their gene flow has important conservation and evolutionary implications. If urban carnivores are primarily dispersing within urban areas, localized adaptation to the urban environment may be facilitated (Meek et al. 2023). Over time, this insularity could also lead to decreased genetic diversity and higher influence of genetic drift (Allendorf 1986, Miles et al. 2019). Our understanding of how urbanization affects evolution and gene flow is still nascent but has relevance for ensuring the persistence of rare urban species (Allendorf et al. 2012). By studying gene flow of common species such as coyotes, we may be able to apply lessons to these rarer species where obtaining a sufficient sample size may be challenging (Golding et al. 2024). This study supports island biogeography as a relevant framework for urban wildlife, which will facilitate our understanding of gene flow and animal movement in a rapidly urbanizing world.

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5. Chapter 5 – Feast or famine: How food deserts, wealth, and greenspace influence coyote diets in Seattle, Washington

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5.1 Abstract

Urban environments create unique challenges for wildlife, where dietary habits may reflect both ecological and societal disparities. In this study, we explored how social-ecological factors, such as household income and human population density, and ecological factors such as tree canopy shape coyote (*Canis latrans*) diets in Seattle, Washington. We hypothesized that coyotes in lower-income and food insecure neighborhoods (i.e., "food deserts") would consume more anthropogenic foods, while those in wealthier, greener areas would have diets richer in non-anthropogenic foods. We used non-invasive fecal metabarcoding to obtain a high-resolution dataset on population-level coyote diet. Using generalized linear mixed models and multivariate analyses, we found significant variation in diet among city parks, but socioeconomic factors explained limited variance in dietary composition. Although socioeconomic factors explained limited variance in diet composition, illegal dumping was a strong predictor of non-native rodent and rabbit consumption. Surprisingly, tree canopy cover was negatively associated with diet diversity and household income did not significantly affect dietary diversity, suggesting that coyote diet is influenced by individual behavior and localized prey availability to a greater degree than human-driven socioeconomic patterns. These findings suggest that coyotes utilize a wide range of food resources, both non-anthropogenic and anthropogenic, depending on site-specific conditions. High-resolution spatial and dietary data can thus reveal the ways that ecological and socioeconomic factors combine to shape wildlife behavior in cities.

5.2 Introduction

Life for urban animals is inextricably intertwined with human society and the ways our cities are developed and maintained (Des Roches et al. 2020, Schell et al. 2020). In addition, many studies

have shown that diets of urban wildlife are different, and often more diverse, than those of their rural counterparts (Murray et al. 2015, Sugden et al. 2021). This is unsurprising given the relative ease of access to domestic animals, pet food, gardens, trash, and compost in urban settings (Oro et al. 2013). Yet, access to these resources is likely heterogeneous across the urban landscape (Schell et al. 2020), just as access to non-anthropogenic prey is likely spatially varied across a city. The diet of these animals can strongly affect their health, fecundity, decision making, and likelihood of conflict with people (Poessel et al. 2017). However, research has predominantly focused on how the ecological landscape influences diet without integrating socioeconomic processes that can also shape diet.

Dietary resources for people across a city may influence what foods are available to wildlife in the same areas (Lawson et al. 2018, Standin et al. 2018). For people, access to healthy food options, grocery stores, and proper trash disposal is not equitable across a landscape, and is highly influenced by historic racialized policies, corporate profitability, and income (Beaulac et al. 2009). In addition to often being congregated in specific areas of a city as a result of these discriminatory policies, Black, Brown, and Indigenous people in the United States are disproportionately located within 'food deserts'—areas with limited access to fresh, healthy food, and supermarkets (Cooksey Stowers et al. 2020). These neighborhoods also have increased rates of illegal dumping and improper waste disposal due to lack of access to proper disposal services and less oversight by government officials (Hohl et al. 2023). Additionally, the era of 'white flight' during the 1950s and 60s saw the movement of grocery stores to suburban communities of predominantly white residents, further limiting access to grocery stores for communities of color (Leslie et al. 2022). In addition to being subjected to inequitable access to food and higher environmental disamenities such as urban pollutants, communities of color are

disproportionately lacking access to environmental amenities such as greenspaces and trees (Schwarz et al. 2015, Nardone et al. 2021**)**. Such greenspaces often host higher levels of biodiversity and non-anthropogenic resources for wildlife (Magle et al. 2021).

Here, we propose the "food desert" hypothesis, whereby the consumption of anthropogenic food by urban wildlife leads to diets that reflect these societal disparities. We test the food desert hypothesis by examining the effects of socioeconomic and ecological variables on the diet of coyotes (*Canis latrans*) in Seattle, Washington. The coyote is an adaptable, mid-sized carnivore whose range has expanded this century to include every major metropolitan area in North America (Hody & Kays 2018). Coyotes represent an apex predator in most North American urban spaces and can potentially deliver ecosystem services through predation, but understanding the extent of these services and their role in an ecosystem begins with understanding what drives their foraging choices. Despite their role as top predators in urban systems, coyotes also eat a variety of plants (Murray et al. 2015). Much of their summer diet is composed of fruit, which may increase their ecosystem services through seed dispersal (Gelmi-Candusso et al. 2024a, Jensen et al. 2024). Though many studies have documented dietary differences between urban and rural coyotes (Murray et al. 2015, Larson et al. 2020, Sugden et al. 2021) and among individual coyotes within urban systems (Caspi et al. 2025), it is unknown to what extent socioeconomic and environmental variables drive prey consumption across a heterogeneous city.

Seattle is one of the fastest growing cities in the United States, with a population of nearly 750,000 (US Census Bureau 2023). Like most major urban areas, Seattle is composed of a matrix of dense housing, industrial centers, and natural land cover types. Coyotes inhabit all of these areas, making it an ideal location to examine neighborhood-level dietary patterns. To

understand factors influencing coyote diet across Seattle's diverse neighborhoods, we collected coyote scats and obtained detailed dietary information using fecal metabarcoding. Fecal metabarcoding facilitates non-invasive, high resolution, and snapshots of both vertebrate and plant taxa consumed by coyotes (Massey et al. 2019). We then acquired and developed detailed spatial layers of socio-economic and environmental predictors, such as access to greenspace, restaurant density, illegal dumping, access to grocery stores, human population density, and household income. If coyotes are eating anthropogenic foods, their diets should reflect those of the people living in the vicinity of a coyote's home range. For coyotes living in lower-income neighborhoods, and/or neighborhoods located within food deserts, we would expect a higher proportion of highly processed, corn- and grain-based items within their diet, reflecting the lack of access to healthy foods in these neighborhoods (i.e., the food desert hypothesis). We would also expect that coyotes living in areas of higher human density would have diets consisting of more anthropogenic food. For coyotes that primarily spend time in greenspaces, and subsequently have home ranges within higher income, whiter neighborhoods, we'd expect a higher amount of non-anthropogenic prey within their diet, given the increased availability of these species. Additionally, Seattle strongly exhibits the luxury effect, a phenomenon in which more affluent, and often whiter, neighborhoods support higher levels of biodiversity (Magle et al. 2021). Thus, we may expect coyotes residing in these areas to have higher dietary diversity.

5.3 Methods

5.3.1 Study Region

We collected coyote scats within the city limits of Seattle, Washington (latitude: *c.* 47.500° to 47.734°; longitude: *c*. -122.436° to -122.236°; Figure 5.1). Seattle is bordered by the Puget Sound to the west and Lake Washington to the east (Figure 5.1).

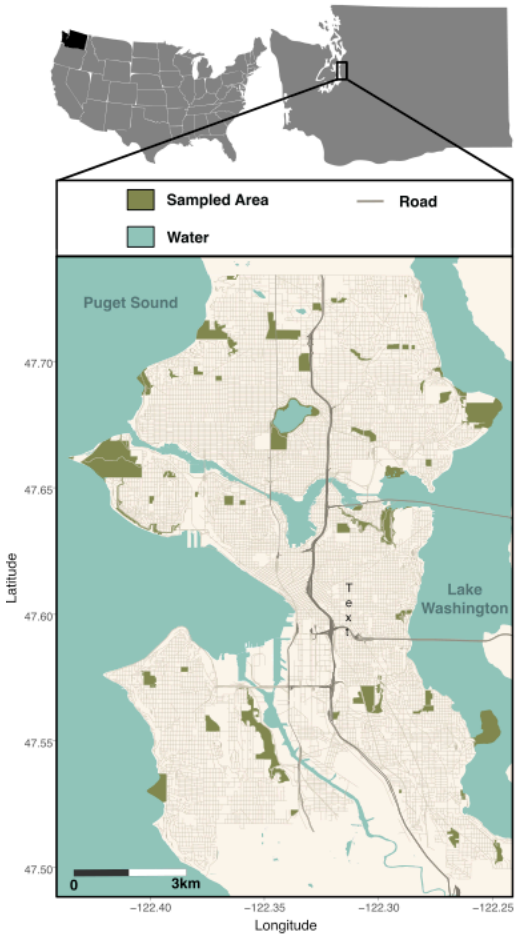

Figure 5.7: Map of the study area, the city of Seattle, with major bodies of water labeled and sampled parks highlighted in green. Above the inset are the locations of the state of Washington within the United States and the location of Seattle within the state of Washington.

5.3.2 Scat Collection

Scats were predominantly collected from municipal parks within Seattle city limits between January 2021 and December 2022. Between May and September 2021, 34 parks were routinely sampled approximately every 2 weeks and an additional 16 parks were opportunistically sampled. Additional scats were opportunistically collected across a subset of the 50 parks that had regular coyote activity during 2021, before and after the main collection period as well as during 2022-2023. Each scat was collected into a Ziploc bag and then placed within a Whirlpak. Scats were then frozen at -80ºC for at least two weeks to

ensure any potential pathogens were rendered inert and then were either kept in this freezer or moved to a -20ºC freezer for storage until DNA extraction.

5.3.3 DNA Extraction & Microsatellite Genotyping

All laboratory procedures, excluding metabarcoding sequencing and DNA fragment analysis, were performed at the University of Washington's School of Environmental and Forest Sciences (SEFS) Genetics Lab. To extract DNA, scat samples were thawed and segmented into four parts. We first swabbed the outer surface of each scat, focusing on the ends where epithelial cells are likely to be found, to identify individual coyotes. Subsequently, we used the same swab to rub both sides of each internal section to gather DNA from ingested items. Each scat was swabbed with a flocked swab moistened with phosphate-buffered saline solution. We employed a modified version of the QIAmp DNA Investigator Kit protocol (Qiagen, Hilden, Germany) for DNA extraction.

5.3.4 Library Preparation & Sequencing

To determine the diet of coyotes, we PCR-amplified samples using a multiplex of two primer pairs: 12SV5 for vertebrates (Riaz et al. 2011) and trnL g/h for plants (Taberlet et al. 2007). Each sample was processed in triplicate. The resulting PCR amplicons were tagged with IDT8 indices (Itegrated DNA Technologies, United States), SPRI bead cleaned, quantified with a Qubit fluorometer, normalized, and pooled according to established protocols (de Barba et al. 2013). The samples were then sent to the Northwest Genomics Center for sequencing on an Illumina NextSeq machine.

5.3.5 Bioinformatics

After receiving the raw files from the sequencing center, we decompressed them using the ‘tar’ command in the terminal. We used custom Bash scripts to combine reads from the same samples across the four sequencing lanes.

We used FastQC (Andrews 2010) to assess the quality of the raw forward and reverse reads for all samples. MultiQC (Ewels et al. 2016) was then used to summarize the FastQC reports across all samples. After confirming that the quality metrics were normal, we used cutadapt to trim primers and

overhangs from both the forward and reverse reads. Another round of FastQC and MultiQC was conducted to verify successful trimming.

Post-trimming, the reads were imported into R (R Core Team 2023)and processed through the standard DADA2 workflow (Callahan et al. 2016). Initially, we plotted quality profiles from a subset of the forward and reverse reads to ensure normal sequence reads. We then filtered and trimmed the reads using the 'filterAndTrim' function from the *DADA2* package, removing reads with expected errors greater than EE = 2 (where EE = ∑(10^(-Q/10)), with Q being the quality score at a base pair). Reads were truncated at the point of a quality score ≤ 2.

Next, we used the 'learnErrors' command to assess error rates within our sequence reads. The error rates and filtered forward and reverse reads were then processed through the 'dada' command, which removes erroneous reads and identifies the true sequenced community. Corresponding forward and reverse reads were merged using the 'mergePairs' command.

We created a sequence table using the 'makeSequenceTable' command and removed inferred chimeras with the 'removeBimeraDenovo' command. The sequence table was then converted into a matrix and saved as a CSV file. Additionally, a fasta file was generated from the sequence table using the 'dataframe2fas' command from the *phylotools* package in R (Zhang 2017).

5.3.6 NCBI Blast

We imported the fasta files into NCBI's web based Blastn tool (Madden 2002) and manually reviewed the blast matches for each sequence. Creating a custom reference database for urban areas, particularly for plants, is not effective due to the numerous species imported as ornamental plants, human food, or pet species from various regions worldwide. We recorded sequence matches to the lowest taxonomic unit when we had high confidence in the BLAST matches (high 'Percent Identity' and high 'Query Cover') and when the species were reasonably expected to be present in our study area. For species unlikely to occur in our study area, we either classified them by genus or selected a close relative known to inhabit our study area. For instance, if a read matched white-tailed deer (*Odocoileus virginianus*), we classified it

as black-tailed deer (*Odocoileus hemionus*), as this is the only deer species in our study system. For sequences that confidently matched multiple species, we maintained the lowest consensus taxonomic unit and noted the possible species. Lastly, we recorded instances where reads did not match any records in NCBI's nucleotide collection.

5.3.7 Additional Read Filtering

We excluded samples that failed to amplify in at least two PCR replicates or had fewer than 100 total reads. For each sample, we retained only the prey items that appeared in at least two of the three PCR replicates. We then averaged the number of reads across the available replicates for each prey item. To remove contamination, we used extraction blanks and PCR negatives. We averaged the number of reads across the replicates for both extraction blanks and PCR negatives and subtracted these from the total reads for each identified sequence in the corresponding samples (de Barba et al. 2013). We also removed any human (*Homo sapiens*) reads from the samples. Next, we calculated the total number of reads in each sample, excluding contamination and canid reads. In line with other studies, we considered only reads that represented at least 1% of the total reads once canid and contamination reads were removed for plants and 0.05% for vertebrates (Caspi et al. 2025**)**. Additionally, we excluded any scats that had fewer canid reads compared to other carnivore reads. These other carnivores included feline species (*Lynx rufus*, *Felis catus*) and raccoons (*Procyon lotor*). For example, if a scat had more feline reads than canine, we excluded it from the analysis as it likely came from a bobcat.

5.3.8 Diet Classifications

We classified diet items into eight categories, with five human-associated categories and three categories that are not clearly associated with humans. Human-associated categories consisted of anthropogenic proteins (i.e., livestock), anthropogenic grains (including corn), garden fruit, domestic cats, and pest rodents (rats and mice). Non-anthropogenic categories consisted of rabbits, other vertebrates (excluding rabbits, black and brown rats, and house mice), and berries (Table 5.1). We chose these categories based on public interest (cats), commonality (rabbits), and groupings we hypothesized to be affected by socio-

economic variables (anthropogenic foods, pest rodents, fruits). We separated eastern cottontail rabbits (*Sylvilagus floridianus*) from other vertebrate prey because they were present in most scats and could swamp out patterns of consumption of other vertebrate species, which were mostly native. Plants were limited to those we were confident were from diet items rather than environmental contamination such as pollen or substrate contamination. Plant taxa were identified at the genus level when possible, and otherwise were identified at the family level. For corn, trnL cannot specify species, but when *Zea* and closely related grass taxa were the most likely in NCBI Blast, we counted these reads as corn. Other *Poaceae* reads that did not have *Zea* as a top option were discarded as contamination or incidental consumption. Though grass is frequenly found in coyote scats in this system, they pass straight through the digestive system without sufficient digestion and are likely not a significant dietary source of calories (Johnson & Hansen 1977). The berries included genera that are commonly found as native and non-native plants in the greenspaces around Seattle. The street fruit category included genera of plants that produce fruit and are commonly planted as ornamentals or cultivated in people's yards.

Table 5.1: Table of taxa listed in each of the 8 dietary group classifications. Taxa are listed at the highest possible taxonomic unit. Common names are listed in the same order as scientific names. Scientific names are listed alphabetically.

Food Item Classifications	**Scientific Names**	**Common Names**
Anthropogenic Grains and Legumes	*Avena, Chenopodium, Glycine, Hordeum, Lens, Linum, Oryza, Sorghum, Zea*	Oats, Quinoa, Soy, Barley/Wheat, Lentil, Flax, Rice, Sorghum, Corn
Anthropogenic Protein	*Bos taurus, Gallus gallus, Meleagris gallopavo, Ovis aries, Sus scrofa*	Cow, Chicken, Turkey, Sheep, Pig
Cats	*Felis catus*	Domestic Cat
Garden Fruit	*Actinidia, Crataegus, Cornus, Juglanaceae, Malus, Prunus, Pyrus, Viburnum*	Kiwis, Hawthorns, Dogwoods, Walnuts, Apples, Cherries & Plums, Pears, Viburnums
Pest rodents	*Mus musculus, Rattus norvegicus, Rattus rattus*	House Mouse, Brown Rat, Black Rat
Berries	*Ericaceae, Ribes, Rubus, Sambucus, Symphoricarpos, Vaccinium*	Heath Family, Currants, Brambles, Elderberry, Snowberry, Huckleberry
Rabbits	*Sylvilagus floridanus*	Eastern Cottontail
Other Vertebrates	*Aix sponsa, Anarhicas, Anas, Aplodntia rufa, Bombycilla cedrorum, Bucephala, Buteo, Castor canadensis, Catharus ustaulatus, Charadrius vociferus, Colaptes*	Wood Duck, Wolffish, Ducks, Mountain Beaver, Cedar Waxwing, Goldeneye, Hawks, Beaver, Swainson's Thrush,

	auratus, Corvus, Cricetidae, Cyprinus, Didelphis virginiana, Fulica americana, Gasterosteus aculeatus, Haleaeetus leucocephalus, Ixoreus naevius, Junco hyemalis, Larus, Lepomis, Leptocottus armatus, Micropterus salmoides, Microtus longicaudus, Neurotrichus gibbsii, Odocoileus hemionus, Paridae, Passerellidae, Patagionas fasciata, Perca flavescens, Pipilo maculatus, Poecile atricapilla, Procyon lotor, Regulus, Scapanus orarius, Scapanus townsendii, Sciurus carolinensis, Sorex, Strix varia, Sturnus vulgaris, Tamiasciurus douglasii, Trachemys scripta, Troglodytes, Turdus	Killdeer, Northern Flicker, Crows, Voles, Carp, Virginia Opossum, American Coot, Three-spined Stickleback, Bald Eagle, Varied Thrush, Dark-eyed Junco, Gulls, Bluegill, Pacific Staghorn Sculpin, Largemouth Bass, Long-tailed Vole, American Shrew Mole, Black-tailed Mule Deer, Tits, New World Sparrows, Band-tailed Pigeon, Yellow Perch, Spotted Towhee, Black-capped Chickadee, Raccoon, Kinglets, Coast Mole, Townsend's Mole, Eastern Gray Squirrel, Long-tailed Shrews, Barred Owl, Starling, Douglas Squirrel, Red-eared Slider, Wrens, True Thrushes

5.3.9 Spatial Data

We created a 1-km diameter buffer around each collected scat and extracted values for nine spatial covariates from within this buffer (Table 5.2). We also extracted all spatial covariates within a 1-km buffer around each park scats were collected from. We chose this 1-km buffer, a similar size to buffers used in comparable studies (Larson et al. 2020, Caspi et al. 2025), as a smaller buffer would be less likely to capture movements made between the location of food consumption and defecation.

We created a custom map of grocery stores within our study area. We classified grocery stores into 4 separate types – general large chains, bargain markets, expensive local chains, and specialty stores (those selling products of a specific global region or cuisine; Appendix C Table S1). To create this map, we used Google Maps and searched the following terms: “QFC”, “Safeway”, “PCC”, “Trader Joes”, “Metropolitan Market”, “Fred Meyers”, “Sprouts”, “Whole Foods”, “Grocery Outlet”, “Dollar Tree”, “Target”, “Market”, “Grocery”, “Mexican Market”, “Mercado”, “African”, “Indian”, “Latin”, “Mediterranean”, “Halal”, “Ethiopian”, “Mexican”, “South American”, “European”, “Asian”, “Chinese”, “Vietnamese”, “Thai”. For each location that came up, we verified that they sold groceries and were not just restaurants. Grocers just outside of Seattle were included to ensure all markets were identified within our 1-km buffers. We then extracted the density of grocers in total, and of each of the four grocer categories within the 1-km buffers.

We obtained restaurant locations from King County Public Health’s inspection data. In Seattle, restaurants are inspected twice yearly. We subset the list of restaurants to those inspected in 2021-2022, representing the restaurants in business during our main sampling periods. We identified unique restaurants based on name and location (King County Public Health 2024). We obtained socio-economic and demographic data from the United States Census Bureau’s at the census block group level from the 2020 census and American Community Survey (US Census Bureau 2020). From this dataset we obtained average household income, percentage of People of Color, and population density.

We obtained all reported illegal dumping instances within the city of Seattle between 2021-2022 from Seattle Public Utilities via a public information request. These were mainly reported through the “Find it, Fix it” app, or the City of Seattle’s online illegal dumping reporting form. Types of illegal dumping records ranged from needles and trash to furniture and televisions. We retained all categories of illegal dumping except for needles, which we did not think would influence coyote diet. We retained all other categories including ‘furniture’ as this may have created habitat for pest rodents such as black rats.

We quantified access to greenspace using NLCD Land Cover Classification data (NLCD 2021). We reclassified NLCD classes “Developed, Open Space”, “Barren Land”, “Deciduous Forest”,

"Evergreen Forest", "Mixed Forest', "Shrub/Scrub", "Grassland/herbaceous", and "Woody Wetlands", as greenspaces using the 'classify' function from the *terra* package (Hijmans 2023). We then calculated percentage of greenspace within each of our buffers. Finally, we calculated percentage of tree cover using the NLCD Tree Canopy dataset (NLCD 2011) and percentage of impervious surface using the NLCD Urban Imperviousness dataset (NLCD 2021).

Prior to modeling, we assessed the correlation among our covariates using the 'corrplot' function from the *corrplot* package (Wei & Simko 2021, Appendix C Table S1). Any variables that had a correlation value 0.6 or greater were not used within the same model to prevent overfitting to multicollinearity (Dormann et al. 2013; Appendix C Figure S2). We then normalized covariates for both the scat buffers and park buffers using the 'scale' function in R.

Table 5.2: Justification, variable type (socioeconomic or ecological), and implications of each covariate tested within the study.

Variable	**Variable Type**	**Justification/Implication**
# Grocers	Socioecomic	Low income and communities of Color are often within food deserts and lack adequate access to healthy food options (Jiao et al. 2012, Cooksey Stowers et al. 2020). Diets in these communities are often low-nutrition and high in grains and corn (Leslie 2022). For coyotes in these areas, we may expect them to have higher proportions of low-nutrition, high grain and corn foods in their diet.
Household Income	Socioecomic	Lower income neighborhoods are often located within food deserts and have decreased access to healthy food options and greenspaces (Jiao et al. 2012, Leslie 2022). Coyotes in these neighborhoods may have an increased percentage of anthropogenic food in their diet and a decreased percentage of non-anthropogenic foods.

# of Reported Illegal Dumping Incidents	Socioecomic	Illegal dumping is often a symptom of decreased access to proper waste disposal and government oversight/resources (Hohl et al. 2023). As a proxy for waste management, areas with increased illegal dumping may have increased habitat for vertebrate pests such as rats or mice. Coyotes in areas with high levels of illegal dumping may have increased percentages of anthropogenic foods and/or pest rodents in their diet.
% Impervious Surface	Socioecomic	As a proxy for population and building density, coyotes in areas with high percentages of impervious surface may have increased proportions of anthropogenic resources and rodent pests in their diets.
% People of Color	Socioecomic	Communities of color often have lower access to greenspaces, tree canopy, and grocery stores because of past and ongoing racialized policies (Cooksey Stowers et al. 2020, Pickett et al. 2023). In addition, due to lower government oversight, resource allocation, and access to waste disposal, these communities may have higher amounts of illegal dumping (Hohl et al. 2023) Coyotes in areas with higher percentages of People of Color may thus have increased proportions of Anthropogenic resources, rodent pests, and low-nutrition foods that are high in grains and corn.
Population Density	Socioecomic	In areas where there are higher densities of people, coyotes are likely to have increased access to anthropogenic resources.
# Restaurants	Socioecomic	Some studies have found increased rat presence in areas with more restaurants (Murray et al. 2020, Sánchez et al. 2021). Coyotes in areas with more restaurants may thus have increased proportion of rodents in their diet. Additionally, because of discarded restaurant waste, coyotes may have increased proportions of anthropogenic foods as well.

% Greenspace	Ecological	Neighborhoods with increased access to greenspace often host higher biodiversity and native species (Magle et al. 2021). Coyotes in neighborhoods with high percentages of greenspace may have increased non-anthropogenic foods in their diet and decreased anthropogenic foods.
% Tree Canopy	Ecological	In Seattle, tree canopy is not directly correlated with greenspace. If native prey need only tree canopy rather than full greenspaces, then tree canopy may buffer the effects of impervious surface and human density (Van Thaden et al. 2021, Zhang et al. 2024). For coyotes in areas with increased tree canopy, we may expect them to have increased proportions of native prey in their diets.

5.3.10 Diet Analyses

We summarized the data using relative read abundance (RRA) and frequency of occurrence (FOO). RRA is the number of reads assigned to a given prey item divided by the number of total reads within that sample. FOO is the number of scats that contain a given prey item divided by the total number of scats collected. Both methods have pros and cons, with RRA suggested to be more accurate, but biased by primer binding affinities and other amplification biases and FOO known to overinflate importance of rare diet items (Mallott et al. 2018, Deagle et al. 2019, Massey et al. 2019, Walker et al. 2023). We chose to proceed with the FOO dataset only as it allowed us to make direct comparisons between vertebrates and plants in their diet. To understand if there were significant differences in diet among collection parks, we calculated a Jaccard dissimilarity matrix for the FOO dataset using the 'vegdist' function from the *vegan* package in R and conducted a permutation-based multivariate analysis of variance (PERMANOVA) for every dyad of scats collected and successfully sequenced using the 'adonis2' function from the *vegan* package. To account for uneven sample sizes among parks, we first narrowed our dataset to the parks with at least 5 scats and then conducted 1000 PERMANOVA runs while sampling 5 scats from each park

for each run with 999 permutations (Appendix C Figure S2). We then averaged the R^2 and p-values across these runs. While a sample of 5 scats likely underestimates diet diversity, we choose this threshold to maximize the variation in covariates across parks (Appendix C Table S2).

We also ran a separate PERMANOVA model to understand if coyote diet was significantly different among neighborhoods with different racial demographics, because many of our variables were correlated with the percentage of non-white inhabitants in each area. To do this, we extracted the percentage of People of Color from a 1km buffer of each park location. We then classified this into three bins, high (>40%), medium (25-40%), and low (<25%). Seattle's population is 61% white, so we based our bins off of these numbers and a reasonable distribution of parks in each bin (n = 9 high, 17 medium, 8 low). We then resampled for even numbers of scats in each bin, with the 'low' bin having the fewest scats (n = 57) and conducted the same 1000 PERMANOVA runs with 999 permutations, then averaged R^2 and p-values across runs. Finally, we calculated Hill-species richness and Hill-Shannon diversity for the parks. We then used hypothesis-driven linear regression modeling with a Gaussian distribution to understand if diversity of prey consumed in each park was driven by any of our selected socioeconomic or environmental variables. These models evaluated whether overall diet, rather than just individual diet items or categories, varied with environmental and socioeconomic factors across Seattle.

We then modeled occurrences of each diet category as the response variables compared to environmental and socio-economic covariates using generalized linear mixed models (GLMM) with a binomial distribution. Separate GLMMs were run for each diet category of interest. To help account for autocorrelation, we added collection site as a random effect (Kreling et al. 2024). Models were run using the 'glmmTMB' function from the *glmmTMB* package in R. We used a hypothesis-driven modeling approach comparing the null model, a full model of variables we predicted could influence each diet category, and combinations of variables within that full model based on specific hypotheses. We selected the best model by lowest AIC value and presented results of models within 2 AIC values of the best model (Akaike 1981). We did not model individual variation in theses analyses because most coyotes were found only within one or two parks in close vicinity (Kreling et al. 2024).

5.4 Results

5.4.1 Scat Collection

We collected and sequenced 870 coyote scats. After all bioinformatic filtering steps, we retained and analyzed 687 scats from 34 parks across Seattle, ranging from 1 to 95 scats per park (mean = 19.63, *SD* = 26.00). Scats were collected from locations that spanned a broad range of socioeconomic and environmental values (Appendix C Table S2). Human population density was negatively correlated with amount of green space (r = -0.74) and positively correlated with amount of impervious surface (r = 0.75; Appendix C Figure S1). Impervious surface was negatively correlated with amount of water (r = -0.9) and green space (r = -0.65). Number of grocery stores and restaurants were negatively correlated with median household income (r = -0.15, -0.39 respectively). Green space was positively correlated with household income and tree canopy (r = 0.22, 0.32 repsectively).

5.4.2 Dietary Patterns

After filtering for parks with at least 5 samples, 641 samples remained across 18 parks. Frequency of occurrence (FOO) and relative read abundance (RRA) were largely in agreement, although RRA appears to include smaller-bodied prey such as voles and rats as higher percentages of the total samples than FOO (Figure 5.2, S3). Overall, we identified 65 families (24 plant, 41 vertebrate; Appendix C Table S3) and 98 genera within coyote scats (35 plant, 63 vertebrate). Of the 63 vertebrate genera, 41 diet items were identified to species. Average Hill-species richness across parks was 10.10 (*SD* = 1.68), and Hill-Shannon diversity was 8.82 (*SD* = 1.62). We found significant differences in diet among parks (PERMANOVA R_{Park}^2 = 0.30, df = 17, p = 0.002). Coyote diets varied among neighborhoods with varying racial makeup, but this factor explained only 2% of dietary variance among parks (R_{POC}^2 = 0.02, df = 2, p = 0.04). Dietary species richness and diversity declined as percent tree canopy increased (richness β = -0.70, p = 0.046; diversity β = -0.62, p = 0.045), but household income, human population density, percent impervious surface, and percent greenspace were not significant predictors of diversity or richness.

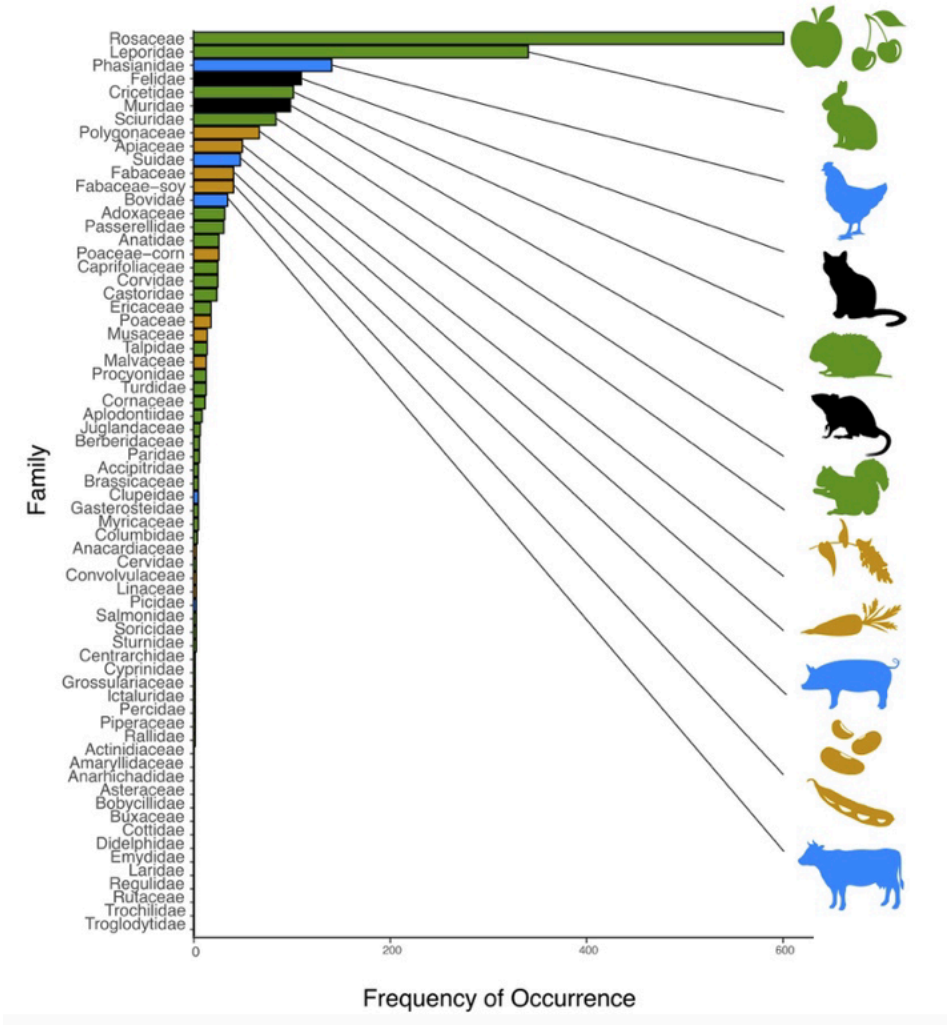

Figure 5.2: Dietary frequency of occurrence (FOO) bar graph at the family level across all coyote scats (*n* = 687) within Seattle. Top FOO plant and vertebrates families are depicted to the right of the bar graph. Families are coded into diet categories: anthropogenic proteins (blue), anthropogenic plants (yellow), naturally-occurring foods (green; excluding pest rodents and cats), and human-associated vertebrates (cats, rats, and house mice; black).

5.4.3 Drivers of coyote diets

For each of the 8 dietary categories (Table 5.1), we constructed generalized linear mixed models with a variety of hypothesis-driven socioeconomic and ecological predictors (Table 5.2). In general, occurrences of dietary categories were not well-predicted by any of our covariates, with most coefficients overlapping 0 (Figure 5.3). However, two socioeconomic predictors – illegal dumping and human population density

– were significant predictors of several human-associated diet categories, and non-anthropogenic categories had several significant socioeconomic and ecological predictors (Appendix C Tables S4-S12).

Of the 5 human-associated diet categories, illegal dumping was a significant predictor of the presence of pest rodents and anthropogenic proteins (Appendix C Tables S4&S7), and human population density was a significant predictor of pest rodents and garden fruits (Appendix C Tables S4&S12). All of these were positive associations. Several other anthropogenic diet categories had covariates in top models, but coefficient values were not significant. The top models for presence of anthropogenic grains and legumes indicated a positive association with the number of grocers and number of restaurants, and negative association with the number of illegal dumping incidents (Appendix C Table S5). However, the null model for anthropogenic grains and legumes was within 2 AIC of the top model, and none of the covariates were statistically significant. The top model for domestic cat presence in scat was the null model (Appendix C Table S6). The null model was also the top model for anthropogenic protein presence in scats, but models within 2 AIC values models included a significant effect of illegal dumping. When anthropogenic grains, corn, and proteins were combined, the top models included a positive association with population density and impervious surface, and a negative association with household income and tree canopy, but none of the predictors were statistically significant (Appendix C Table S7). Garden fruit presence in scats was positively associated with household income and population density and negatively associated with tree canopy, but only population density was statically significant (Appendix C Table S11).

Diet categories that were not clearly human-associated (rabbits, other vertebrate prey, and berries) had a mix of socioeconomic and ecological predictors. Rabbit presence in scats was significantly negatively associated with illegal dumping incidents (Appendix C Table S8). The top model for other vertebrate prey included a significant negative association with human population density, and models within 2 AIC included a significant positive association with green space and a significant negative associatioin with imperivous (Appendix C Table S9). The sole top model for berry presence included

household income, tree canopy, and population density, which were all statistically significant (Appendix C Table S10).

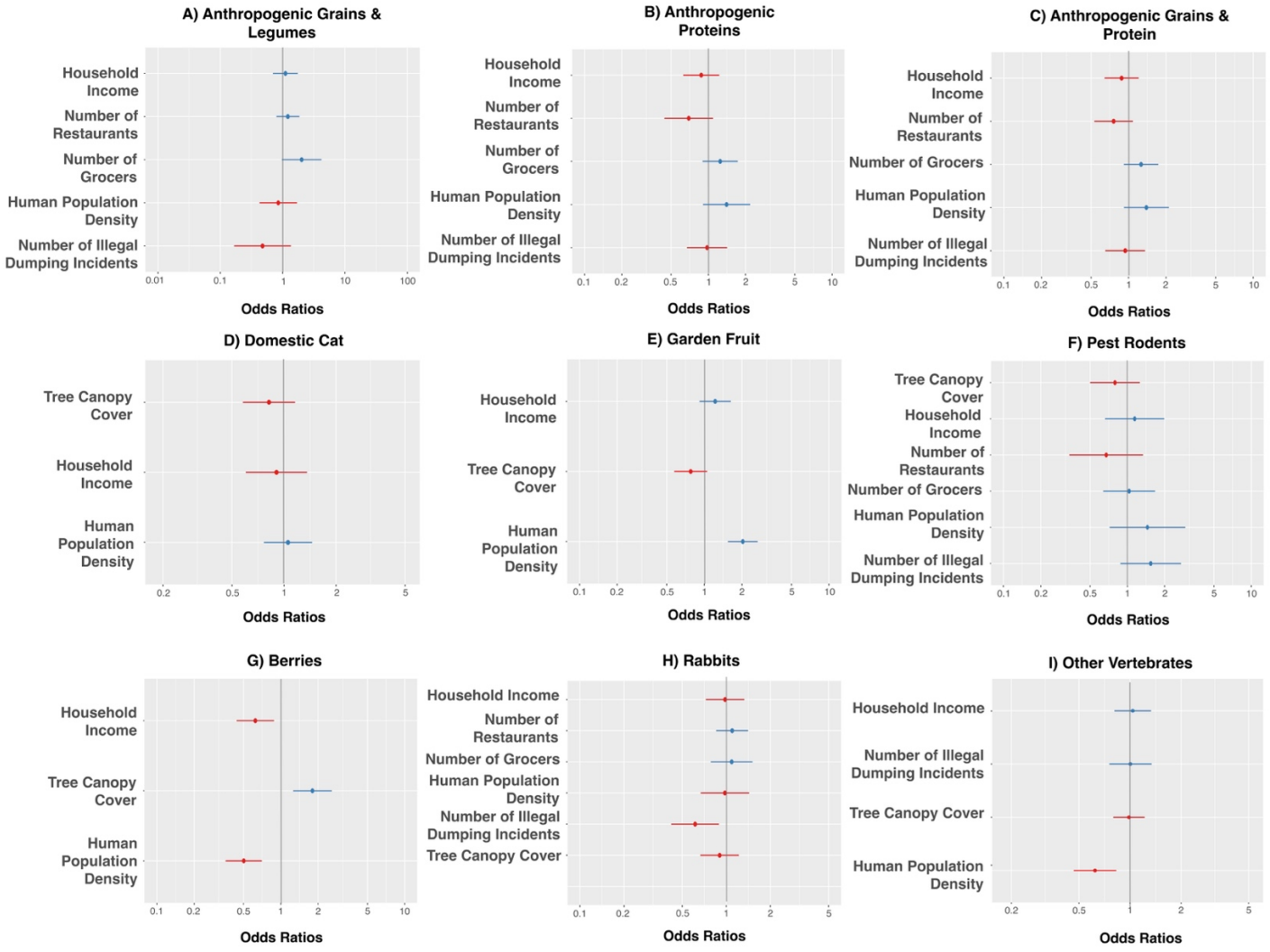

Figure 5.3: Coefficient plots for all full models for the 8 diet categories and the combined anthropogenic grains and proteins: A) Anthropogenic Grains & Legumes, B) Anthropogenic Proteins, C) Anthropogenic Grains and Proteins, D) Domestic Cat, E) Garden Fruit, F) Pest Rodents, G) Berries, H) Rabbits, I) Other Vertebrates. Dark vertical gray lines indicate odds ratio = 1 (no effect). Low odds ratios (< 1) are shown in red and indicate negative association, and high odds ratios (> 1) are shown in blue and indicate positive association.

5.5 Discussion

Urban regions are inherently social-ecological systems (Frank et al. 2017, Arnaiz-Schmitz et al. 2018). Cities are shaped by their policies, governance, and management, which influences outcomes such as who lives where, and who has access to healthy food or greenspaces (Wolch et al. 2014, Nardone et al. 2020). The ways in which cities are shaped, however, do not only affect the human residents, but the wildlife residents as well (Schell et al. 2020). While the theory around how human social factors affect wildlife is well-developed, empirical testing is still lacking (Liu et al. 2007, Schell et al. 2020). Additionally, most empirical work has focused on the effects of wealth on biodiversity (i.e., the "luxury effect"; Leong et al. 2018, Magle et al. 2021). Here we sought to elucidate how socioeconomic factors as well as ecological variables influence dietary choices of coyotes in Seattle, Washington. Despite high resolution in our predictor variables and dietary data, we found surprisingly few relationships between diet and these variables. Our results indicate that drivers of diet choice for urban coyotes may be highly context dependent and difficult to quantify. Contrary to our 'food desert' hypothesis, we found generally weak effects of income, human demographics, grocery store, and restaurant density on coyote diet diversity and composition. Despite the low performance of most predictors, illegal dumping appears to be an important link between socioeconomic factors and consumption of some prey species such as pest rodents and rabbits. Overall, the surprisingly weak influence of these habitat and socioeconomic predictor variables suggests that variation in non-anthropogenic prey among city parks may be a primary driver of diet that varies independently of the spatial predictors we examined.

We found significant differences in diet among city parks. Surprisingly, tree canopy cover was negatively correlated with diet diversity. This negative effect may indicate that there is a larger variety of potential food items in areas with open spaces and/or areas with high quantities of impervious surface compared to areas with high canopy coverage. Some of the parks in Seattle have low tree canopy cover but substantial open space, such as lawns, which could host a diversity of small mammals and birds in addition to plentiful anthropogenic foods from trash and nearby neighborhoods. While some studies have found correlations between biodiversity and human household income (Leong et al. 2018, Magle et al.

2021), impacts on predator-prey interactions and diet remain poorly understood. Our analysis indicates that diet diversity of coyotes and human household income are unrelated in Seattle, suggesting the luxury effect does not necessarily extend to trophic interactions. Instead, individual's preference for different food items may limit the diversity in their diet regardless of the biodiversity within their home ranges, or diversity of food items may vary considerably by home range.

Pest rodents were more frequently found in coyote scats in areas with more illegal dumping incidents. Illegal dumping incidents, therefore, may be a proxy for improper—or lack of access to—waste management, which is consistent with increased rodent presence in scat (Murray et al. 2018). Neighborhoods that have been historically and currently under-resourced and invested in may thus have higher present of pest rodents and may garner increased benefit from coyote presence and predation on pest rodents. Impervious surface is strongly associated with the number of illegal dumping incidents ($r = 0.66$) and may also be an important influence on pest rodent distribution. However, in univariate testing, illegal dumping was always a stronger predictor than impervious surface. Illegal dumping may be more likely to occur in areas with higher impervious surface and population density, but likely captures some other combination of socioeconomic variables that impervious surface alone does not account for. Models of pest rodent occurrences that had strong support also included human population density. The three rodent species included in our pest rodent category are human commensals and thrive with access to human food resources and human structures for shelter (Puckett et al. 2020). Thus, where there are more people there may be more rats and house mice, though this might be mediated by access to soil and greenspace for burrow establishment (Easterbrook et al. 2005, de Cock et al. 2024).

Unlike with pest rodents, presence of rabbits in scats was negatively associated with illegal dumping. Rabbits may be avoiding areas with high densities of rats or could be selecting for areas with larger lawn spaces where there would be ample forage, which is negatively associated with impervious surface. Consumption of other vertebrate prey was negatively associated with human population density and impervious surface, and positively associated with greenspace, likely indicating higher availability of native rodents and birds in parks. Intuitively, we would expect to find a higher diversity and abundance of

other vertebrate prey species in areas with large greenspaces where a variety of wildlife prey may be able to persist (Clucas & Marzluff 2015).

Berry occurrences were negatively associated with household income and human population density and positively associated with tree canopy. Himalayan blackberry (*Rubus armenicus*) is a highly prolific, invasive species and is a common food source for coyotes (Jensen et al. 2024). While our trnL primers fail to differentiate among *Rubus* species, which also includes native trailing blackberry (*Rubus ursinus*) and salmonberry (*Rubus spectabilis*), most of the visible berries in scats were blackberries (indicated by the dark purple coloration and *Rubus*-type seeds). A negative association with human population density likely indicates increased availability of these food sources in open spaces, parks, and locations with high tree canopy. Native berry species are also found primarily within these forest fragments (S. Kreling, personal observation). Parks located within more affluent neighborhoods often possess active volunteer teams who work to restore the urban forests, planting native species, and removing invasive plants (Romolini et al. 2013). In contrast, individuals in lower income neighborhoods may not have the time or capacity to conduct this kind of volunteer work (Sundeen et al. 2007, Kremer et al. 2013). Such areas, therefore, may have higher levels of invasive plants such as Himalayan blackberry. Additionally, these areas are more likely to have vacant lots that can easily become overrun with invasive blackberries (Anderson & Minor 2017). The increase in garden fruit with human population density and income may indicate that wealthier areas where people are living may be more likely to have these trees growing in their neighborhoods or have discarded fruits in areas that are accessible to coyotes, such as backyard compost piles (McLain et al. 2012). The variety of predictors explaining different dietary groups highlights the importance of collecting high-resolution spatial data and exploring both socioeconomic and environmental correlates of urban wildlife diet. Our results also suggest that predictors are context dependent, with some food groupings more likely to be predicted by environmental effects alone, socioeconomic effects alone, or a combination of the two.

Carnivores comprise a large portion of the literature on human-wildlife conflict, but few studies have quantified the socioeconomic benefits of carnivores on the landscape and the ecosystem services

they provide (Gilbert et al. 2016). Ecosystem services are benefits that biotic components of the landscape contribute to human society (Gilbert et al. 2016). Coyotes may provide ecosystem services via predation of nuisance herbivores, reduction of invasive species, reduction in populations of disease vectors, or even as biotic "street cleaners" (Ćirovićet al. 2016, Sonawane et al. 2021). In Seattle, we found that coyote diet varies by park and is largely comprised of non-native species of both plants and vertebrates. Because coyote diets are varied in their amount of anthropogenic food items across neighborhoods, there are likely large variations in potential benefits from individual coyotes or coyote family groups. For instance, in areas with a lot of illegal dumping, coyotes may provide benefits by offering a no cost, toxin-free means of rodent pest control, especially in neighborhoods where access to pest control is limited. Additionally, over a million dollars in Seattle each year is spent in collaboration with the Green Seattle Partnership on working to restore and increase the quality and quantity of urban forests (Daniels et al. 2016). Thus, coyotes who frequently consume native plants may provide ecosystem services via seed dispersal. Future research should investigate and quantify these potential services at both a population-wide and individual level.

While we anticipated strong associations between human socioeconomic factors and coyote food consumption, our results were likely tenuated by the lack of knowledge of coyote's home ranges. Sampling occurred only in greenspaces, which likely did not represent a coyote family's full home range and thus access to resources. Additionally, scat location is not necessarily the location of food consumption and likely led to weaker associations. Food deserts and inequitable access to greenspace for humans are still prominent in the Seattle landscape (Jiao et al. 2012, Abel & White 2015), and may have increasing disparities with gentrification (White & Abel 2019) and impact wildlife (Schell et al. 2020). We suggest that future researchers combine movement data from collared coyotes with dietary metabarcoding to better understand the ties between access to green space, quality of food, and access to human food and coyote diet.

5.6 Conclusion

Our study reveals that coyote diets in Seattle are influenced by both socio-economic and ecological factors, reflecting the complex interplay between human activities and wildlife diet. While coyotes are adaptable in their dietary choices, we found pest rodent consumption to be positively associated with illegal dumping and human population density, suggesting that human-mediated resources can shape coyote foraging in densely populated, underserved neighborhoods. Contrary to the food desert hypothesis, however, we found limited evidence linking socio-economic disparities to increased consumption of anthropogenic foods. Habitat features including tree canopy cover significantly influenced dietary diversity, with greener areas supporting less diverse, non-anthropogenic diets. Additionally, berry consumption was linked to lower household income and tree canopy cover, indicating that berries may be more likely to be consumed in areas where there is less time to remove invasive species such as Himalyan blackberry. These findings highlight the need for integrative approaches that consider both ecological and socio-economic drivers in urban wildlife management, especially as cities continue to expand. Understanding dietary patterns can help mitigate human-wildlife conflicts and promote biodiversity in urban spaces, particularly in communities with limited access to greenspaces and waste management services. Future work should combine known home ranges of individuals with dietary information for a more precise understanding of the drivers of food consumption.

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6. Chapter 6 – Urbanization drives patterns of resource partitioning among coyotes

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6.1 Abstract

Niche partitioning and resource specialization can lead to spatial heterogeneity in trophic dynamics, but little is known about how these dynamics may change across landscapes of varying complexity. Urban ecosystems are often characterized by a wider array of both natural and anthropogenic food sources, which can increase predator densities compared with exurban and wildland areas. Urbanization could thus intensify intraspecific competition and facilitate individual specialization, or relax intraspecific competition if resources are not limiting, leading to more generalized diets. To investigate how urbanization affects dietary niche breadth and individual specialization, we used fecal metabarcoding to analyze coyote (*Canis latrans*) diets across three landscapes in Washington, USA: the Greater Seattle Metropolitan Area, Bainbridge Island, and two wildland study sites. As expected, we found lower dietary species richness in the wildland areas, but dietary diversity was surprisingly consistent across regions, with minor differences in Hill-Shannon diversity and niche breadth. Urban coyotes had a broad population-level diet and limited individual specialization, whereas wildland coyotes exhibited the highest individual specialization and lowest dietary overlap. Dietary specialization may be constrained by home range size and the diversity of prey types available to an individual coyote, highlighting the importance of landscape and prey heterogeneity in urban landscapes. These findings indicate that urbanization can relax intraspecific competition among predators, whereas individual dietary specialization may have a greater influence on predator-prey dynamics and human-wildlife conflict in more natural ecosystems with less diverse resources.

Key Words: Canis latrans, diet, eDNA, metabarcoding, specialization

6.2 Introduction

Niche partitioning and resource specialization are key mechanisms by which species reduce inter and intraspecific competition (Alley 1982, Bolnick et al. 2003). Where individuals occur in high densities or resources are limited, the ways in which these resources are partitioned can define the success of the population (Bolnick et al. 2003). In an urbanizing world, human disturbance and food supplementation may substantially alter these coexistence mechanisms (Sévêque et al. 2020). Urban regions are generally characterized by diverse, abundant food resources (Smith et al. 2018, Gámez et al. 2022), which can support high densities of predators (Fischer et al. 2012) and may thus facilitate strong resource partitioning. However, the degree to which anthropogenic influences alter such resource specialization is not well understood and may be highly context dependent (Newsome et al. 2015, Sévêque et al. 2020, Manlick & Newsome 2021).

Coyotes (*Canis latrans*) are one of the most well-studied carnivore species in North America and function as an important apex predator in urban ecosystems (Gallo et al. 2019). Coyotes are classic dietary generalists at the population level, consuming a wide variety of plants and animals across a range of urbanization (Murray et al. 2015, Poessel et al. 2017a, Sugden et al. 2021, Jensen et al. 2022). It is unclear, however, if such population-level generalism arises because individuals are generalists, or because individuals specialize on different dietary items across the population. Coyote populations in urban regions tend to have higher diet diversity compared to non-urban coyotes because of access to a larger number of native or non-native prey, ornamental plants, and human food (Murray et al. 2015, Sugden et al. 2021). However, understanding how dietary diversity and population density contribute to individual specialization remains poor. This knowledge is important for carnivore conservation, because conflicts related to diet may occur due to a handful of "problem" individuals rather than systemic population-wide behaviors. For instance, sheep depredation northern California was found to be almost entirely carried out by breeding male coyotes rather than entire family groups or transients (Blejwas et al.

2006). To facilitate human-coyote coexistence, it is imperative to understand whether individuals specialize on different items, and how the degree of specialization varies across landscape contexts.

In urban areas where the density of coyotes and other mesocarnivores is inflated due to increased food availability (Fischer et al. 2012, Šálek et al. 2014), alternative strategies for coexistence could emerge (Sévêque et al. 2020). While dietary niche breadth is expected to be wider in urban areas due to increased resource variety, individuals may specialize on certain resources to decrease intraspecific competition. For example, coyotes in Chicago had greater dietary variation among individuals than coyotes in surrounding exurban regions (Newsome et al. 2015). Alternatively, if resources are plentiful enough that the increased density of coyotes and other mesocarnivores does not sufficiently limit the population through competition, coyotes may consume the most abundant items within their home ranges and have low degrees of individual specialization. Indeed, Larson et al. (2020) found reduced individual variation in urban coyotes and widest niche breadth in suburban areas of Los Angeles, California. In wildland areas with a lower diversity of available resources, individuals should theoretically have more generalized diets and reduced levels of resource partitioning. However, inferences regarding effects of urbanization on niche partitioning and individual specialization have been limited by traditional methods of scat analysis that identify diet items based on identification of undigested remains. Advances in genomic methods such as fecal metabarcoding allow for high-resolution dietary construction, which is especially advantageous in urban areas where carnivores may consume foods that are difficult or impossible to identify using traditional methods (De Barba et al. 2013, Forin-Wiart et al. 2018).

To understand how urbanization affects the niche breadth and individual specialization of coyotes, we used fecal metabarcoding to generate a comprehensive diet dataset among three distinctive landscape contexts in Washington, USA. Our samples come from the Greater Seattle Metropolitan Area, an offshore exurban island, and two wildland study sites in northern Washington. If urbanization intensifies intraspecific competition, we expect coyotes to have increased individual specialization in the Seattle area compared with wildland regions. If urbanization relaxes competition, we expect coyotes to be more generalized with wider niche breadths in urban areas.

6.3 Methods

6.3.1 Study Region

Non-invasive scat collection was conducted in three separate landscape types within the state of Washington. Our urban samples were collected from the Greater Seattle Metropolitan Area, Washington (latitude: *c.* 47.500° to 47.734°; longitude: *c*. -122.593° to -122.479°; ~1200 km^2; Figure 6.1). Our island data comes from Bainbridge Island, an island just off the coast of Seattle within the Puget Sound (latitude: *c.* 47.574° to 47.721°; longitude: *c*. -122.436° to -122.236°; ~72 km^2). Our wildland samples were collected as part of the Washington Predator Prey Project (https://predatorpreyproject.weebly.com/) in the Methow Valley (latitude: *c.* 48.045° to 49.000°; longitude: *c*. -121.393° to -119.593°) and northeastern Washington (latitude: *c.* 47.954° to 48.686°; longitude: *c*. -118.399° to -117.039°). Most of these wildland study areas were comprised of natural land cover, with 3.5% agricultural cover and 1.1% developed areas (Ganz et al. 2024).

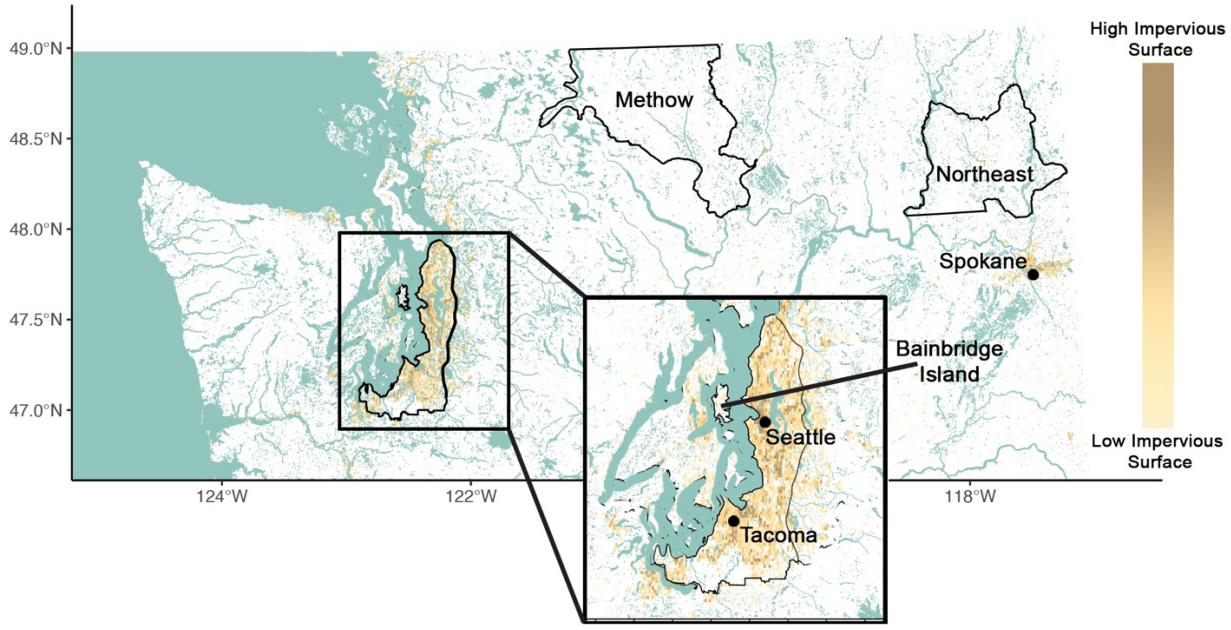


Figure 6.8: On the top, a map of the Northern half of the state of Washington state with all study areas outlined in black. In the lights blue are waterways. The gradient of yellow and brown represents the percent of impervious

surface (NLCD 2019) across the region depicted. In the middle, an inset of the Bainbridge Island and Greater Seattle Metropolitan study areas. Major cities are located across both maps.

6.3.2 Scat Collection

Scats were collected with a Ziploc bag and then placed within a Whirlpak, and they were stored at -20 to -80 degrees Celsius. Scats were collected in the Greater Seattle Metropolitan Area between 2021-2023, with emphasis on months May – October when weather was less rainy and more likely to yield scats with both coyote genotype and diet data. In 2021, green spaces within the city of Seattle were repeatedly sampled on a roughly 2 week basis as reported in Kreling et al. (2024). Scats within the Methow and Northeast study regions were semi-systematically collected with dedicated scat transects walked or driven once per month during summer and winter 2018 - 2020 as part of the Washington Predator Prey Project. Additional scats were opportunistically collected during other aspects of field work.

6.3.3 DNA Extraction & Microsatellite Genotyping

All laboratory steps, except for metabarcoding sequencing and DNA fragment analysis, were conducted in the School of Environmental and Forest Sciences (SEFS) Genetics Lab at the University of Washington's. To extract DNA, scat samples were thawed and divided into four sections. We swabbed the outer surface of each scat, focusing on the ends where epithelial cells are most likely to be present, to identify individual coyotes. Using the same swab, we then sampled both sides of each internal section to gather DNA from ingested items. Each scat was swabbed with a flocked swab moistened with phosphate-buffered saline solution. DNA extraction followed a modified version of the QIAmp DNA Investigator Kit protocol (Qiagen, Hilden, Germany).

For individual coyote identification, we amplified DNA from the samples at 12 microsatellite loci using two multiplexed PCR panels. To minimize genotyping errors, each sample was replicated three times, following Prugh et al. (2005). The first panel amplified nuclear microsatellite loci FH2001, FH2010, FH2054, FH2088, and FH2328. The second panel amplified CXX2235, FH2096, FH2137,

FH2140, FH2159, and two additional loci on the X and Y chromosomes for sex determination (DBX6, DBY7; Prugh et al., 2005; Seddon, 2005). Both panels were processed under identical cycling conditions using touchdown-PCR: an initial 15-minute denaturation at 95°C, followed by 10 touchdown cycles at 94°C for 30 seconds, 68°C decreasing by 1°C per cycle for 30 seconds, and 72°C for 45 seconds. This was followed by 30 cycles at 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds, with a final extension at 60°C for 15 minutes. After amplification, the PCR plates were frozen and sent to Yale's Keck DNA Sequencing Core for fragment analysis using an Applied Biosystems 3130 Series Genetic Analyzer via capillary electrophoresis. Allele sizes were quantified using GeneMapper (Curie-Fraser & Shah, 2010), and consensus genotypes were created from the replicates following Prugh et al. (2005) and imported into GeneAlEx in Excel (Peakall & Smouse, 2006). To ensure no dog scats were collected, genotypes were compared with alleles from domestic dog reference samples. Scats collected in the wildland sites were additionally screened with a mitochondrial DNA panel to ensure they were of coyote origin.

6.3.4 Library Preparation & Sequencing

To analyze coyote diet, we performed PCR amplifications using a multiplex of two primer pairs: 12SV5 for vertebrates (Riaz et al., 2011) and trnL g/h for plants (Taberlet et al., 2007). Each sample was processed in triplicate. The PCR amplicons were tagged with custom sample-specific indices, quantified with a Qubit DNA quantifier, normalized, bead cleaned, and pooled following established protocols (de Barba et al. 2013). The pooled samples were then submitted to the Northwest Genomics Center for sequencing on an Illumina NextSeq platform.

6.3.5 Bioinformatics

Upon receiving the raw sequence files, we decompressed them using the 'tar' command in the terminal. Using custom bash scritps, we merged reads from the same samples across the four sequencing lanes. These merged files were exported as forward and reverse reads in fasta format for each sample and replicate. For the third run, we created a custom Bash script to merge our reads from across sequencing lanes. FastQC (Andrews, 2010) was employed to assess the quality of the raw reads, and MultiQC (Ewels

et al., 2016) was used to summarize the results across all samples. After confirming acceptable quality metrics, we used cutadapt to trim primers and overhangs from both forward and reverse reads, followed by another round of FastQC and MultiQC to ensure successful trimming. Trimmed reads were then processed through the *DADA2* package in R (Callahan et al., 2016). We plotted the quality profiles of a subset of reads to ensure normal distribution. Reads were filtered and trimmed using DADA2's 'filterAndTrim' function, with reads having expected errors greater than EE = 2 being discarded. The 'learnErrors' function was used to assess error rates, and the filtered reads were denoised using the 'dada' command, which eliminates erroneous reads to reconstruct the true community composition. Forward and reverse reads were merged using 'mergePairs'. A sequence table was generated using the 'makeSequenceTable' function, and chimeras were removed with the 'removeBimeraDenovo' command. The resulting sequence table was converted to a matrix and saved as a CSV file.

6.3.6 NCBI Blast

The fasta files were uploaded to NCBI's Blastn tool (Madden, 2002) for manual review of sequence matches. Given the large number of ornamental and non-native species in urban areas, creating a custom reference database was not feasible. We identified matches to the lowest taxonomic unit when we had high confidence based on high 'Percent Identity' and high 'Query Cover' scores, and when the species were likely to be found in our study area. For unlikely species, we either classified them at the genus level or assigned a closely related species known to exist in the area. For instance, reads matching European beaver (*Castor fiber*) were identified as American beaver (*Castor canadensis*). When sequences matched multiple species, the lowest common taxonomic unit was recorded. We also documented instances where no sequences matched the NCBI nucleotide database.

6.3.7 Additional Read Filtering

We excluded any samples that failed to amplify in at least two PCR replicates or had fewer than 100 total reads. For each sample, we retained only prey items that were detected in at least two out of three PCR replicates and averaged the number of reads for each prey item across those replicates. To account for contamination, we used extraction blanks and PCR negatives, averaging the reads from these controls and

subtracting them from the total reads for each sequence in the respective samples. Additionally, we removed any human (*Homo sapiens*) reads from the dataset. We then calculated the total number of reads per sample, excluding both contamination and canid reads. Following standard practices, we considered only the reads representing at least 1% of the total for plants and 0.05% for vertebrates, after excluding canid and contamination reads (Caspi et al. 2025). Furthermore, we excluded scats that had more non-canid carnivore reads than canid reads. These non-canid carnivores included species such as bobcats (*Lynx rufus*), domestic cats (*Felis catus*), raccoons (*Procyon lotor*), and river otters (*Lontra canadensis*). For example, if a scat identified as coyote had more feline than canid reads, it was excluded as it likely originated from a bobcat. Bobcat scats could have been misidentified as coyote scats, and raccoon or river otter scats may have been inadvertently included in the sequencing process if collected for a different project.

6.3.8 Taxonomic Resolution

Plants were limited to those we were confident were from diet items rather than environmental contamination such as pollen or substrate contamination. Plants taxa were identified at the genus level when possible, and otherwise were identified at the family level. For corn, trnL cannot specify species, but when *Zea* and closely related grass taxa were the most likely in NCBI Blast, we counted these reads as *Zea*. Other *Poaceae* reads that did not have *Zea* as a top option were discarded as contamination unless representing another genus that likely represented a purposefully consumed food item (e.g., *Avena, Hordeum*). Vertebrates were largely identified to species but were represented at the genus level where it was not possible to differentiate between species (e.g., shrews, voles). We ensured that no samples contained a species or genus and a higher level classification (genus or family) that could represent the same food item.

6.3.9 Niche Breadth & Individual Specialization

To control for the effect of sample size on diet diversity, we first created species accumulation curves for two diversity metrics using the *iNEXT* package in R (Chao et al. 2014, Hsieh et al. 2020). Based on these

curves, we set a minimum cutoff of 8 samples per individual for inclusion in analyses (Figure 6.2). We then resampled by drawing 8 random samples from each coyote with more than 8 scats. Resampling was repeated 1,000 times, and the average frequency of occurrence values across all dietary items for each study area were recorded. We chose to use frequency of occurrence values as they have been found to be highly correlated with relative read abundance diets (ratio of taxa-specific reads to all reads) and allow the direct incorporation of dietary information across plant and vertebrate primers (Shively et al. in press).

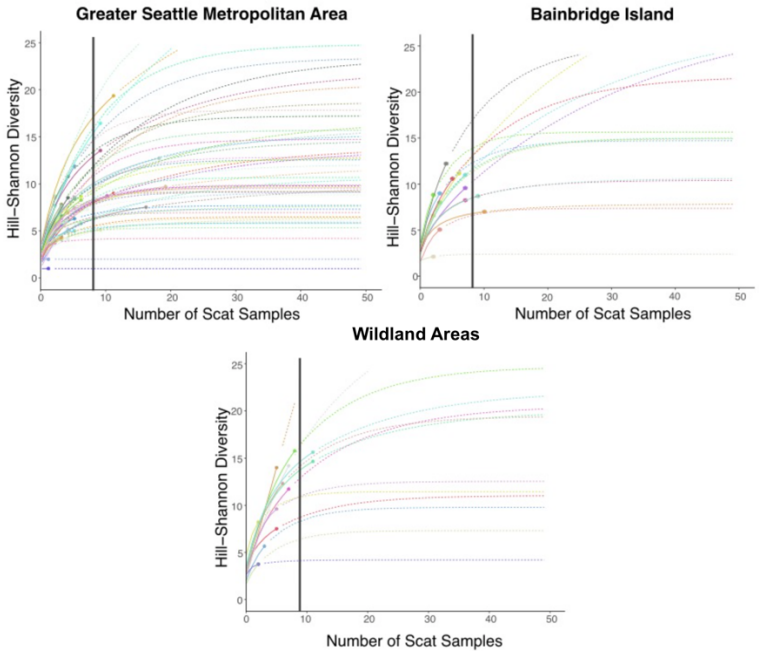


Figure 6.2: Species accumulation curves for all individuals identified with more than 1 scat sample against Hill-Shannon Diversity for all three study regions. Each line represents an individual. Where the line is solid is the observed value, the dashed component is interpolated by the *iNEXT* package in R. A vertical black bar is drawn at the 8-scat cutoff for each graph.

Next, we calculated Hill-species richness and Hill-Shannon diversity across the diet of individual coyotes using the 'estimateD' function from the *iNEXT* package in R based on presence/absence of dietary items in each coyotes' scat samples. This function rarefies to the lowest sample size (n = 8) to account for differences in diversity related to uneven sampling among individuals but allows us to assess if the assumed difference in prey diversity among urbanization levels is reflected among the diets of

individuals. To determine how urbanization affected population-level niche breadth, we calculated Levin's niche breadth index (*B*) using the rarified frequency of occurrence dataset for each study area and the formula $B = \frac{1}{\Sigma p_i^2}$ where p_i is the proportion of resources used (Levin 1968). To meet assumptions of this index, we normalized the data so that each row summed to one prior to calculating *B*. Then we used Wilcoxon Signed Rank tests to test for differences in individual niche breadth among the different study areas (Wilcoxon 1945).

Then, to assess individual specialization, we computed a Jaccard dissimilarity matrix using the 'vegdist' function from the *vegan* package (Oksanen et al., 2022). We then used the 'Eindex' function from the *RInSp* package in R (Zaccarelli et al., 2013) to calculate a jackknife cross-validated estimate of the E index for diet specialization and mean dietary overlap among individuals. These indices are derived from the DIETA1 software and based on Araújo et al. (2008). The E index measures the degree of individual variation in diet, ranging from 0 (all individuals have identical diets) to 1 (each individual has a unique diet). Mean overlap (O_{mean}) also ranges from 0 to 1, with 0 indicating no dietary overlap across the population and 1 representing complete overlap across the population. We then used a permutation-based multivariate analysis of variance (PERMANOVA) on the dissimilarity matrices to determine if diet varied among individuals and among our study areas. PERMANOVA's were conducted in R using the 'adonis2' function from the *vegan* package. To account for unequal sampling sizes between individuals, we ran 1000 separate PERMANOVA's drawing 8 samples from each individual and averaged across the results.

6.4 Results

6.4.1 Sampling

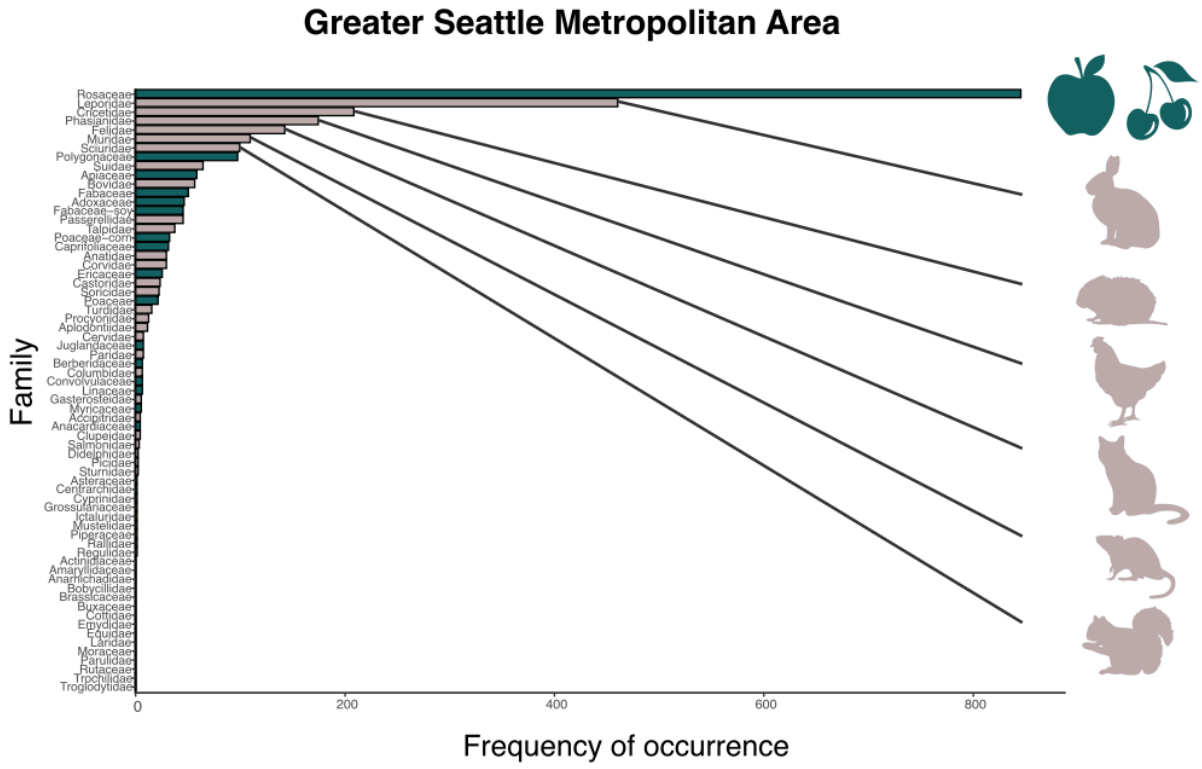
We conducted diet analysis on 1,220 scats from the Greater Seattle Metropolitan Area. This resulted in 948 scats successfully sequenced and deemed of coyote origin. Of these 948 scats, 827 were successfully genotyped (*n* = 147 individuals). On average we collected 5.59 scats per individual (*SD* = 6.42, range = 1-36), with 35 individuals having at least 8 scat samples assigned to their ID ($\bar{x}$ =14.57, *SD* = 7.57). Of 145

Bainbridge Island scats run for diet, 106 were successfully sequenced, and 99 were assigned to one of 26 coyotes (x = 3.96 scats per individual, SD = 3.17, range = 1-12 scats). Five individuals were represented by at least 8 scat samples ($\bar{x}$ = 9.4, SD = 1.67). Of 175 scats from the combination of our two wildland study areas, we successfully obtained dietary information for 146 scat samples (n = 106 from the Methow Valley and 35 from the Northeast). Of the 146 scat samples, 105 were assigned to a genotyped coyote (n = 15 individuals), with an average of 9.6 scat samples collected per individual (SD = 4.14, range = 1-18). Ten individuals (7 from MV, 3 from NE) had at least 8 samples assigned to them ($\bar{x}$ =11.8 scat samples/individual, SD = 2.82).

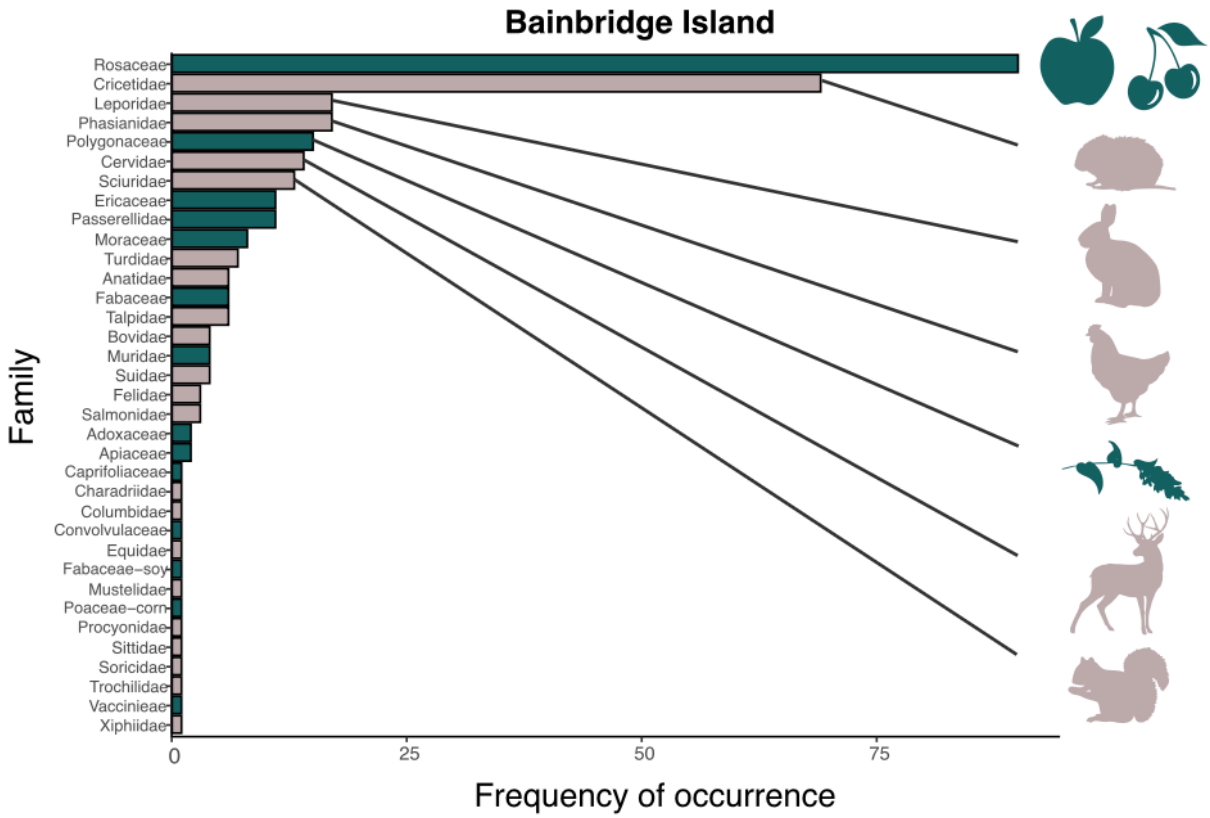
6.4.2 Dietary Diversity & Niche Breadth

Within scats from the Greater Seattle Metropolitan Area, we identified 23 vertebrate families (40 species; 10 additional genera) and 14 plant families consumed with frequency of occurrence values ≥ 0.01 (Figure 6.3, Appendix D Table S1 & S2). In the Bainbridge Island study area, we identified 23 vertebrate families (22 species, 4 additional genera) and 9 plant families. Lastly, for the wildland study areas we identified only 14 vertebrate families (13 species, 4 additional genera) and 10 plant families across all of our scat samples.

A)


Greater Seattle Metropolitan Area
Family
Rosaceae
Leporidae
Cricetidae
Phasianidae
Felidae
Muridae
Sciuridae
Polygonaceae
Suidae
Apiaceae
Bovidae
Fabaceae
Adoxaceae
Fabaceae−soy
Passerellidae
Talpidae
Poaceae−corn
Caprifoliaceae
Anatidae
Corvidae
Ericaceae
Castoridae
Soricidae
Poaceae
Turdidae
Procyonidae
Aplodontiidae
Cervidae
Juglandaceae
Paridae
Berberidaceae
Columbidae
Convolvulaceae
Linaceae
Gasterosteidae
Myricaceae
Accipitridae
Anacardiaceae
Clupeidae
Salmonidae
Didelphidae
Picidae
Sturnidae
Asteraceae
Centrarchidae
Cyprinidae
Grossulariaceae
Ictaluridae
Mustelidae
Piperaceae
Rallidae
Regulidae
Actinidiaceae
Amaryllidaceae
Anarrhichadidae
Bobycillidae
Brassicaceae
Buxaceae
Cottidae
Emydidae
Equidae
Laridae
Moraceae
Parulidae
Rutaceae
Trochilidae
Troglodytidae
0
200
400
600
800
Frequency of occurrence

B)


Bainbridge Island
Family
Rosaceae
Cricetidae
Leporidae
Phasianidae
Polygonaceae
Cervidae
Sciuridae
Ericaceae
Passerellidae
Moraceae
Turdidae
Anatidae
Fabaceae
Talpidae
Bovidae
Muridae
Suidae
Felidae
Salmonidae
Adoxaceae
Apiaceae
Caprifoliaceae
Charadriidae
Columbidae
Convolvulaceae
Equidae
Fabaceae−soy
Mustelidae
Poaceae−corn
Procyonidae
Sittidae
Soricidae
Trochilidae
Vaccinieae
Xiphiidae
0
25
50
75
Frequency of occurrence

C)

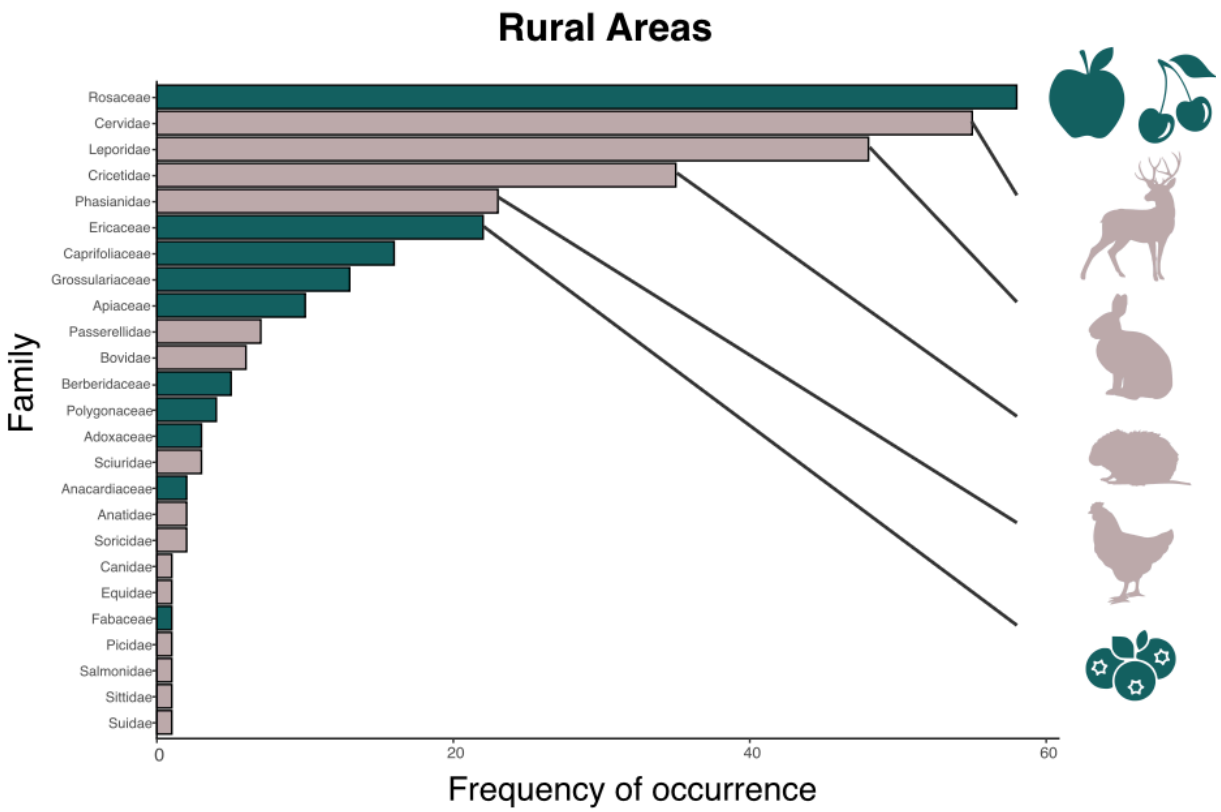


Figure 6.3: Bar graphs of frequency of occurrence of different taxonomic families identified across all coyote scats for each study region: A) Greater Seattle Metropolitan Area, B) Bainbridge Island, C) Rural Areas. The top dietary items are depicted with silhouettes to the right of the bars. Vertebrate prey are shown in light brown, while vegetative foods are depicted in dark green.

Dietary diversity was remarkably similar among the three study areas. For the Greater Seattle Metropolitan Area coyotes, Hill-Shannon diversity was 9.19 (*SD* = 2.91) and Hill-species richness was 11.56 (*SD* = 3.23). Similarly, Hill-Shannon diversity was 9.15 (*SD* = 1.71) and Hill-species richness was 11.99 (*SD* = 2.85) for Bainbridge Island diets. And lastly, for wildland coyotes, Hill-Shannon diversity was 7.96 (*SD* = 3.01) and Hill-species richness was 9.28 (*SD* = 2.98). Population-wide Levin's niche was also similar among all three study areas, but widest by a slight margin for the Greater Seattle Metropolitan

Area coyotes ($\bar{x}$=8.19, *SD* = 3.05), followed by the wildland coyotes ($\bar{x}$ = 7.99, *SD* = 2.80), and lastly the Bainbridge Island coyotes ($\bar{x}$ = 7.51, *SD* = 1.15).

6.4.3 Individual Specialization

The degree of dietary overlap varied by study region (Figure 6.4). The Greater Seattle Metropolitan Area coyotes (O_{mean} = 0.43) had lower overlap across individuals than Bainbridge Island coyotes (O_{mean} = 0.55), but higher overlap than wildland coyotes (O_{mean} = 0.34). Thus, individuals on Bainbridge Island had more similar diets across the population than the other two study areas.

PERMANOVA results confirmed significantly different diets among individuals within each region (Greater Seattle Metropolitan Area: R_{ID}^2 = 0.33, p = 0.001; Bainbridge Island: R_{ID}^2 = 0.22, p = 0.010; Wildland: R_{ID}^2 = 0.29, p = 0.001). Based on these results, 22-33% of diet variation within each study area was explained by individual coyote ID. The E index of specialization was highest for individuals in the wildland study areas (E = 0.65, Var = 2.74x10^{-5}), intermediate for the Greater Seattle Metropolitan Area (E = 0.57, Var = 2.22 x 10^{-7}), and lowest in the Bainbridge Island study area (E = 0.45, 5.43x10^{-5}), consistent with the population-wide overlap values showing highest dietary overlap among the Bainbridge Island coyotes.

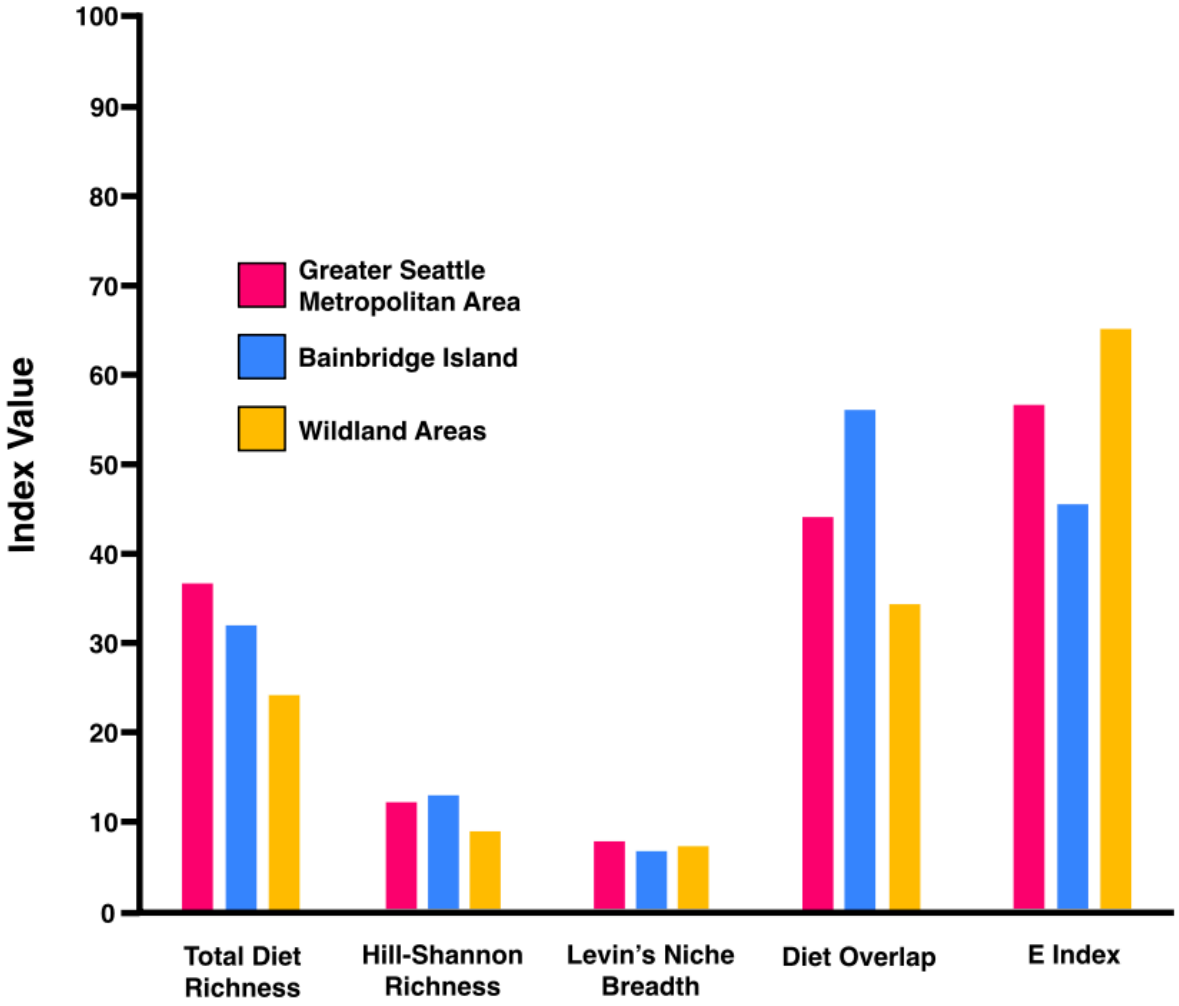


Figure 6.4: Bar graph of number of taxonomic families identified, average E-index of individual specialization, average mean network overlap (O_{mean}), average Levin's niche breadth, and average Hill-Shannon richness results across all three study areas. E-index and mean network overlap (O_{mean}) range between 0 and 1 and are rescaled to 0-100 for visualization. Greater Seattle Metropolitan Area results are displayed in red, Bainbridge Island in Blue, and the wildland areas in yellow.

6.5 Discussion

Coyotes are one of the most successful species in the Anthropocene, doubling their historic range to occupy virtually every ecosystem available across North America, including metropolitan areas (Hody & Kays 2018). While their success is generally attributed to their adaptability and generalist nature (Hody &

Kays 2018), understanding whether their populations are comprised of individual generalists or specialists with substantial individual behavioral variation has important management implications across urbanization gradients. While theory would suggest high variability in niche partitioning and individual specialization as a result of differences in resource diversity and intra and interspecific competition, we found surprisingly similar niche widths among coyotes occupying areas with varying degrees of urbanization in Washington. Despite similar levels of diet diversity, we found evidence for specialization among individuals that varied substantially across these diverse landscape contexts. Specialization was lower in our most urban study area compared to our wildland area and lowest in the exurban area, suggesting that coyotes in urban and exurban areas may experience reduced competition for resources.

Our wildland study areas had the highest degree of individual specialization and the lowest degrees of dietary overlap. These study areas were much larger (~5,000 km^2 each) than Bainbridge Island (~72 km^2) and the Greater Seattle Metropolitan Area (~1200 km^2), and coyotes likely occurred at much lower densities in the wildland areas (Fischer et al. 2012, Šálek et al. 2014*).* Coyotes in more urbanized regions have significantly smaller home ranges, which may constrain the resources available to them and reduce the potential for specialization (Atwood et al. 2010*,* Šálek et al. 2014, Ward et al. 2018). While we do not know home range sizes of coyotes in the Greater Seattle Metropolitan Area, studies in other urban regions such as Chicago have recorded coyote home ranges as small as 5 km^2 (Gehrt et al. 2009), whereas coyotes in our wildland areas had average home ranges of 35 km^2 (n = 34 coyotes; Prugh et al. 2023). Likewise, on a relatively small island such as Bainbridge Island, prey availability is likely to be more homogenous than across a larger wildland area with diverse topography and land cover (Tobler 1970). Thus, home ranges and territoriality may strongly influence coyote diet and access to individual resources throughout our study areas. Our findings suggest that coyotes in more urban areas may face lower degrees of competition due to prolific anthropogenic resources and abundant non-native prey despite having increased intraspecific density and conspecific densities. While we do not have estimates of resource availability in our study regions, other works have demonstrated that resource availability is generally higher in urban regions than in non-urban regions (Hansen et al. 2020, Thompson et al. 2021). The

increased specialization among wildland coyotes may reflect increased intraspecific competition due to lower diversity and abundance of resources, as well as increased interspecific competition with other carnivores like bobcats (*Lynx rufus*), wolves (*Canis lupus*), and mountain lions (*Puma concolor*; Prugh et al. 2023).

Dietary richness is often found to be or assumed to be greater in urban regions as a result of access to both native and anthropogenic food sources (Murray et al. 2015, de Souza Laurindo & Vizentin-Bugoni 2020, Larson et al. 2020). However, little research has uncovered if this diversity is reflected at the population level or at the individual level. While we identified more vertebrate and plant taxa within our urban study system across all individuals, rarified individual diet diversity was only slightly lower in our wildland study areas compared to Bainbridge Island and Greater Seattle Metropolitan Area coyotes. While increased number of taxa were found in our urban region across the population, the lack of difference in individual dietary diversity highlights that territorial animals like coyotes are constrained to the resources in their immediate vicinity. With significantly smaller home ranges, urban predators may have a similar number prey species available to any given individual compared to their wildland counterparts, despite having a greater diversity of resources available across the urban landscape.

Predators like coyotes can have high levels of perceived conflict stemming from their dietary choices (Poessel et al. 2017b). The degree of individual diet specialization across diverse landscape contexts therefore has important management implications, because individual "specialist" predators can have tremendous effects on prey populations. For instance, individual mountain lions that have learned to hunt bighorn sheep (*Ovis canadensis*) have caused major localized sheep declines (Festa Bianchet et al. 2006, Ross et al. 1997). Similarly, extirpation of the Stephen's Island wren (*Xenicus lyalli*) was the result of predation by a single domestic cat (*Felis catus*; Greenway 1967). In these cases, removing the few individuals involved in conflicts instead of population-wide removal efforts may be effective in reducing conflict (Conradie & Piesse 2013, Peebles et al. 2013, Blackwell et al. 2016, Swan et al. 2017). We generally found lower degrees of individual diet specialization in the urban and island areas than in wildlands, indicating that effective conflict mitigation strategies may differ among these landscape types.

Removal of 'problem' individuals who may be depredating domestic animals could be effective in wildland areas (Blejwas et al. 2002), but this strategy may not be effective in cities. For example, consumption of domestic cats is a major source of human-coyote conflict in urban areas (Draheim et al. 2019), but our findings indicate that removal of specific "cat-killing" coyotes would not be an effective conflict mitigation strategy. Of the coyotes sampled within the Greater Seattle Metropolitan Area, 74% consumed cats, though the degree of cat predation varied substantially (range = 0 – 63% of scats contained cat remains, mean = 15%; n = 35 coyotes). These findings suggest that lethal removals may not be as effective for mitigating conflicts for dietary items consumed evenly across the population, and thus other strategies such as keeping domestic cats indoors are essential to promoting co-existence (Poessel et al. 2017a,b).

While dietary richness can give us an idea of how varied an individual's diet may be, niche breadth can indicate the dispersion of dietary diversity and point to either widespread consumption of many items or heavy consumption on relatively few taxa. Niche breadth was surprisingly similar across study areas and individuals. The relatively narrow values for niche breadth compared to the number of resources available to coyotes indicate that coyote diet is largely made up of a few key species such as Eastern cottontail rabbits in the Greater Seattle metropolitan Area or cervids in the wildland study areas. This indicates that despite finding 40 unique species and 10 additional genera in the Greater Seattle Metropolitan Area versus 13 species and 4 genera across our wildland study areas, the bulk of their diets are often made up of a few common species. Across our study areas, coyotes appear to be eating what is easily available to them within their home ranges and acting predominantly as dietary generalists at both the individual- and population-level. Thus, the trophic effects of coyotes may be relatively consistent across diverse landscapes, whereby coyotes may function as keystone predators reducing the dominance of the most abundant prey species locally available (Henke and Bryant 1999). Using our data, however, we could not assess prey distribution and availability on the landscape. To better understand the relationship between prey availability and niche breadth, further research that estimates prey availability and how it varies across coyote home ranges should be conducted.

Because scats represent a snapshot of an individual's consumption, multiple samples from the same individual are required to accurately characterize diets (Prugh et al. 2008). In our case, species accumulation models indicated that at least 8 scat samples per individual were required before dietary diversity began to level off. Longitudinal sampling of the same individual can be challenging, especially across large study areas or in areas with high turnover rates. In our ~5,000 km^2 wildland study areas, for example, the number of coyotes with at least 8 successfully genotyped scats was limited because field efforts were not sufficient to repeatedly collect scats from the same individuals across such broad landscapes. Additionally, while metabarcoding offers a much finer resolution of diet—capable of identifying most vertebrate species and many plant genera—a significant drawback is the need for repeated sampling of individuals. Other methods, such as stable isotope analysis, offer inferior taxonomic resolution but have the advantage of integrating dietary information over longer periods from single samples (Crawford et al. 2008). Future research should consider combining lower-resolution information such as stable isotope analysis with metabarcoding to increase understanding of long-term dietary trends, while still being able to assess individual diet variation (Bonin et al. 2020).

Understanding dietary specialization and diversity has important ecological implications. Patterns of prey consumption by individual predators can alter prey distribution, behavior, and population size (Packer et al. 2003, Salo et al. 2010), which can have cascading effects on the vegetative landscape (Schmitz et al. 2000). Many studies have pointed to dietary differences between urban and non-urban populations (Sugden et al. 2021), but few have investigated the individual behaviors and choices that contribute to these population-level differences, or how these differences may arise (Romero-Vidal et al. 2023). Urbanization often comes with abundant resources and supports high densities of mesocarnivores. Yet, we have little understanding of how mesocarnivore densities interplay with these abundant resources to shape the landscape of competition. Romero-Vidal et al. (2023) found that dietary patterns were largely driven by intraspecific competition and individual behavior for birds, while urbanization generally did not contribute to these trends. Our results indicate resources in urban areas may be sufficiently abundant that

coyote densities are limited by territoriality rather than resources, thereby relaxing the intensity of intraspecific competition and thus dietary partitioning. While lethal removal of coyotes in urban areas can be an effective management tool for some conflicts (Breck et al. 2017), lack of individual specialization may render this management option ineffective when conflict pertains to dietary items that are shared evenly across the population.

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7. Chapter 7 – Conclusions

From 2017 through 2023, across all study sites, this research collected a tremendous amount of data, totaling over 5,300 scats and nearly 80 coyote carcasses. Combined, I was able to genetically identify 728 individual coyotes, facilitating dietary and genetic comparisons across three diverse ecosystems – Greater Seattle Metropolitan Area, offshore oceanic islands, and wildlands in northern Washington. While the first chapter provides a framework for understanding how urbanization may alter evolutionary constraints for wildlife, this rich dataset allowed me to address potential eco-evolutionary differences that may arise as a result of urbanization and landscape modification in the chapters that followed.

In the preceding four chapters, I focus on the coyote, an adaptable mesocarnivore that has seen great success in the Anthropocene as other species struggle to persist. Understanding what has led to the coyote's success can help elucidate what makes other species struggle to cope and facilitate conservation efforts for these species. Here I have found that coyotes in Seattle and the surrounding urban regions show differences in diet and gene flow compared to wildland areas. In chapter 3, I address gene flow within the city of Seattle using private alleles and STRUCTURE analyses. I found that coyotes in Seattle are roughly divided into four quadrants, each divided by two linear barriers – Interstate-5 which runs vertically through the city and the Lake Washington Ship Canal which runs horizontally through the city. Despite being a highly mobile species, these linear barriers appear to have significant effects on animal movement and subsequent breeding. When I expanded the gene flow analyses across our three study systems, I found that the Seattle-Tacoma metropolitan area has similar genetic patterns to Whidbey and Bainbridge Islands, two offshore islands with a small bridge connecting them to the mainland. This suggests that urban areas may act as semi-permeable genetic islands, with reduced genetic diversity and short dispersal distances. While Chapter 3 found Interstate-5 to act as a linear barrier to coyote movement at a city-wide scale, the state-wide analysis did not reveal highways to be acting as significant barriers. This highlights the variability that can come from anlayzing genetic and spatial data at different scales

and indicates that the correct spatial scale is important to inform management practices such as the building of highway overpasses or designing habitat corridors.

When assessing diet within the city of Seattle, I found that presence of diet items in scats were driven by both natural and anthropogenic variables, but the individual drivers varied by specific dietary group. Expanding across the state, this research suggests that individual specialization is highest in wildland areas, though the differences in specialization across the three ecosystem types varied much less than expected. Diet diversity at a population-level was highest in the urban region and lowest in the wildland areas, though at the individual-level dietary diversity was relatively even across systems. This suggests that diet may mainly be constrained by home ranges and what food items are present within this constrained space. For urban coyotes, despite having access to more types of food across the entire city, the smaller home range of urban coyotes compared to wildland coyotes may constrain the actual number of food items available to each individual.

In addition to the analyses included in this dissertation, the dietary data produced can also be used by wildlife managers to understand where conflict may be most likely to occur within the city based on dietary trends. For instance, coyotes eating more anthropogenic food may be in closer proximity to humans or may be being fed (intentionally or otherwise), increasing the risk of human-wildlife conflict (Murray et al. 2015). Similarly, this data can also point to which areas of the city are subjected to the highest domestic animal depredation rates. Genetic data can help facilitate understanding of the effects of urbanization and anthropogenic infrastructure on gene flow for wildlife, allowing us to build increasingly wildlife-friendly cities.

As urbanization continues to expand globally, understanding its effects on wildlife becomes increasingly important to ensure spaces function for both humans and for maintaining biodiversity. Urban regions offer unique advantages and challenges for wildlife. Species able to cope and persist may become fundamentally different than their wildland counterparts. However, understanding these potential differences begins by understanding the ecological differences that come with urbanization.

7.1 Future Research

One of the limitations of non-invasive methods is not having a concrete home range for the animals studied. I suggest that future research couple non-invasive scat collection with collaring in order to better understand drivers of dietary choices. While I was able to conduct a variety of analyses using buffers around scats or parks, without knowing true home ranges, these buffers likely include a variety of used and unused spaces giving less precision and accuracy to our models. Collaring would additionally help elucidate gene flow and movement of coyotes. I also suggest combining high-resolution fecal metabarcoding with stable isotope analysis to better understand long-term trends in diet across a population. While stable isotope analysis is at a much courser resolution, it offers a long-term glimpse of dietary trends that require many fecal samples to be collected over time for the same individual.

8. Appendices

8.1 Appendix A – Supplemental Material for Chapter 3

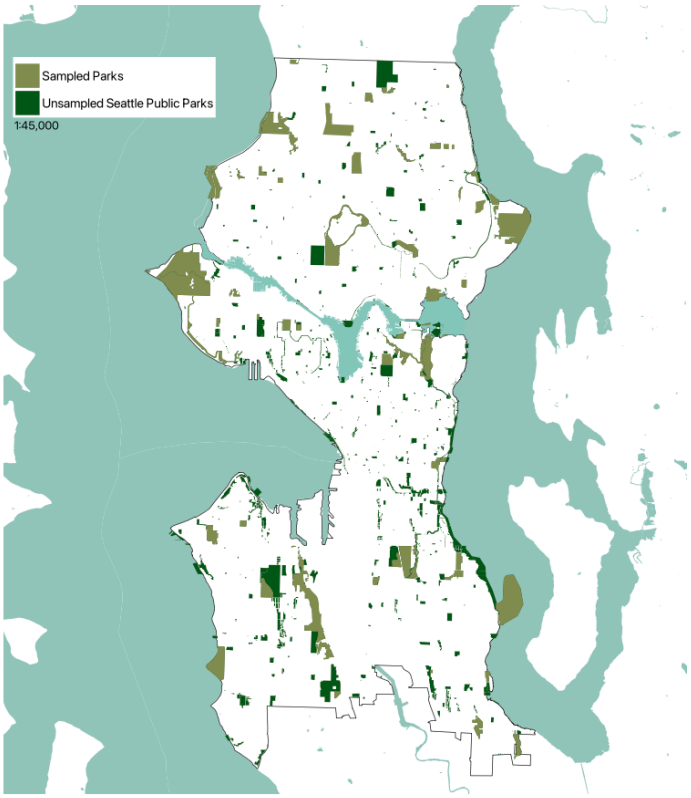


Figure S1: A map of Seattle with all sampled locations in light green and all unsampled Seattle Public parks in dark green.

Table S1: Rarified allelic richness values for all loci for quadrant groups with related individuals retained.

Locus	NE	NW	SW	SE
FH2001	6.49	4.30	5.00	6.07
FH2010	2.00	2.00	2.00	2.39
FH2054	3.43	2.78	2.00	3.39
FH2088	3.00	5.96	5.79	5.10
FH2328	5.63	5.96	5.79	5.10
CXX2235	5.01	4.67	3.99	4.20
FH2096	2.98	2.98	2.99	1.87
FH2137	7.46	5.00	4.80	5.52
FH2140	5.33	6.71	5.00	5.12
FH2159	4.05	5.41	4.90	5.61
Mean Richness	4.54	4.37	4.05	4.22

Table S2: Private alleles across all quadrant groups. The 'Individuals' column gives the number of coyotes included in each group. The following columns are genotyped loci, and each value is the rarified number of private alleles at each locus for each group, corrected for the lowest sampling size.

	Individuals	FH2001	FH2010	FH2054	FH2088	FH2328	CXX2235	FH2096	FH2137	FH2140	FH2159	Sum
NE	17	0.268	0.000	0.322	0.000	1.401	0.613	0.000	1.096	0.082	0.000	3.782
NW	23	0.000	0.000	0.000	0.000	0.068	1.379	0.003	0.027	1.389	0.645	3.511
SE	23	1.139	0.391	0.185	0.000	0.393	0.635	0.000	0.768	0.880	1.763	5.802
SW	10	0.003	0.000	0.000	0.003	1.052	0.039	0.995	0.022	1.143	0.959	4.216

8.2 Appendix B – Supplemental Material for Chapter 4

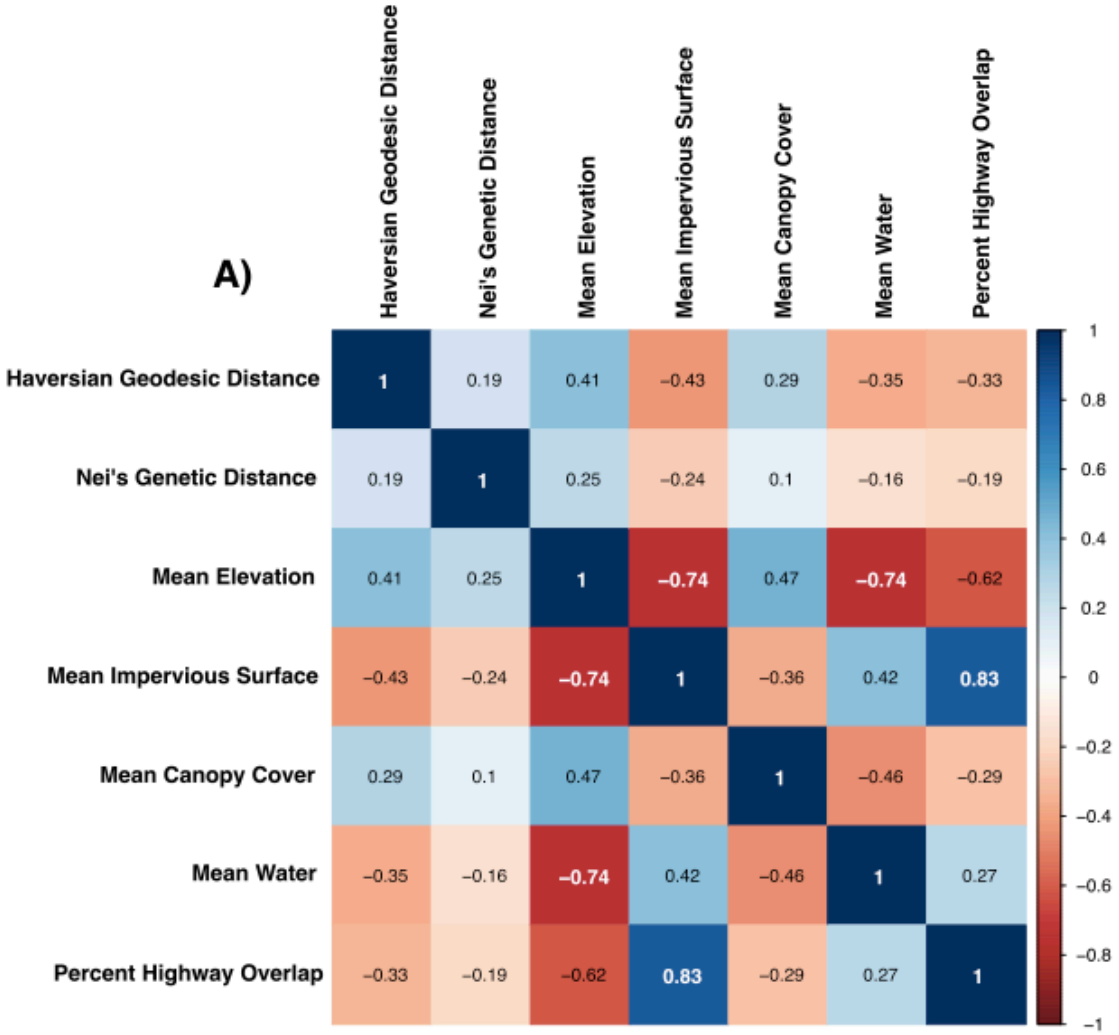


Figure S1: Correlation values for all variables for all coyote dyads.

8.3 Appendix C – Supplemental Material for Chapter 5

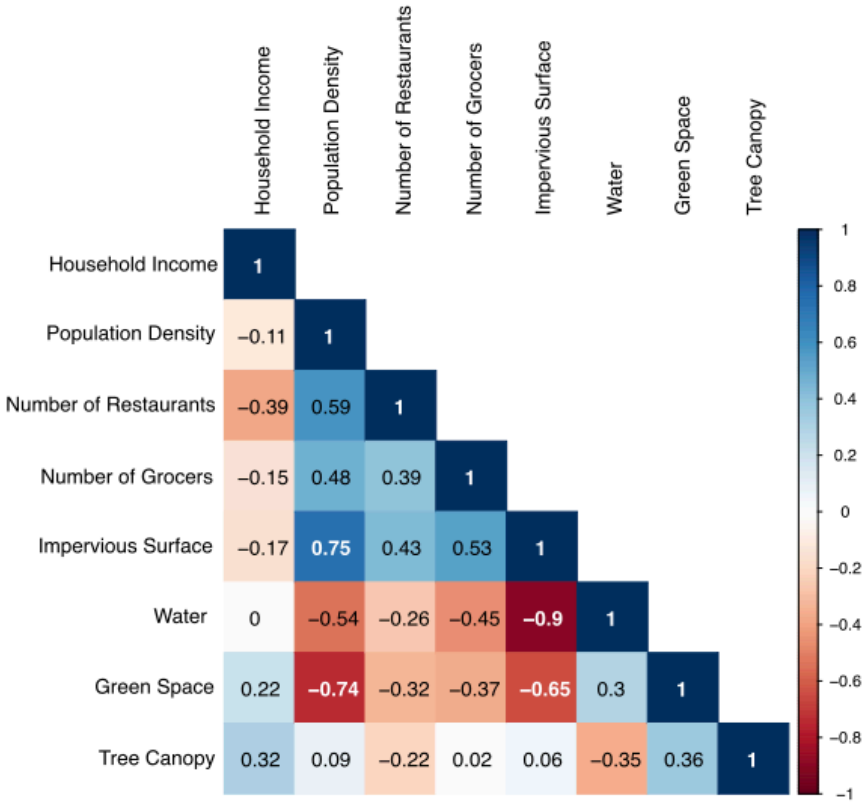


Figure S1: Correlation plot between all potential variables with Pearson's correlation coefficient indicated for every dyad of variables extracted from the scat buffers. Variables above the 0.6 correlation threshold are bolded and in white font.

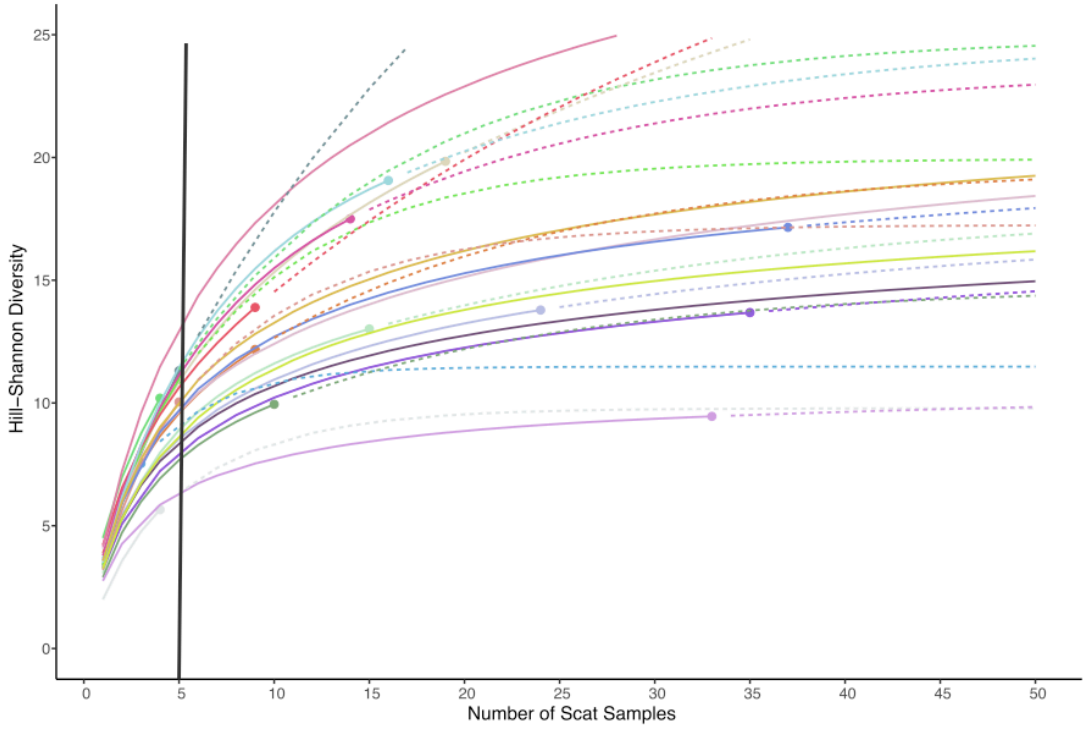
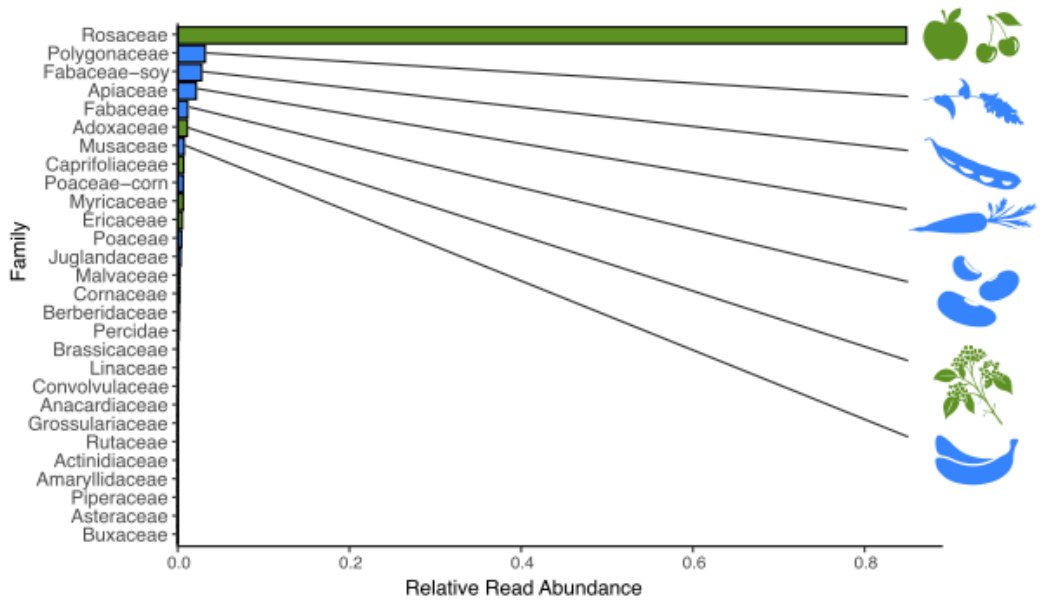


Figure S2: Species accumulation curves for Hill-Shannon and Hill-Simpson Diversity across city parks. Each line represents a separate park.

A)



B)

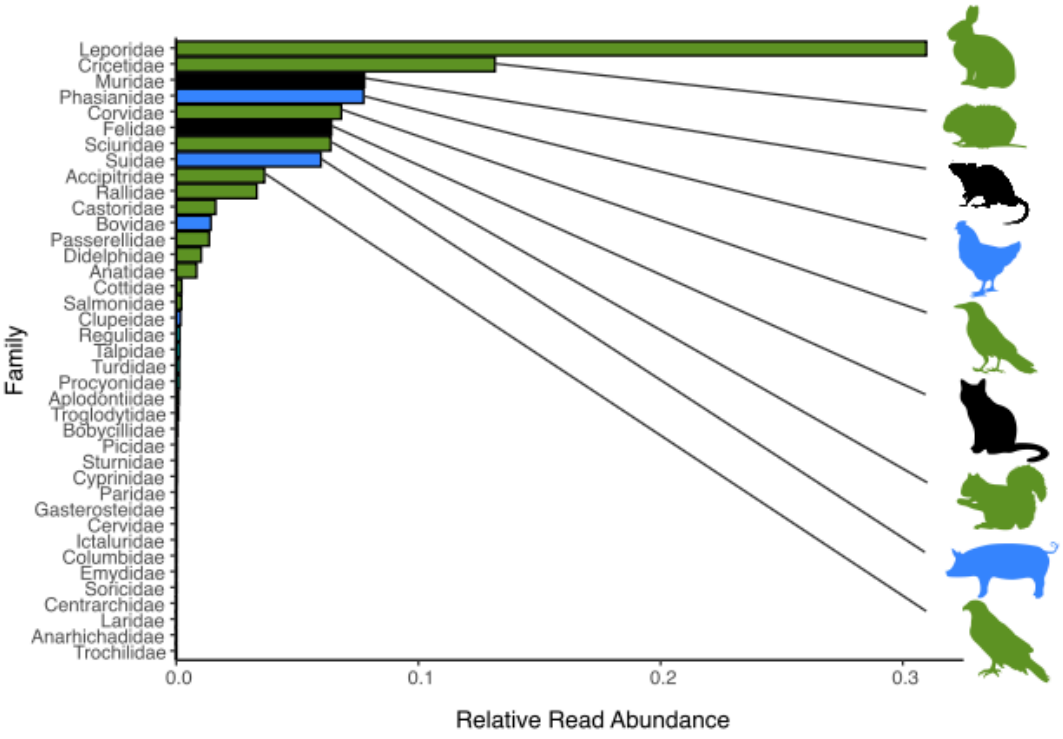


Figure S3: Relative read abundance (RRA) bar graphs for vertebrates (A) and plants (B). Families are color-coded by being anthropogenic in source (blue), non-anthropogenic in source (green), and pest rodents and cats (black).

Table S1: Listed below are the four grocer type classifications and a list of the name of every grocer included in each classification.

Grocer Classification	Included Grocers
Budget	Dollar Tree, Grocery Outlet
Expensive	Bert's Red Apple Market, Ken's Market, Kitchen & Market, Leschi Market, Marketime Foods, Metropolitan Market, PCC Community Markets, Town & Country Market,
Specialty	99 Ranch Market, Abay Market, Abyssinia Market, Addis Market Seattle, Asian Family Market, Byblos Mediterranean Halal Arabi, Castillos Supermarkets, Continental Spices & Grocery, Dong Hing Market, Dong Sing Market, Dur Dur Groceries, East African Grocery, East African Habesha Market, East Africa Imports & Restaurant, Ebenezer Tienda Latina, El Paso Supermarket, Enat Souk, Ethio Mini-Market, European Foods, Fou Lee Market & Deli, Gambia International Inc., Golden Hong Market, Goodies Mediterranean Market, Guadalupe Market, H Mart, H-Mart Grab & Go, Hau Hau Market, Hung Long Grocery Store, Ilyas West African Market, Indian Sweets & Spices, J & B African Market, La Conasupo Taqueria & Snack Shop, La Herradura, Latin Market, Latina Real, M2M Mart, Mana Market, Masha Allah Grocery Store, Mediterranean Oasis, Mekong Asian Market, Mercado Latina, Mesob Habesha Grocery, Moga's Market Halal Grocery & Clothing, Nazareth Market, New Golden Village Market, Plaza Latina, Roba's African Store, Ruhamah Grocery, Sammis Halal Market, Selam Market, Taftan Bazaar and Halal Meat, The Souk, Tienda Latina El Amiguito, Uwajimaya Seattle, Yesler Grocery.
Standard	Albertson's, Belltown Market, Fred Meyer, QFC, Safeway, Sprouts Farmers Market, Target, Trader Joe's, Whole Foods Market

Table S2: Range and mean values for parks and individuals across all covariates. Variables were extracted from a 1km buffer from scats and parks. For individuals the mean is weighted by the number of samples each individual contributed.

	Individual	Park

	Min	Mean	Max	Min	Mean	Max
% Greenspace	2.36	13.13	33.97	1.22	9.95	22.71
# Grocers	0	0.31	3	0	2.14	7
Household Income	37.95	332.14	504.49	183.57	340.37	467.53
# of Reported Illegal Dumping Incidents	0	331	1814	154	896	1976
% Impervious Surface	0.28	38.00	68.00	20.76	48.48	66.64
% People of Color	17.88	37.52	74.09	18.04	39.85	72.76
Population Density	2.46	9.46	27.56	6.98	12.39	16.86
# Restaurants	0	12.70	83	5	32.64	103
% Tree Canopy	8.26	17.20	34.35	11.66	20.22	26.63

Table S3: Table of scientific and common names of taxa found within coyote scats to the highest resolution. Where 'Other' is listed in the genus column, we identified multiple potential genera aside from other genera listed in that family within the table but were not able to identify which one with confidence. These reads thus were grouped at the family level.

	Family	**Genus**	**Species**	**Common Name**

Bird	*Accipitridae*	*Buteo*	—	Hawks
	Accipitridae	*Haliaeetus*	*Haliaeetus leucocephalus*	Bald Eagle
	Anatidae	*Aix*	*Aix sponsa*	Wood Duck
	Anatidae	*Anas*	—	Dabbling Ducks
	Anatidae	*Bucephala*	—	Diving Ducks
	Bombycillidae	*Bombycilla*	*Bombycilla cedrorum*	Cedar waxwing
	Columbidae	Patagioenas	Patagioenas fasciata	Band-Tailed Pigeons
	Covidae	*Corvus*	*Corvus*	Crows
	Laridae	*Larus*	—	Gulls
	Paridae	*Poecile*	*Poecile Atricapilla*	Black-capped Chickadee
	Passerellidae	*Junco*	*Junco hyemalis*	Dark-eyed Junco
	Passerellidae	*Pipilo*	*Pipilo maculatus*	Spotted Towhee
	Phasianidae	*Coturnix*	*Coturnix japonica*	Japanese Quail
	Phasianidae	*Gallus*	*Gallus gallus*	Domestic Chicken
	Phasianidae	*Meleagris*	*Meleagris gallopavo*	Turkey
	Phasianidae	*Phasianus*	*Phasianus colchicus*	Ring-necked Pheasant
	Picidae	*Colaptes*	*Colaptes auratus*	Northern Flicker

	Rallidae	*Fulica*	*Fulica americana*	American Coot
	Regulidae	*Regulus*	—	Kinglets
	Sittidae	—	—	Nuthatches
	Strigidae	*Strix*	*Strix varia*	Barred Owl
	Sturnidae	*Sturnus*	*Sturnus vulgaris*	Common Starling
	Trochilidae	—	—	Hummingbirds
	Troglodytidae	*Troglodytes*	—	Wrens
	Turdidae	*Catharus*	*Catharus ustaulatus*	Swainson's Thrush
	Turdidae	*Ixoreus*	*Ixoreus naevius*	Varied Thrush
	Turdidae	*Turdus*	—	True Thrushes
Fish	*Alosidae*	*Brevoortia*	—	Brevoortia
	Anarhichadidae	*Anarhichas*	—	Wolffish
	Centrarchidae	*Lepomis*	—	Sunfish
	Centrarchidae	*Micropterus*	*Micropterus salmoides*	Large-mouth Bass
	Clupeidae	—	—	Herring & Sprat
	Cottidae	*Leptocottus*	*Leptocottus armatus*	Pacific Staghorn Sculpin
	Cyprinidae	*Cyprinus*	—	Carps
	Cyprinidae	*Mylocheilus*	*Mylocheilus caurinus*	Peamouth Chub
	Gasterosteidae	*Gasterosteus*	*Gasterosteus aculeatus*	Three-spined Stickleback
	Ictaluridae	*Ameiurus*	—	Bullheads
	Percidae	*Perca*	*Perca flavescens*	Perch

	Salmonidae	*Oncorhynchus*	*Oncorhynchus kisutch*	Coho Salmon
	Salmonidae	*Salmo*	Salmo salar	Atlantic Salmon
Mammal	*Aplodontiidae*	*Aplodontia*	*Aplodontia rufa*	Mountain Beaver
	Bovidae	*Bos*	*Bos taurus*	Domestic Cattle
	Bovidae	*Ovis*	*Ovis aries*	Domestic Sheep
	Castoridae	*Castor*	*Castor canadensis*	North American Beaver
	Cervidae	*Odocoileus*	*Odocoileus hemionus*	Black-tailed Deer
	Cricetidae	Other	—	Voles
	Cricetidae	*Microtus*	*Microtus longicaudus*	Long-tailed vole
	Didelphidae	*Didelphis*	*Didelphis virginiana*	Virginia Opossum
	Felidae	*Felis*	*Felis catus*	Domestic Cat
	Muridae	*Mus*	*Mus musculus*	House Mouse
	Muridae	*Rattus*	*Rattus norvegicus*	Norway Rat
	Muridae	*Rattus*	*Rattus rattus*	Black Rat
	Leporidae	*Oryctolagus*	*Oryctolagus cuniculus*	European Rabbit
	Leporidae	*Sylvilagus*	*Sylvilagus floridianus*	Eastern Cottontail
	Procyonidae	*Procyon*	*Procyon lotor*	Raccoon

	Sciuridae	*Sciurus*	*Sciurus carolinensis*	Eastern Gray Squirrel
	Sciuridae	*Tamiasciurus*	*Tamiasciurus douglasii*	Douglas Squirrel
	Soricidae	*Sorex*	—	Long-tailed Shrews
	Suidae	*Sus*	*Sus scrofa*	Domestic Pig
	Talpidae	*Neurotrichus*	*Neurotrichus gibbsii*	American Shrew Mole
	Talpidae	*Scapanus*	*Scapanus orarius*	Coast Mole
	Talpidae	*Scapanus*	*Scapanus townsendii*	Townsend's Mole
Plant	*Actinidiaceae*	*Actinidia*	—	Kiwi
	Adoxaceae	*Sambucus*	—	Elderberries
	Adoxaceae	*Viburnum*	—	Honeysuckles
	Amaranthaceae	*Chenopodium*	—	Quinoa
	Amaryllidaceae	*Allium*	—	Onion
	Anacardaceae	*Anacardium*	—	Cashews
	Apiaceae	—	—	Carrot Family
	Asteraceae	*Lactuca*	—	Lettuces
	Berberidaceae	—	—	Barberry Family
	Brassicaceae	*Brassica*	—	Brassicas
	Brassicaceae	*Hesperis*	—	Hesperids
	Caprifoliaceae	*Symphoricarpos*	—	Snowberries
	Cornaceae	*Cornus*	—	Dogwoods
	Convolvulaceae	*Ipomoea*	—	Sweet Potatoes
	Ericaceae	Other	—	Heath Family

	Ericaceae	*Vaccinium*	—	Huckleberries
	Fabaceae	Other	—	Legume Family
	Fabaceae	*Arachis*	—	Peanuts
	Fabaceae	*Lens*	—	Lentils
	Fabaceae – Soy	*Gylcine* likely	—	Soy, Glycine
	Fabaceae	*Trifolium*	—	Clovers
	Grossulariaceae	*Ribes*	—	Currants
	Juglandaceae	—	—	Walnut Family
	Linaceae	*Linum*	—	Flaxes
	Malvaceae	*Theobroma*	—	Chocolate
	Moraceae	*Morus*	—	Mulberries
	Musaceae	*Musa*	—	Bananas
	Myricaceae	—	—	Sweet Gales
	Piperaceae	*Piper*	—	Pepper
	Poaceae	*Avena*	—	Oats
	Poaceae	*Hordeum*	—	Barley/Wheat
	Poaceae	*Oryza*	—	Rice
	Poaceae	*Sorghum*	—	Sorghums
	Poaceae – Corn	*Zea* likely	—	Corn
	Polygonaceae	*Rumex*	—	Dock
	Rosaceae	*Crataegus*	—	Hawthorns
	Rosaceae	*Malus*	—	Apples
	Rosaceae	*Prunus*	—	Plums
	Rosaceae	*Pyrus*	—	Pears
	Rosaceae	*Rubus*	—	Brambles
	Rutaceae	—	—	Citrus Family

Reptile	*Emydidae*	*Trachemys*	*Trachemys scripta*	Red-eared Slider

Table S4: Hypothesis-driven models for pest rodents (Norway rat, black rat, house mouse) presence in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate. *p < 0.05, **p < 0.01

Model Name – 1km buffer	**Model**	**AIC**	**ΔAIC**
Waste 1	Rodents ~ Number of Illegal Dumping Incidents (β = 0.51)** + (1|Collection Park)	**509.5**	
Waste 2	Rodents ~ Number of Illegal Dumping Incidents (β = 0.42) + Population Density (β = 0.13) + (1|Collection Park)	511.2	1.7
Socioeconomic 3	Rodents ~ Population Density (β = 0.43)* + (1|Collection Park)	511.5	2.0
Socioeconomic1	Rodents ~ Household Income + Population Density* + (1|Collection Park)	513.3	3.8
Habitat 1	Rodents ~ Impervious Surface + (1|Collection Park)	514.0	4.5
Null	Rodents ~ (1|Collection Park)	514.2	4.7
Habitat 2	Rodents ~ Impervious Surface + Tree Canopy + (1|Collection Park)	515.5	5.0
Socioeconomic 2	Rodents ~ Household Income + (1|Collection Park)	515.5	5.0
Full	Rodents ~ Tree Canopy + Household Income + Number of Restaurants + Number of Grocers + Population Density + Number of Illegal Dumping Incidents + (1|Collection Park)	515.7	5.2
Restaurant	Rodents ~ Number of Restaurants + (1|Collection Park)	516.2	6.7

Table S5: Hypothesis-driven models for anthropogenic grains and legumes presence in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate. ·p<0.1

Model Name	**Model**	**AIC**	**ΔAIC**
Waste 2	Anthro Grains & Legumes ~ Number of Grocers (β = 0.67)· + Number of Illegal Dumping Incidents (β = -0.73) + (1|Collection Park)	**189.4**	
Null	Anthro Grains & Legumes ~ (1|Collection Park)	190.3	0.9
Waste 1	Anthro Grains & Legumes ~ Number of Restaurants (β = 0.11) + Number of Grocers (β = 0.67)· + Number of Illegal Dumping Incidents (β = -0.78)· + (1|Collection Park)	191.2	1.8
Habitat 2	Anthro Grains & Legumes ~ Impervious Surface (β = -0.20) + (1|Collection Park)	191.4	2.0
Habitat 3	Anthro Grains & Legumes ~ Tree Canopy + (1|Collection Park)	191.9	2.5
Socioeconomic 2	Anthro Grains & Legumes ~ Population Density + (1|Collection Park)	192.0	2.6
Socioeconomic 3	Anthro Grains & Legumes ~ Household Income + (1|Collection Park)	192.3	2.9
Habitat 1	Anthro Grains & Legumes ~ Tree Canopy + Impervious Surface (1|Collection Park)	193.1	3.7
Socioeconomic 1	Anthro Grains & Legumes ~ Household Income + Population Density + (1|Collection Park)	194.0	4.6
Full	Anthro Grains & Legumes ~ Household Income + Number of Restaurants + Number of Grocers· + Population Density + Number of Illegal Dumping Incidents + (1|Collection Park)	194.8	5.4

Table S6: Hypothesis-driven models for domestic cat presence in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate.

Model Name	**Model**	**AIC**	**ΔAIC**
Null	Cat ~ (1|Collection Park)	**568.4**	
Socioeconomic 3	Cat ~ Household Income (β = -0.16) + (1|Collection Park)	569.7	1.3
Habitat 1	Cat ~ Tree Canopy (β = -0.22) + Impervious Surface (β = 0.10) + (1|Collection Park)	570.3	1.9
Socioeconomic 2	Cat ~ Population Density (β = 0.05) + (1|Collection Park)	570.4	2.0
Habitat 2	Cat ~ Greenspace + Impervious Surface + (1|Collection Park)	570.9	2.5
Socioeconomic 1	Cat ~ Household Income + Population Density + (1|Collection Park)	571.6	3.2
Full	Cat ~ Tree Canopy + Household Income + Population Density + (1|Collection Park)	572.3	3.9

Table S7: Hypothesis-driven models for anthropogenic proteins (beef, chicken, pork, sheep, turkey) presence in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate. *$p < 0.05$

Model Name	**Model**	**AIC**	**ΔAIC**
Null	Anthro Proteins ~ (1|Collection Park)	**745.4**	
Socioeconomic 2	Anthro Proteins ~ Population Density (β = 0.17) + (1|Collection Park)	745.9	0.5
Waste 1	Anthro Proteins ~ Number of Restaurants (β = -0.20) + Number of Grocers (β = 0.24) + Number of Illegal Dumping Incidents (β = 0.11)*+ (1|Collection Park)	747.2	1.8

Habitat 3	Anthro Proteins ~ Tree Canopy (β = -0.07) + (1|Collection Park)	747.2	1.8
Socioeconomic 3	Anthro Proteins ~ Household Income (β = -0.05) + (1|Collection Park)	747.4	2.0
Habitat 2	Antrho Proteins ~ Impervious Surface (β = 0.03) + (1|Collection Park)	747.4	2.0
Waste 2	Anthro Proteins ~ Number of Grocers + Number of Illegal Dumping Incidents*+ (1|Collection Park)	747.5	2.1
Socioeconomic 1	Anthro Proteins ~ Household Income + Population Density + (1|Collection Park)	747.8	2.4
Full	Anthro Proteins ~ Household Income + Number of Restaurants + Number of Grocers + Population Density + Number of Illegal Dumping Incidents*+ (1|Collection Park)	748.6	3.2
Habitat 1	Anthro Proteins ~ Tree Canopy + Impervious Surface + (1|Collection Park)	749.1	3.7

Table S8: Hypothesis-driven models for anthropogenic proteins and anthropogenic grain presence in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate.

Model Name	**Model**	**AIC**	**ΔAIC**
Socioeconomic 2	Anthro Grains, Corn, & Proteins ~ Population Density (β = 0.19) + (1|Collection Park)	**766.0**	
Socioeconomic 1	Anthro Grains, Corn, & Proteins ~ Household Income (β = -0.06) + Population Density (β = 0.19) + (1|Collection Park)	767.8	1.8
Socioeconomic 3	Anthro Grains, Corn, & Proteins ~ Household Income (β = -0.07) + (1|Collection Park)	767.8	1.8

Habitat 2	Anthro Grains, Corn, & Proteins ~ Impervious Surface (β = 0.05) + (1|Collection Park)	767.9	1.9
Habitat 3	Anthro Grains, Corn, & Proteins ~ Tree Canopy (β = -0.03) + (1|Collection Park)	768.0	2.0
Full	Anthro Grains, Corn, & Proteins ~ Household Income + Number of Restaurants + Number of Grocers + Population Density + Number of Illegal Dumping Incidents + (1|Collection Park)	769.4	3.4
Habitat 1	Anthro Grains, Corn, & Proteins ~ Tree Canopy + Impervious Surface + (1|Collection Park)	769.9	3.9
Null	Anthro Grains, Corn, & Proteins ~ (1|Collection Park)	771.9	5.9
Waste 2	Anthro Grains, Corn, & Proteins ~ Number of Grocers + Number of Illegal Dumping Incidents + (1|Collection Park)	773.7	7.7
Waste 1	Anthro Grains, Corn, & Proteins ~ Number of Restaurants + Number of Grocers + Number of Illegal Dumping Incidents + (1|Collection Park)	774.4	8.4

Table S9: Hypothesis-driven models for rabbits (*Sylvilagus floridanus*) presence in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate. *p < 0.05

Model Name	**Model**	**AIC**	**ΔAIC**
Waste 1	Rabbit ~ Number of Illegal Dumping (β = -0.28)* + (1|Collection Park)	**887.4**	
Waste 2	Rabbit ~ Number of Restaurants + Number of Grocers + Number of Illegal Dumping* + (1|Collection Park)	889.7	2.3
Null	Rabbit ~ (1|Collection Park)	890.2	2.8
Habitat 3	Rabbit ~ Tree Canopy + (1|Collection Park)	892.2	4.8

Socioeconomic 2	Rabbit ~ Household Income + (1|Collection Park)	892.2	4.8
Habitat 1	Rabbit ~ Impervious Surface + Tree Canopy + (1|Collection Park)	893.1	5.7
Habitat 2	Rabbit ~ Impervious Surface + Greenspace + (1|Collection Park)	893.1	5.7
Socioeconomic 1	Rabbit ~ Household Income + Population Density + (1|Collection Park)	893.1	5.7
Full	Rabbit ~ Household Income + Number of Restaurants + Number of Grocers + Population Density + Number of Illegal Dumping Incidents* + Tree Canopy + Water + (1|Collection Park)	896.6	9.2

Table S10: Hypothesis-driven models for other vertebrate prey (excluding rabbits and pest rodents) in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate.

Model Name	**Model**	**AIC**	**ΔAIC**
	Other Vertebrate ~ Population Density (β = -0.48)***	**866.0**	
Socioeconomic 1	Other Vertebrate ~ Household Income (β = 0.03) + Population Density (β = -0.47)*** + (1|Collection Park)	867.9	1.9
Habitat 3	Other Vertebrate ~ Greenspace (β = 0.43)*** + (1|Collection Park)	867.9	1.9
Habitat 4	Other Vertebrate ~ Impervious (β = -0.46)*** + (1|Collection Park)	868.7	2.7
Habitat 1	Other Vertebrate ~ Tree Canopy + Impervious+ (1|Collection Park)	870.7	4.7

Full	Other Vertebrate ~ Household Income + Number of Illegal Dumping Incidents + Tree Canopy + Population Density** + (1|Collection Park)	871.9	5.9
Socioeconomic 2	Other Vertebrate ~ Household Income + (1|Collection Park)	881.9	15.9
Habitat 2	Other Vertebrate ~ Tree Canopy + (1|Collection Park)	882.6	16.6
Null	Other Vertebrate ~ (1|Collection Park)	932.3	66.3

Table S11: Hypothesis-driven models for berries in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate.

Model Name	**Model**	**AIC**	**ΔAIC**
Full	Berry ~ Household Income (β = -0.47)** + Tree Canopy (β = 0.59)** + Population Density (β = -0.69)*** + (1|Collection Park)	**851.1**	
Habitat 3	Berry ~ Greenspace*** + (1|Collection Park)	861.4	10.3
Habitat 4	Berry ~ Greenspace*** + Tree Canopy + (1|Collection Park)	862.2	11.1
Socioeconomic 1	Berry ~ Household Income· + Population Density*** + (1|Collection Park)	864.4	13.3
Habitat 1	Berry ~ Tree Canopy** + (1|Collection Park)	871.2	20.1
Habitat 2	Berry ~ Impervious Surface** + (1|Collection Park)	874.3	23.2
Null	Berry ~ (1|Collection Park)	880.4	29.1

Table S12: Hypothesis-driven models for garden fruit in scats with AIC and ΔAIC values from the top model. Models are listed in ascending AIC order and the top model AIC is bolded. Regression coefficients (β) for models within 2 AIC of the top model are reported next to each covariate.

Model Name	**Model**	**AIC**	**ΔAIC**

Full	Garden Fruit ~ Household Income (β = 0.20) + Tree Canopy (β = -0.25) + Population Density (β = 0.71)*** + (1|Collection Park)	**846.3**	
Socioeconomic 3	Garden Fruit ~ Population Density (β = 0.68)*** + (1|Collection Park)	846.5	0.2
Socioeconomic 2	Garden Fruit ~ Population Density (β = 0.69)*** + Household Income (β = 0.13) + (1|Collection Park)	847.5	1.2
Habitat 3	Garden Fruit ~ Greenspace*** + (1|Collection Park)	849.9	3.6
Socioeconomic 1	Garden Fruit ~ Household Income + Greenspace*** + (1|Collection Park)	850.3	4.0
Habitat 4	Garden Fruit ~ Greenspace*** + Tree Canopy + (1|Collection Park)	851.6	5.3
Habitat 2	Garden Fruit ~ Impervious Surface*** + (1|Collection Park)	859.6	11.3
Habitat 1	Garden Fruit ~ Tree Canopy* + (1|Collection Park)`	866.0	19.7
Null	Garden Fruit ~ (1|Collection Park)	869.6	23.3

8.4 Appendix D – Supplemental Material for Chapter 6

Table S1: Taxonomic families present within each study area with frequency of occurrence greater than 0.01.

Scientific Name	**Taxa Type**	**Common Name**	**Greater Seattle Area FOO**	**Bainbridge Island FOO**	**Methow FOO**	**Northeast FOO**	**Wildland Combined FOO**
Accipitridae	Bird	Hawks	0.01	--	--	--	--
Adoxaceae	Plant	Elderberry Family	0.05	0.02	0.02	--	0.02
Anacardiaceae	Plant	Cashew Family	0.01	--	0.02	--	0.01
Anatidae	Bird	Ducks	0.03	0.06	0.02	--	0.01
Apiaceae	Plant	Carrot Family	0.06	0.02	0.09	--	0.07
Aplodontidae	Mammal	Mountain Beavers	0.01	--	--	--	--
Berberidaceae	Plant	Barberry Family	0.01	--	0.02	0.09	0.04
Bovidae	Mammal	Cattle	0.06	0.04	0.06	--	0.04
Caprifoliaceae	Plant	Honeysuckle Family	0.03	0.01	0.08	0.23	0.11

Castoridae	Mammal	Beavers	0.03	--	--	--	--
Cervidae	Mammal	Deer & Elk	0.01	0.13	0.28	0.71	0.38
Charadriidae	Bird	Plovers & Lapwings	--	0.01	--	--	--
Clupeidae	Fish	Herrings & Sprats	0.01	--	--	--	--
Columbidae	Bird	Doves & Pigeons	0.01	0.01	--	--	--
Convolvulaceae	Plant	Morning Glory Family	0.01	0.01	--	--	--
Corvidae	Bird	Crows	0.03	--	--	--	--
Cricetidae	Mammal	Voles	0.22	0.65	0.30	0.09	0.24
Equidae	Mammal	Horses	--	0.01	0.01	--	0.01
Ericaceae	Plant	Heath Family	0.03	0.10	0.15	0.14	0.15
Fabaceae	Plant	Legume Family	0.05	0.06	--	0.03	0.01
Fabaceae – Soy	Plant	Soy	0.05	0.01	--	--	--
Felidae	Mammal	Cats	0.15	0.03	--	--	--
Gasterosteidae	Fish	Stickleback	0.01	--	--	--	--
Grossulariaceae	Plant	Currant Family	--	--	0.05	0.23	0.09
Juglandaceae	Plant	Walnut Family	0.01	--	--	--	--
Leporidae	Mammal	Rabbits	0.49	0.16	0.32	0.37	0.33
Linaceae	Plant	Flax Family	0.01	--	--	--	--
Moraceae	Plant	Mulberry Family	--	0.08	--	--	--
Muridae	Mammal	Rats	0.12	0.04	--	--	--
Mustelidae	Mammal	Weasels	--	0.01	--	--	--
Myricaceae	Plant	Sweet Gale Family	0.01	--	--	--	--
Paridae	Bird	Tits & Chickadees	0.01	--	--	--	--
Passerellidae	Bird	Passerines	0.05	0.10	0.06	0.03	0.05
Phasianidae	Bird	Pheasants, Chickens, & Turkeys	0.18	0.16	0.09	0.37	0.16
Picidae	Bird	Woodpeckers	--	--	0.01	--	0.01
Poaceae	Plant	Grass Family	0.02	--	--	--	--
Poaceae – Corn	Plant	Corn	0.03	0.01	--	--	--
Polygonaceae	Plant	Buckwheat Family	0.10	0.14	0.03	0.03	0.03
Procyonidae	Mammal	Raccoons	0.01	0.01	--	--	--
Rosaceae	Plant	Rose Family	0.89	0.85	0.26	0.83	0.40
Salmonidae	Fish	Salmon	--	0.03	0.01	--	0.01
Sciuridae	Mammal	Squirrels	0.11	0.12	0.03	--	0.02
Sittidae	Bird	Nuthatches	--	0.01	0.01	--	0.01
Soricidae	Mammal	Shrews	0.02	0.01	0.02	--	0.01
Suidae	Mammal	Pigs	0.07	0.04	0.01	--	0.01
Talpidae	Mammal	Moles	0.04	0.06	--	--	--
Trochilidae	Bird	Hummingbirds	--	0.01	--	--	--
Turdidae	Bird	Robins & Thrushes	0.02	0.07	--	--	--
Xiphiidae	Fish	Swordfishes	--	0.01	--	--	--

Table S2: Table of higher resolution taxonomy sorted alphabetically within taxa type. Each taxon is listed with its scientific and common name. This table represents all species that were detected, even at low levels after filtering. An 'X' denotes presence within that study system while a '—' denotes absence. For the wildland study area deer are listed under *Odocoileus* as both *Odocoileus hemionus* and *Odocoileus virginianus* are present in parts of the wildland study system and are not distinguishable using these methods. On Bainbridge Island and in the Greater Seattle Area, only *Odocoileus hemionus* occurs.

Taxa Type	Scientific Name	Common Name	Greater Seattle Area	Bainbridge Island	Wildland Combined
Bird	*Aix sponsa*	Wood Duck	**X**	—	—
	Anas	Ducks	**X**	**X**	**X**
	Bombycilla cedrorum	Cedar Waxwing	**X**	—	—
	Bonasa umbellus	Ruffed Grouse	—	—	**X**
	Bucephala	Goldeneyes	**X**	—	—
	Buteo	Owl	**X**	—	—
	Catharus ustaulatus	Swainson's Thrush	**X**	**X**	—
	Charadrius vociferus	Killdeer	—	**X**	—
	Colaptes auratus	Northern Flicker	**X**	—	**X**
	Columbidae	Doves	**X**	—	—
	Corvus	Crows	**X**	—	—
	Coturnix japonica	Japanese Quail	**X**	—	—
	Dendragapus fuliginosus	Sooty Grouse	—	—	**X**
	Gallus gallus	Domestic Chicken	**X**	**X**	—
	Haliaeetus leucocephalus	Bald Eagle	**X**	—	—
	Ixoreus naevius	Varied Thrush	—	**X**	—
	Junco hyemalis	Dark-eyed Junco	**X**	**X**	**X**
	Larus	Gulls	**X**	—	—
	Meleagris gallopavo	Turkey	**X**	**X**	**X**
	Neurotrichus gibbsii	American Shrew Mole	**X**	**X**	—
	Paridae	Tits	**X**	—	—
	Patagioenas fasciata	Band-tailed Pigeon	**X**	**X**	—
	Phasianus colchicus	Ring-necked Pheasant	**X**	—	—
	Pipilo maculatus	Spotted Towhee	**X**	**X**	**X**
	Poecile Atricapilla	Black-capped Chickadee	**X**	—	—
	Regulus	Kinglets	**X**	—	—
	Sittidae	Nuthatches		**X**	**X**
	Sturnus vulgaris	Common Starling	**X**	—	—
	Trochilidae	Hummingbirds	**X**	**X**	—
	Troglodytes	Wrens	**X**	—	—
	Turdus	True Thrushes	**X**	**X**	—
Fish	*Ameiurus*	Bullheads	**X**	—	—

	Anarhichas	Wolffish	**X**		
	Brevoortia	Brevoortia	**X**	—	—
	Cyprinus	Carps	**X**	—	—
	Gasterosteus aculeatus	Three-spined Stickleback	**X**	—	—
	Lepomis	Sunfish	**X**	—	—
	Leptocottus armatus	Pacific Staghorn Sculpin	**X**	—	—
	Micropterus salmoides	Large-mouth Bass	**X**	—	—
	Mylocheilus caurinus	Peamouth Chub	**X**	—	—
	Oncorhynchus	Pacific Salmon & Trout	**X**	**X**	—
	Oncorhynchus kisutch	Coho Salmon	**X**	—	**X**
	Perca	Perch	**X**	—	—
	Salmo salar	Atlantic Salmon	**X**	—	—
	Xiphias gladius	Swordfish	—	**X**	—
Mammal	*Alces alces*	Moose	—	—	**X**
	Aplodontia rufa	Mountain Beaver	**X**	—	—
	Bos taurus	Domestic Cattle	**X**	**X**	**X**
	Castor canadensis	North American Beaver	**X**	—	—
	Clethrionomys	Slender Voles	—	—	**X**
	Cricetidae	Voles	**X**	**X**	**X**
	Didelphis virginiana	Virginia Opossum	**X**	—	—
	Equus caballus	Domestic Horse	**X**	**X**	**X**
	Felis catus	Domestic Cat	**X**	**X**	—
	Lepus americanus	Snowshoe Hare	—	—	**X**
	Microtus longicaudus	Long-tailed vole	**X**	—	**X**
	Mus musculus	House Mouse	**X**	—	—
	Neogale vison	American Mink	—	**X**	—
	Neurotrichus gibbsii	American Shrew Mole	**X**	**X**	—
	Odocoileus	Deer (Both black and white tail occur in parts of wildland study areas)	Odocoileus hemionus	Odocoileus hemionus	**X**
	Odocoileus hemionus	Black-tailed Deer	**X**	**X**	—
	Oryctolagus cuniculus	European Rabbit	**X**	—	—
	Ovis aries	Domestic Sheep	**X**	**X**	—
	Procyon lotor	Raccoon	**X**	**X**	—
	Rattus norvegicus	Norway Rat	**X**	**X**	—
	Rattus rattus	Black Rat	**X**	**X**	—
	Scanapus orarius	Coast Mole	**X**	**X**	—

	Scanapus townsendii	Townsend's Mole	**X**	—	—
	Sciurus carolinensis	Eastern Gray Squirrel	**X**	**X**	—
	Sorex	Long-tailed Shrews	**X**	**X**	**X**
	Sus scrofa	Domestic Pig	**X**	**X**	**X**
	Sylvilagus floridianus	Eastern Cottontail	**X**	**X**	—
	Tamias	Chipmunks	—	**X**	—
	Tamiasciurus douglasii	Douglas Squirrel	**X**	**X**	**X**
Plant	*Actinidia*	Kiwi	**X**	—	—
	Allium	Onions	**X**	—	—
	Anacardium	Cashews	**X**	—	—
	Apiaceae	Carrot Family	**X**	**X**	**X**
	Arachis	Peanuts	**X**	—	—
	Avena	Oats	**X**	—	—
	Berberidaceae	Barberry Family	**X**	—	**X**
	Brassica	Brassicas	**X**	—	—
	Canabis	Canabis	—	**X**	—
	Cornus	Dogwoods	**X**	—	—
	Crataegus	Hawthorns	**X**	—	**X**
	Ericaceae	Heath Family	**X**	**X**	—
	Fabaceae	Legume Family	**X**	—	—
	Fabaceae – Soy	Soy, Glycine	**X**	**X**	—
	Hesperis	Rockets	**X**	—	—
	Ipomoea	Sweet Potatoes	**X**	**X**	—
	Juglandaceae	Walnut Family	**X**	—	—
	Lactuca	Lettuces	**X**	—	—
	Lens	Lentils	**X**	—	—
	Linum	Flaxes	**X**	—	—
	Mangifera	Mangoes	**X**	—	—
	Morus	Mulberries	**X**	**X**	—
	Musa	Bananas	**X**	**X**	—
	Oryza	Rice	**X**	—	—
	Piper	Pepper	**X**	—	—
	Poaceae – Corn	Corn	**X**	**X**	—
	Prunus	Plums	**X**	**X**	**X**
	Pyrus	Pears	**X**	—	—
	Rhus	Sumacs	—	—	**X**
	Ribes	Currants	**X**	—	**X**
	Rubus	Brambles	**X**	**X**	**X**
	Rumex	Dock	**X**	**X**	**X**
	Rutaceae	Citrus Family	**X**	—	—
	Sambucus	Elderberries	**X**	**X**	**X**
	Sorghum	Sorghums	**X**	—	—
	Symphoricarpos	Snowberries	**X**	**X**	**X**
	Theobroma	Chocolate	**X**	—	—
	Trifolium	Clovers	**X**	**X**	**X**
	Vaccinium	Huckleberries	**X**	—	**X**
	Viburnum	Honeysuckles	**X**	—	—
Reptile	*Trachemys scripta*	Red-eared Slider	**X**	—	—

VITA

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