Urban Landscape Complexity as a Driver of Urban Evolution in White Clover (*Trifolium repens*)

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Abstract

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Cities are influencing evolution on contemporary timescales, but the mechanisms driving evolutionary change are not well understood. Previous studies on urban eco-evolutionary dynamics generally assume that urban structures predictably evolve from a dense core to less intensive peripheries, discounting their spatial complexities. Cities are mosaics of patches, each governed by a unique set of parameters that interact to create a unique set of ecological conditions and stressors distributed unevenly across the landscape. Using an urban-rural transects approach, prior studies identified variance in Hydrogen Cyanide (HCN) production in *Trifolium repens* (white clover) contingent on the distance from city centers. This investigation introduces a refined approach via the urban complexity framework, incorporating measures of landscape heterogeneity, connectivity, and historical contingency to explicate variability in HCN production across 20 North American cities. A model selection approach was employed to comparatively evaluate the explanatory power of these predictors to distance from city center along an urban-rural transect in multi-city and single city models. The results reveal that multivariate models incorporating urban complexity variables, notably the connectivity of

cropland, demonstrated higher adjusted R^2 values than univariate distance-based models in multi-city contexts. Although this finding does not significantly augment the predictive efficacy of single city models, it underscores the shared explanatory contribution of these variables. In conclusion, this investigation posits urban complexity as a critical determinant of urban eco-evolutionary dynamics, warranting further exploration to effectively inform the practice of urban planning.

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Chapter 1 Introduction

Cities are influencing evolution on contemporary timescales, yet a comprehensive understanding of the underlying mechanisms remains elusive. In particular, we do not know how the spatial and temporal complexity of urban landscapes might play a role in the evolutionary dynamics (Alberti & Wang, 2022; Santangelo, Ness, et al., 2022; Swan et al., 2021). For example, Santangelo, Ness, et al. (2022) used urban-rural transects to show that cities are driving the global parallel evolution of hydrogen cyanide (HCN) production in white clover (*Trifolium repens*). Urban evolutionary studies typically assume that urban exposure changes predictably with distance from the city center (Alberti & Wang, 2022; Ramalho & Hobbs, 2012). However, distance from city center only accounted for 26.7% of variation in HCN production. While 39% of cities showed increasing HCN production with distance from city center, 8% showed the opposite trend (Santangelo, Ness, et al., 2022).

Such variability in results is common among studies investigating urban ecology and evolution because of the inherent properties of cities (Santangelo, Roux, et al., 2022; Swan et al., 2021). Cities are complex mosaics of patches, each governed by a unique set of parameters—including ownership, use, regulation, and historical circumstance—which interact to create a unique set of ecological conditions and stressors distributed unevenly across the landscape (Swan et al., 2021). Urban-rural transects cannot account for this variability (Alberti & Wang, 2022). Recent studies propose a bio-complexity framework to elucidate the dynamics of urban environmental change through an examination of urban landscape heterogeneity, connectivity, and historical contingencies (Alberti & Wang, 2022; Cadenasso et al., 2006). This multidimensional framework captures the variation in social, ecological, and built factors that characterize cities and drive urban eco-evolutionary dynamics (Alberti & Wang, 2022). The role of urban complexity in driving eco-evolutionary dynamics is not well explored in existing research, and further research could enhance our understanding of urban eco-evolutionary dynamics (Alberti, 2015; Santangelo, Ness, et al., 2022; Santangelo, Roux, et al., 2022).

In order to address this gap, this study investigates how landscape heterogeneity, connectivity, and historical contingency can explain variability in HCN production in white clover compared to distance from city center along an urban-rural transect (Fig. 1.1). The use of these predictors to explain eco-evolutionary phenomena is unique and introduces a new framework for urban complexity into the field of urban eco-evolutionary dynamics.

Research Question: Can landscape heterogeneity, connectivity, and historical contingency improve the explanation variability in HCN production in white clover compared to distance from city center along an urban-rural transect?

Hypothesis: Incorporating metrics of landscape heterogeneity, connectivity, and historical contingency will improve the explanation variability in HCN production in white clover compared to distance from city center along an urban-rural transect by incorporating more of the variability inherent in urban regions.

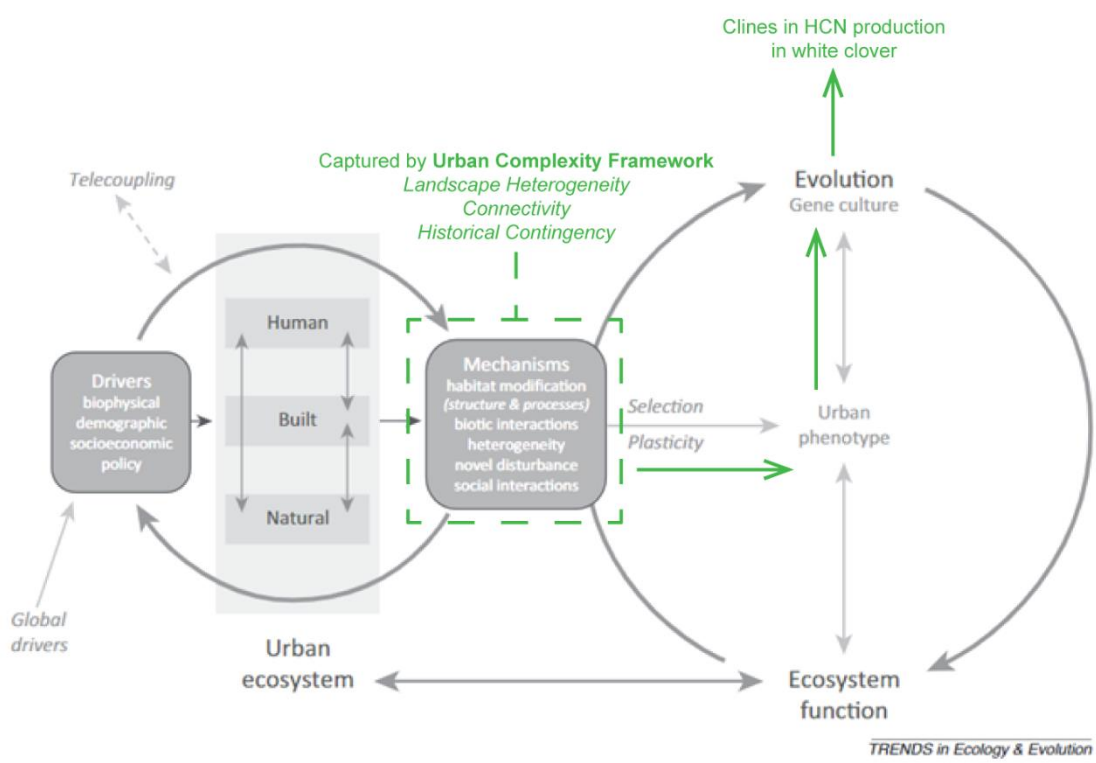


Figure 1.1 Framework of urban eco-evolutionary feedback from Alberti (2015), modified to highlight the pathways investigated in this study (green).

To answer this question, I select a subsample of the data collected by Santangelo, Ness, et al. (2022) in the United States and Canada and analyze it using a model selection approach adapted from the methods used in Santangelo, Ness, et al. (2022). I include predictors representing urban complexity—landscape heterogeneity, connectivity, and historical contingency—and biophysical factors in these models to compare their explanatory power to the distance from city center along an urban-rural transect. Landscape metrics are calculated from 30m resolution Landsat land cover data (Commission for Environmental Cooperation, 2020) using Fragstats version 4.2 (McGarigal et al., 2023).

Subsequent chapters discuss related literature, methods, and the results of the investigation. Chapter 2 reviews literature on urban eco-evolutionary dynamics, urban complexity frameworks, and white clover. Chapter 3 discusses the methods used to investigate the hypothesis that landscape heterogeneity, connectivity, and historical contingency can explain variability in HCN production in urban white clover. The results of this analysis are presented in Chapter 4, and Chapter 5 includes a discussion of the interpretations of top models, the implications of the results, and the limitations of the study. Chapter 6 wraps up with a conclusion and directions for future research.

This study underscores that incorporating measures of urban complexity, particularly connectivity of cropland, can improve the explanatory power of models of urban variation in HCN production in white clover. These results emphasize the importance of urban complexity in driving urban eco-evolutionary dynamics, suggesting that this framework should be investigated further as a driver of urban ecological and evolutionary phenomena. In addition to influencing the design of future research, support for the urban complexity framework can more effectively inform the practice of urban planning by emphasizing the importance of landscape patterns and change in shaping both ecological and evolutionary processes on a human timescale. Designers and planners should consider the connectivity of key habitat patches, as well as the diversity of patches and historical changes to the landscape, when designing for conservation to manage the adaptive trajectory of important species.

Chapter 2 Literature Review

2.1 Urban Eco-Evolutionary Dynamics

A review of experimental studies shows that genetic diversity, like species diversity, can have important implications for ecological functions, such as resilience (A. R. Hughes et al., 2008), and more recent findings indicate that response diversity, which refers to the variation in responses of species to disturbance, is essential to maintaining ecosystem function in the face of environmental change (Alberti, 2015). This can generate reciprocal effects where ecological conditions and evolutionary functions interact, a phenomenon known as eco-evolutionary dynamics (Fig. 2.1) (Alberti, 2015; A. R. Hughes et al., 2008). Urban environments provide new selective pressures and contexts—as a result of human-mediated changes in biotic and abiotic interactions—that have the potential shape evolution, making cities an excellent place to study evolution and eco-evolutionary feedbacks. Studying urban eco-evolutionary dynamics can inform urban planning and conservation efforts by clarifying the limits and causes of adaptation (Alberti, 2015; Donihue & Lambert, 2015).

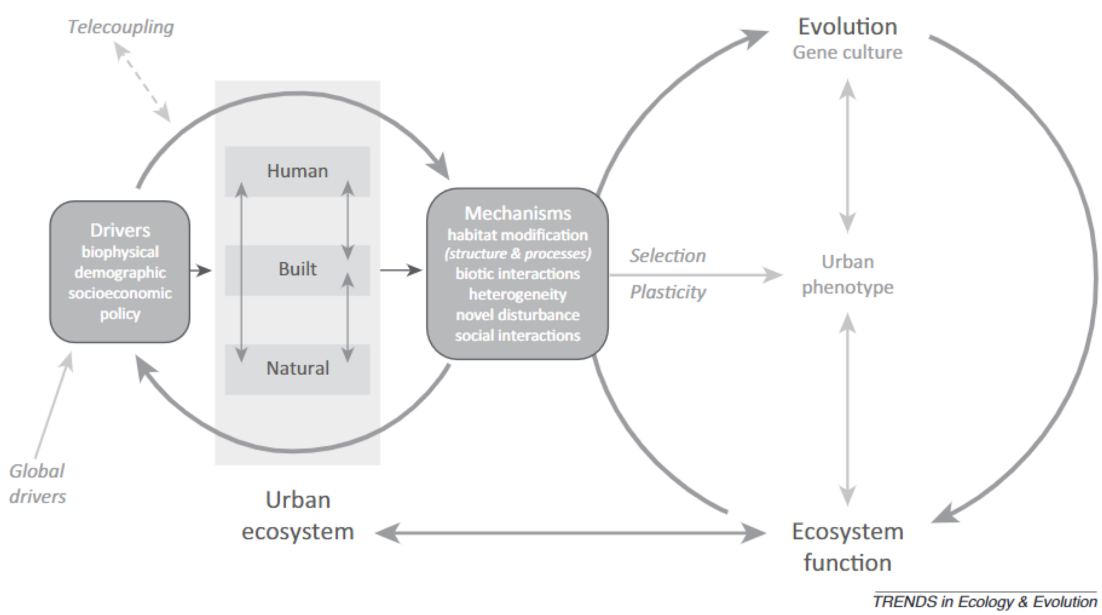


Figure 2.1 Framework of urban eco-evolutionary feedback. Reproduced from Alberti (2015, p. 118).

A wealth of literature has been published discussing urban-driven eco-evolutionary feedbacks in birds, rodents, fish, invertebrates, amphibians, and plants (Alberti, 2015). A metanalysis of observational and experimental studies investigating rates of phenotypic change showed that urbanization is accelerating rates of change compared to natural and non-urban contexts (Alberti, Correa, et al., 2017). Key drivers of urban evolution include change in spatial and temporal heterogeneity, land cover conversion, and the destruction of native habitat (Alberti, Correa, et al., 2017). A variety of other reviews also describe the impacts of urbanization on urban ecology and evolution. Alberti, Marzluff, et al. (2017) hypothesize that urbanization accelerates rates of phenotypic and that genetic differences between urban and nonurban populations may be related changes in spatial and temporal heterogeneity. Johnson and Munshi-South (2017) found that urbanization increases genetic drift and restricts gene flow, but the impact on adaptation is less certain. Beninde et al.'s (2015) meta-analysis on intra urban biodiversity highlights the importance of patch area, corridors, and vegetation structure for maintaining biodiversity. However, many studies are limited to a single city or species, and the field lacks a unified framework that could be used to develop a theory of urban eco-evolutionary dynamics (Alberti et al., 2020; Donihue & Lambert, 2015; Johnson & Munshi-South, 2017).

Historically, urban ecologists have described patterns in cities using broad predictors, such as distance from city center or percent impervious cover, but in order to advance the field and inform planning and conservation action, generalizations about the structure and function of urban ecosystems must be developed and confirmed (McDonnell & Hahs, 2013; Pickett et al., 2016; Ramalho & Hobbs, 2012). Studies should compare between cities, regions, or countries (McDonnell & Hahs, 2013), and increasingly, researchers also advocate for more specific, question-driven predictors that address the causal mechanisms behind urban ecological phenomena, unlike urban-rural comparisons or gradients (Alberti et al., 2020; McDonnell & Hahs, 2013; McPhearson et al., 2016; Pickett et al., 2016; Ramalho & Hobbs, 2012). This new paradigm recognizes cities as heterogenous, dynamic mosaics of patches and seeks to understand how the complex structures inform function (Alberti et al., 2020; McPhearson et al., 2016; Pickett et al., 2016; Ramalho & Hobbs, 2012). Acknowledging the complex social, ecological, and technological nature of cities will improve the applicability of urban ecological knowledge for conserving biodiversity and increasing resilience in cities (Alberti et al., 2020; Alberti & Wang, 2022; McPhearson et al., 2016; Ramalho & Hobbs, 2012), and may account for the seemingly contradictory ecological and evolutionary responses detected in previous studies (Alberti & Wang, 2022; Swan et al., 2021). Further work is needed to understand how the complex interactions in cities shape eco-evolutionary phenomena (Alberti et al., 2020; Alberti & Wang, 2022; McPhearson et al., 2016; Ramalho & Hobbs, 2012).

2.2 Urban Complexity

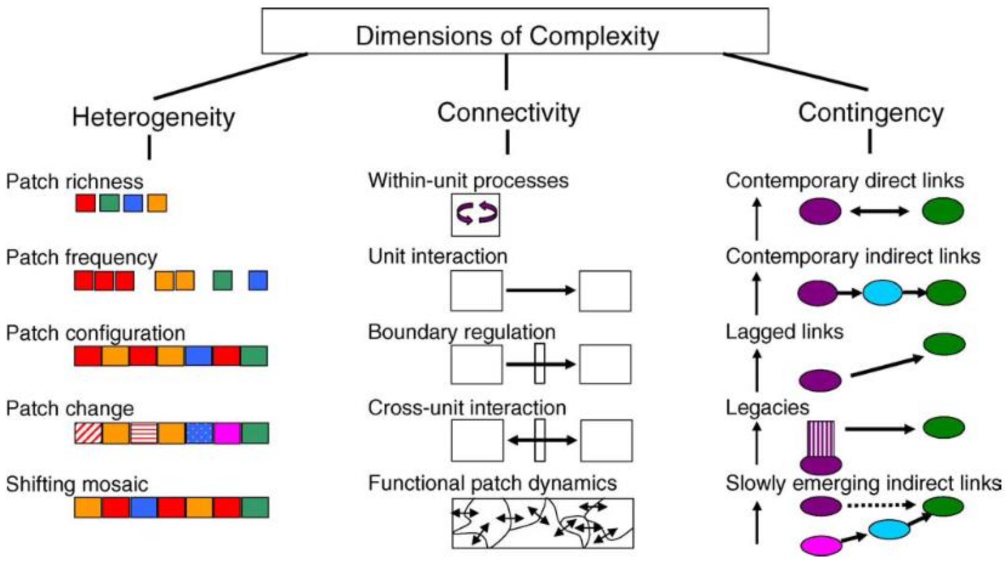
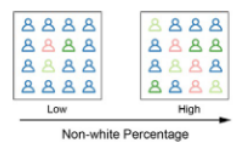
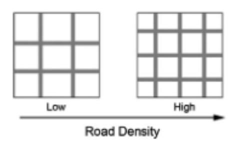
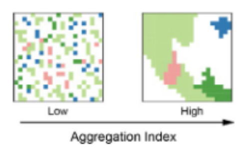
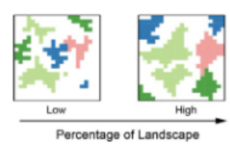


Figure 2.2 Framework for dimensions of biocomplexity. Reproduced from Cadenasso et al. (2006, p. 4).

Cadenasso et al. (2006) proposed heterogeneity, connectivity, and contingency as three dimensions of a structural biocomplexity framework (Fig. 2.2), where heterogeneity represents how pieces are arranged, connectivity represents how they interact, and historical contingency represents how they change over time. These dimensions can be quantified using landscape metrics (Alberti & Wang, 2022): the signatures of urbanization can be accurately captured using a group of several landscape metrics, such as the Shannon's Diversity Index, Contagion, and Landscape Shape Index (Wu et al., 2011). Alberti and Wang (2022) use a suite of landscape metrics representing landscape heterogeneity, connectivity, and complexity (Fig. 2.3) to quantify built, ecological, and social aspects of the urban landscape, using multivariate models to explore their effect on species richness and beta diversity at the metropolitan and 1 km grid cell level. Heterogeneity—which may support a wider array of species—is an important property of cities because they are shaped by both ecological and anthropogenic forces (Alberti, 2015; Alberti et al., 2020; Alberti & Wang, 2022). Cities are also known for their impact on connectivity. Urbanization typically fragments the landscape and leads to habitat isolation, but urban infrastructure may also facilitate dispersal for some species (Alberti, 2015; Alberti & Wang, 2022). Historical contingency reflects the biophysical and socioeconomic changes that have shaped the landscape and may continue to influence the current conditions through time lags and legacy effects or habitat stability (Alberti et al., 2020; Alberti & Wang, 2022). Unlike a gradient, this framework can capture the uneven distribution of stressors across the landscape; patterns of variation in biodiversity were detected across all three axes. This type of multidimensional framework can represent the complexity of urban landscapes and could be used to build towards a mechanistic understanding of urban ecological phenomena, but additional studies are needed to further demonstrate the applications (Alberti & Wang, 2022).

Dimensions	Metrics	Equation	Description
Heterogeneity	Landscape Shannon Diversity Index (SHDI)	$SHDI = -\sum_{i=1}^{m}(P_i * lnP_i)$ P_i = percentage of the land occupied by class i	
	Non-white percentage	P = percentage of the non-white population	
Connectivity	Road density (km/km^2)	$D = \frac{\sum_{i=1}^{m} L_i}{A}$ L_i = length of road i A= unit area	
	Aggregation Index (AI) of Development and AI of Forest	$AI = \frac{g_{ii}}{max \to g_{ii}}$ g_{ii} = number of like adjacencies between grid cells of class i max $\to g_{ii}$ = max number of like adjacencies between grid cells of class i	
Contingency	Percentage of Land (PLAND) of Forest	$PLAND = P_j = \frac{\sum_{i=1}^{n} a_{ij}}{A_j}(100)$ a_{ij}= area of patch i in class j A = total area of class j	
	PLAND change	$PLAND\ change =$ $PLAND\,2016 - PLAND\,2001$	Measured for two classes: forest and development
	AI change	$AI\ change =$ $AI\,2016 - AI\,2001$	

Note: Landscape SHDI measures the diversity of landscape elements, and the non-white population percentage reflects social diversity. Forest PLAND is calculated for both 2001 and 2016, and changes between the 15 years were also calculated as additional metrics to represent contingency. Development AI and Forest AI measure, respectively, how aggregated the impervious surface and the forest cover in cities are. Correlation test among metrics is included as Figure S1 & S2.

Figure 2.3 Metrics used to represent the three dimensions of the biocomplexity framework (Reproduced from Alberti and Wang (2022, p. 1031).

2.3 White Clover

2.3.1 History and Spread

White clover (*Trifolium repens*), a branching creeping perennial, was domesticated in southern Spain in around 1000 and subsequently spread across Europe (Frame & Newbould, 1986; Kjærgaard, 2003). It became increasingly important in the 17th and 18th centuries, when its nitrogen-fixing capacity became essential for supporting the production of cereal crops to feed Europe's growing population. White clover spread to the United States and New Zealand in the 19th century for use as feed for cattle and to provide nitrogen for other crops (Kjærgaard, 2003). It remains an essential pasture legume in temperate regions today, where it is both seeded in pastures and found naturally along roadsides and in fields (Frame & Newbould, 1986).

2.3.2 Cyanogenesis

White clover populations are naturally polymorphic for the production of hydrogen cyanide (HCN), a common plant defense (Poulton, 1990), resulting in plants that are either cyanogenic or completely acyanogenic. This polymorphism is mediated through the epistatic interaction between two independently segregating Mendelian loci (Corkill, 1942). First, the *Ac* locus catalyzes the production of cyanogenic glucosides (Olsen et al., 2008; Olsen & Small, 2018), and the second locus, *Li*, codes for the hydrolyzing enzyme that liberates HCN from the glucoside (Coop, 1940; Olsen et al., 2007). The functional alleles at both loci are partially dominant, so a plant requires at least one functional copy at both alleles to be cyanogenic (Corkill, 1942).

The drivers of HCN production in white clover are not completely clear, but climate and herbivory pressure may be important factors. Cyanogenesis is thought to be a result of local climate adaptation (M. A. Hughes, 1991; Olsen & Ungerer, 2008)—particularly under stressful conditions (Kooyers et al., 2018). Some studies suggest that cyanogenesis may be selected against in cold climates because if cells lyse, HCN may cause self-toxicity (M. A. Hughes, 1991; Thompson et al., 2016). However, others have found that HCN production has a minimal impact on freezing tolerance in white clover (Kooyers et al., 2018; Olsen & Ungerer, 2008), suggesting instead that stressful conditions make energetic costs of HCN production unsustainable, leading to selection against cyanogenesis (Kooyers et al., 2018). There is also evidence for drought stress as a driver of HCN production (M. A. Hughes, 1991; Kooyers et al., 2014; Kooyers & Olsen, 2013; Santangelo, Ness, et al., 2022). This may occur because soil nitrogen is frequently limited during water stress, and cyanogenic glucosides may serve as a source of nitrogen during drought (Kooyers & Olsen, 2013). HCN production in white clover is also an effective defense against herbivory by snails, slugs, insects, mollusks, and voles (M. A. Hughes, 1991; Olsen & Ungerer, 2008; Thompson et al., 2016), and cyanogenesis could be driven by herbivory pressure (Santangelo, Ness, et al., 2022). However, because HCN production has energetic costs, there are fitness tradeoffs, and acyanogenic plants could have an advantage in areas with fewer generalist herbivores (Olsen & Ungerer, 2008).

2.3.3 Urban Eco-Evo Studies on White Clover

White clover has been used as a focal species in many recent studies on urban eco-evolutionary dynamics, including a global comparative study. Thompson et al. (2016) tested for clines in HCN production along urbanization gradients in four cities. Clover was sampled from three transects in Toronto, where HCN production consistently increased with the distance from city center. A term for population density had no effect. Transects were sampled in three additional cities, and two of the three cities showed results consistent with the findings in Toronto, while no cline was detected in the third city, Montreal. Additional investigation of herbivory on temperature differences along the gradients and between cities detected no spatial variation in herbivory but suggest that climatic conditions that lead to increased freeze damage in city centers may be driving the clines.

Next, Santangelo et al. (2018) used spatially explicit simulation models to investigate the influence of genetic drift on HCN production, concluding that this phenotype is susceptible to drift and that drift should be rejected as a null model before assuming selection is occurring. Johnson et al. (2018) quantified the frequency of HCN production across transects from 20 cities, analyzing genetic diversity to conclude that the parallel evolution of clines was likely due to natural selection. Santangelo, Thompson, et al. (2020) then tested if cold climatic conditions are required for the evolution of HCN clines by comparing the frequency of HCN genotypes across a latitudinal gradient of 16 cities. While the coldest climate produced no clines—supporting the hypothesis that thick snow cover insulates clover from freezing—the strongest clines occurred in the warmest climates, suggesting alternative pressures may be selecting for HCN production in different climates. Santangelo, Rivkin, et al. (2020) compared urban and rural clover in a common garden experiment to test for the impacts of urbanization on 14 phenotypic traits. Results suggested urban adaptation may be affecting multiple traits.

The results of these studies informed the development of a study of HCN production across urban-rural gradients in 160 cities globally (Santangelo, Ness, et al., 2022). However, distance from city center only accounted for 26.7% of variation in HCN production. While 39% of cities showed increasing HCN production with distance from city center, 8% showed the opposite trend. Pairwise nucleotide diversity was compared between urban and rural sites in selected cities to compare the strength of genetic drift, and there was no significant difference between urban and rural sites, even when the presence or slope of a cline was considered. Santangelo, Ness, et al. (2022) concluded that herbivory pressure selects for higher HCN in rural areas, but in areas where overall herbivory pressure is low, HCN clines are driven by drought stress—although variation in cline direction is common and may be explained by other ecological and evolutionary factors.

Finally, Santangelo, Roux, et al. (2022) sampled transects across five park-suburban gradients in the Greater Toronto area to test for the impact of environmental heterogeneity on HCN clines, and four out of five clines showed lower HCN production inside parks, increasing towards the suburbs—in the opposite direction of their hypothesis based on the direction of urban-rural clines. However, clines were only present at one of two loci coding for HCN production, suggesting that this locus, rather than the HCN phenotype, may be under selective pressure. Herbivory and temperature could not explain the presence of clines, but increased drought stress in suburban areas may be driving the clines (Santangelo, Roux, et al., 2022). While this study shows that HCN production in white clover responds to local environmental heterogeneity, none of these studies address the complexity of urban landscapes, which cannot be captured by distance from city center, but may ultimately be driving clover evolution.

Chapter 3 Methods

With this study, I explored how variables that reflect the complex spatial and temporal structure of cities account for the variability in urban eco-evolutionary phenomena. Specifically I tested the hypothesis that landscape heterogeneity, connectivity, and historical contingency can explain variability in hydrogen cyanide (HCN) production in urban white clover (*Trifolium repens*). I replicated the methods used in Santangelo, Ness, et al. (2022)—which involved creating multilinear and binomial regressions to assess strength, direction, and consistency of HCN clines—with new variables representing urban complexity. This modification allowed direct comparison of results to assess the ability of urban complexity to explain this variability.

Santangelo, Ness, et al. (2022) sampled clover along urban-rural transects to show that cities are driving the global parallel evolution of HCN production in white clover, estimating the proportion of specimens producing HCN at each sampling location. It is important to note that the original sampling methods employed by Santangelo, Ness, et al. (2022) were not tailored for this complexity analysis, meaning that city selection did not regulate for potential effects such as transect length and city size, which could influence my results. I used a subset of the 110,019 samples collected by Santangelo, Ness, et al. (2022) in 160 cities across the world to control for similarity in historical development and land cover data quality.

3.1 City Selection

I selected 20 out of the 89 cities sampled in the United States and Canada to control for the impacts of culture and governance, as well as data consistency. Because transect length (which ranges from 2.61 km to 71.15 km) may reflect differences in city structure (compact versus sprawling), I used a stratified sampling method to select 10 cities with longer transects and 10 cities with shorter transects. Short and Long Transect cities were defined based on a natural division in the data (Fig. 3.1), with transects under 22.6 km considered Short and transects over 22.6 km considered Long. These 89 cities were distributed across seven different Level I ecoregions (Table 3.1), as defined by the Commission for Environmental Cooperation. Level I ecoregions represent broad patterns to create context at global or intercontinental scales (Commission for Environmental Cooperation, 1997). Sampling was also stratified by ecoregion to ensure they were equally represented.

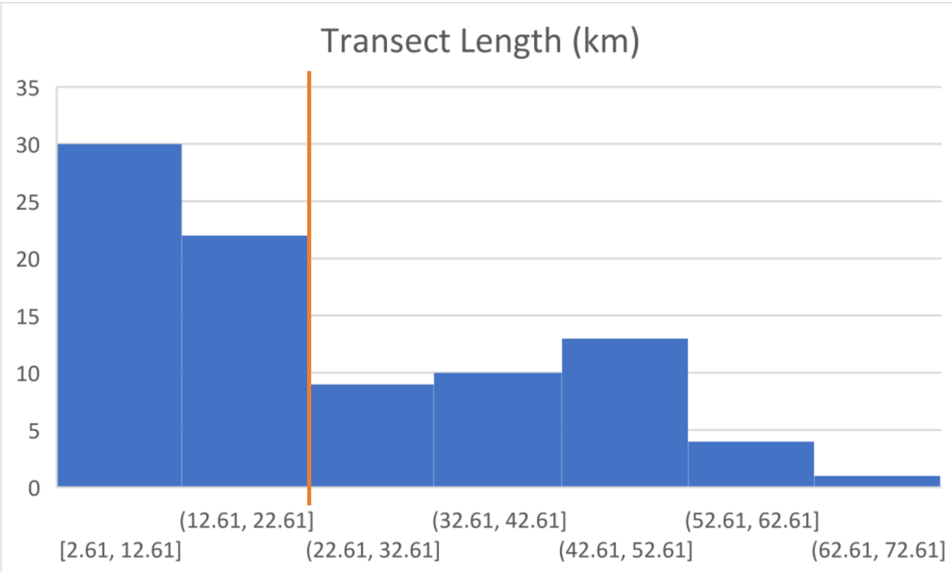


Figure 3.1 Histogram of transect lengths of cities sampled in the United States and Canada. The orange line reflects the break in the data used to categorize cities as either short or long transect.

Table 3.1 Distribution of cities by Level I ecoregion and transect length.

Ecoregion	*Short Transect Cities*	*Long Transect Cities*	*Total*
Taiga	1	0	1
Northern Forests	2	1	3
Marine West Coast Forest	4	3	7
Eastern Temperate Forest	39	26	65
Great Plains	3	6	9
North American Deserts	1	0	1
Mediterranean California	2	1	3
Total	52	37	89

Within each transect length strata, I used a random number generator to randomly select cities from each ecoregion to ensure all were included. To select 10 Short Transect cities, first, one city was randomly selected from each of the seven represented ecoregions, which will left three more cities to be selected. Three of the five ecoregions with more than one short-transect city were randomly selected, and one additional city was randomly selected from each of those three ecoregions. To select 10 Long Transect cities, two cities were randomly selected from each of the five represented ecoregions, unless only one city was represented, leaving two more cities to be selected. Two of the three ecoregions with more than two cities were randomly selected, and one more city was randomly selected from each of those two ecoregions (Fig. 3.2).

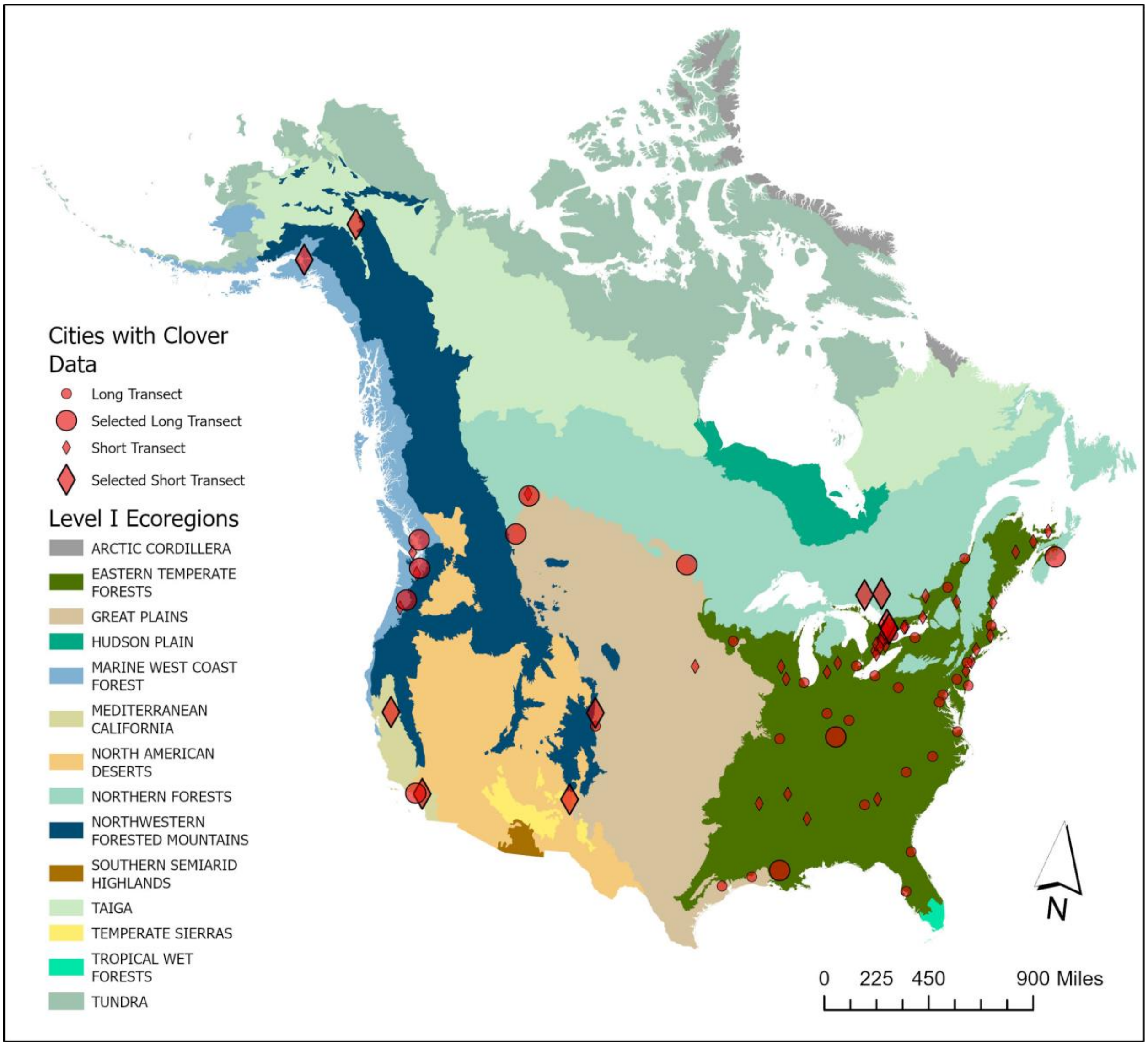


Figure 3.2 Map of the 89 cities in the United States and Canada sampled in Santangelo, Ness, et al. (2022), and the 20 selected for use in this study, by ecoregion. The original 89 cities represent only seven of the 14 ecoregions present across the two countries, but stratification by ecoregion ensured that all seven ecoregions represented were included in this study.

3.2 Metrics

Variables representing the spatiotemporal structure of cities were calculated using 30m resolution Landsat land cover data, which is available at the continental level. The land cover classes have been defined from remotely sensed data according to the Land Cover Classification System developed by the Food and Agriculture Organization of the United Nations (Commission for Environmental Cooperation, 2020); however, land cover was reclassified into five simpler categories for this analysis (Table 3.2).

Table 3.2 North American land cover classes included in the 30m resolution Landsat land cover data, as categorized using the Food and Agriculture Organization of the United Nations' Land Cover Classification System (Commission for Environmental Cooperation, 2020), and the reclassification into simplified categories.

Reclassification	*Commission for Environmental Cooperation Land Cover Classes*
Natural Vegetation	Temperate or sub-polar needleleaf forest Sub-polar taiga needleleaf forest Tropical or sub-tropical broadleaf evergreen forest Tropical or sub-tropical broadleaf deciduous forest Temperate or sub-polar broadleaf deciduous forest Mixed forest Tropical or sub-tropical shrubland Temperate or sub-polar shrubland Tropical or sub-tropical grassland Temperate or sub-polar grassland Subpolar or polar shrubland-lichen-moss Subpolar or polar grassland-lichen-moss Subpolar or polar barren-lichen-moss Wetland
Cropland	Cropland
Built-up	Urban and built-up
Unvegetated	Barren land Snow and Ice
Water	Water

Heterogeneity, connectivity, and contingency are the three dimensions of Cadenasso et al.'s (2006) structural biocomplexity framework, and these dimensions can be quantified using landscape metrics (Alberti & Wang, 2022): the signatures of urbanization can be accurately captured using a group of several landscape metrics, such as the Shannon's Diversity Index, Contagion, and Landscape Shape Index (Wu et al., 2011). Unlike a gradient, this framework can capture the uneven distribution of stressors across the landscape; patterns of variation in biodiversity were detected across all three axes. This type of multidimensional framework can represent the complexity of urban landscapes and could be used to build towards a mechanistic understanding of urban ecological phenomena (Alberti & Wang, 2022).

Using metrics that have been shown to effectively quantify urban landscape patterns for each dimension (Wu et al., 2011), I analyzed heterogeneity, connectivity, and historical contingency within a 250m radius around each sample point (Table 3.3), although the scale relevant to clover evolution is unclear. Variation in HCN production within metropolitan areas may be caused by freeze damage, drought stress, herbivory, or other factors (Santangelo, Roux, et al., 2022; Santangelo, Thompson, et al., 2020; Thompson et al., 2016), which may be impacted by multiple landscape patterns acting at different scales. Sampled populations could be as close as 200m (Santangelo, Ness, et al., 2022), so to maintain independence between the sample points, I identified all sample points that were within 500m of another sample point. I made random selections from those clustered points to include in the analysis until it was not possible to add any more sample points in without creating any overlaps in the 250m radius landscapes.

Table 3.3 Dimensions of urban complexity and the landscape metrics used to represent them.

Dimension	*Metric*	*Equation*	*Description*
Landscape Heterogeneity	Shannon's Diversity Index (SHDI)	$SHDI = -\sum_{i=1}^{m}(P_i * \ln P_i)$ Where P_i is the proportion of the landscape made up by class i	*Landscape-level metric*
Connectivity	Aggregation Index (AI)	$AI = \left[\frac{g_{ii}}{maxg_{ii}}\right](100)$ Where g_{ii} is the number of like adjacencies $maxg_{ii}$ is the maximum possible number of like adjacencies for class i	*Class-level metric* calculated for built-up, cropland, and natural vegetation
Contingency	Percent of Land (PLAND) Change	$PLAND = \frac{\sum_{j=1}^{n} a_{ij}}{A} * 100$ Where a_{ij} is the area of each patch and A is the total landscape area $PLAND_{change} = PLAND_{2015} - PLAND_{2010}$	*Class-level metric* calculated for built-up, cropland, and natural vegetation

3.2.1 Landscape Heterogeneity

Landscape heterogeneity, which refers to the composition of patches, often increases with development because of the addition of anthropogenic driving forces and their interactions with natural ones (Alberti & Wang, 2022). Landscape heterogeneity may impact HCN production because the composition and arrangement of landscape patches can shape important drivers of selection, such as microclimate and herbivory (Erell et al., 2011; Jonsen & Fahrig, 1997; Kruess, 2003; Miles et al., 2019). Heterogeneity was calculated using Shannon's Diversity Index (SHDI) based on the five reclassified covers (Table 3.3) (Alberti & Wang, 2022; Wu et al., 2011). All landscape metrics (Table 3.3) were calculated using Fragstats version 4.2 (McGarigal et al., 2023).

3.2.2 Connectivity

Urbanization tends to affect habitat connectivity by fragmenting or artificially connecting natural habitat, affecting gene flow. Connectivity was represented using three measures: Aggregation Index (AI) of Natural Vegetation, AI of Built-up cover, and AI of Cropland (Table 3.3). AI represents the arrangement and connectivity between pixels of the same cover type (Alberti & Wang, 2022). For landscapes that lack the focal cover type, AI will be considered 0 to eliminate NAs in the dataset.

Natural Vegetation, Built-up cover, and Cropland were included in the analysis because these three cover types are predicted to impact white clover and drivers of HCN production. Areas of natural vegetation serve as sources of insect predators and parasites, reducing herbivory pressure in surrounding areas (Rand et al., 2006; Veres et al., 2013; Woltz et al., 2012), but loss of natural habitat can also reduce insect abundance (Forister et al., 2019). Vegetation also impacts climate by increasing moisture and decreasing temperature (Chen et al., 2022; Erell et al., 2011; Robitu et al., 2006). In addition to impacting habitat connectivity, built-up areas can impact climate through the urban heat island effect (Erell et al., 2011), which may negatively impact some herbivore species, but the overall impacts of urbanization on insects is unclear and may be taxa dependent (Miles et al., 2019). Finally, both built-up areas and cropland may be treated with pesticides, which have a major impact on insect abundance (Forister et al., 2019). Cropland also provides important habitat for white clover, and many farmers grow clover as feed for livestock in pastures or as a nitrogen fixer for other crops (Frame & Newbould, 1986; Kjærgaard, 2003).

3.2.3 Historical Contingency

I quantified historical contingency to account for habitat stability, landscape change over time, and possible legacies, indirect effects, or time lags by measuring change in Percent of Land (PLAND) Natural Vegetation, PLAND Built-up, and PLAND Cropland (Table 3.3) (Alberti & Wang, 2022). PLAND change was calculated using PLAND of each cover near the time of data collection, in 2015, and calculating and subtracting PLAND of each cover from 2010. The five-year interval was selected based on the availability of comparable high resolution land cover data.

3.2.4 Biophysical Factors

Biophysical and climatic factors—particularly aridity—may impact HCN production (Kooyers et al., 2018; Olsen & Ungerer, 2008; Santangelo, Ness, et al., 2022; Thompson et al., 2016), so annual potential evapotranspiration (PET) was also included as a predictor to represent biophysical impacts on trait changes (Alberti & Wang, 2022). I used PET data prepared by Santangelo, Ness, et al. (2022): PET was extracted using a 100m buffer around each sample point from the Global PET dataset provided by the Consultative Group for International Agriculture Research – Consortium for Spatial Information, which provides the annual average PET from the period of 1950-2000.

3.3 Statistical Analysis

I used R to replicate the statistical methods used by Santangelo, Ness, et al. (2022) and create comparable models. The response variable in a binomial generalized linear mixed-effects model was the proportion of plants producing HCN within each population, as calculated using the *glmer* function from the package *lme4* (Bates et al., 2015). After performing normality checks, transformations, and scaling, models were generated for all combinations of predictors—SHDI, Built-Up AI, Cropland AI, Natural Vegetation AI, Built-Up PLAND change, Crop PLAND change, Natural Vegetation PLAND change, PET, and Distance (from city center)—barring correlation. Pairs of predictors with correlation coefficients with absolute values over 0.5 or variance inflation factors (VIF) over 2.5 were not included in the same models (Hair et al., 2010; Zar, 2010). Random effects were also included to allow intercepts and slopes to vary by city (Santangelo, Ness, et al., 2022). Using the *dredge* function from the package *MuMIN*, models were compared using an information-theoretic approach, AICc, and secondarily log-likelihood, to select the most parsimonious models (Barton, 2023). I compared the adjusted R^2 of several top multi-city models generated in this study to Santangelo, Ness, et al.'s (2022) model to see if the multidimensional framework can provide a more complete explanation of variation in HCN production, and I compared the significance of predictors to determine if one predictor is clearly dominant. R^2 was calculated using *r.squaredGLMM* from the package *MuMIn* and then adjusted based on the degrees of freedom and residual degrees of freedom for each model (Nakagawa et al., 2017).

For each of the selected cities, I created three sets of separate binomial regressions of HCN production using the fixed effects from the top three multi-city models. These models were used to assess consistency in the size and direction of clines across cities (Santangelo, Ness, et al., 2022). I compared the consistency in the strength and direction of slopes of single-city models against Santangelo, Ness, et al. (2022). If clines generated by the new models are more consistent in direction and more frequently significant, this would suggest that the variables representing urban complexity are more representative of the forces driving urban evolution in white clover than distance from city center.

Chapter 4 Results

4.1 Preparing Model Inputs

4.1.1 Independent Landscapes

The 20 selected cities initially contained 758 sample points, but 382 of those points were within 500m of each other, meaning that the landscapes created using a 250m radius would not be independent. After making random selections from those clustered points to include until it was not possible to add any more sample points in without creating any overlaps, the total number of sample points included in the study was 542 (Table 4.1). This left 5 cities with less than 20 sample points, the minimum specified in Santangelo, Ness, et al. (2022).

Table 4.1 All 20 selected cities, the initial number of sample points, and the number of sample points included in the study after ensuring the independence of the 250m radius landscapes.

City	Initial Sample Points	Included Sample Points
Albuquerque	40	32
Anchorage	44	21
Barrie	25	13
Baton Rouge	45	36
Bradford	20	8
Calgary	40	35
Edmonton	44	34
Fairbanks	40	20
Fort Collins	23	11
Halifax	40	26
La Verne	40	11
Los Angeles	42	28
Louisville	46	39
North Bay	45	23
Portland, OR	40	39
Sacramento	41	27
Seattle	40	39
Sudbury	20	15
Vancouver	40	38
Winnipeg	43	37
Total	758	542

4.1.2 Distributions and Transformations

The response variable, frequency of hydrogen cyanide (HCN) production in white clover (*Trifolium repens*), was distributed according to the binomial distribution (Fig. 4.1). The distributions of all predictors were observed and transformed as necessary (Table 4.2); however, due to the nature of many of the landscape metric variables, normal distributions could not be achieved. All references to predictors henceforth refer to their transformed distributions.

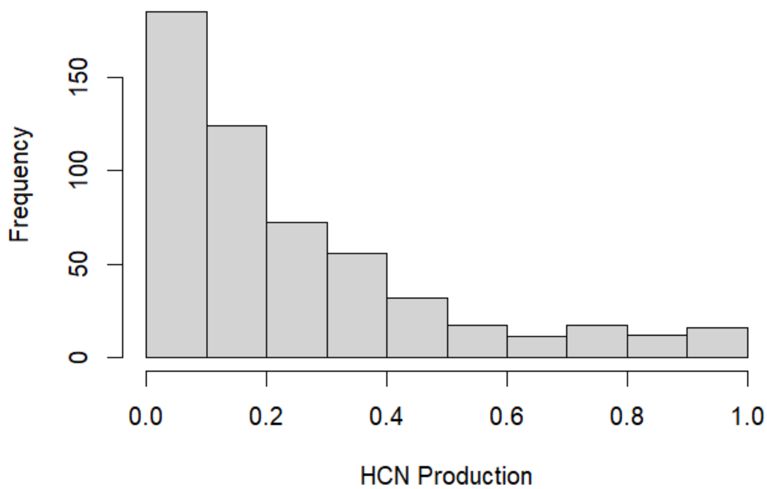
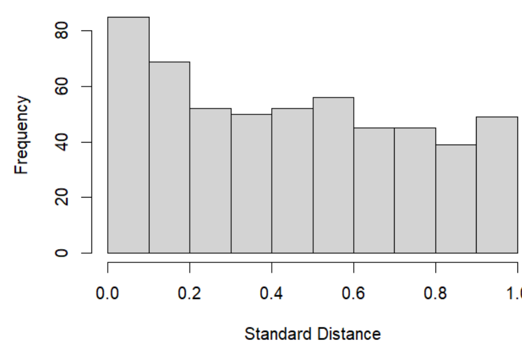
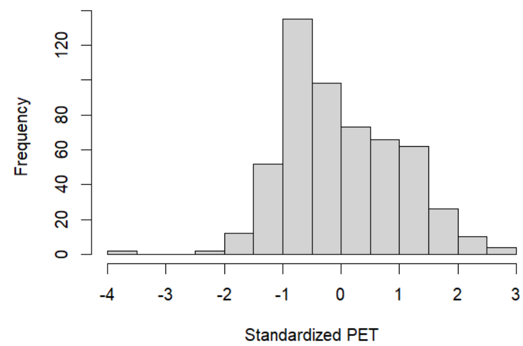
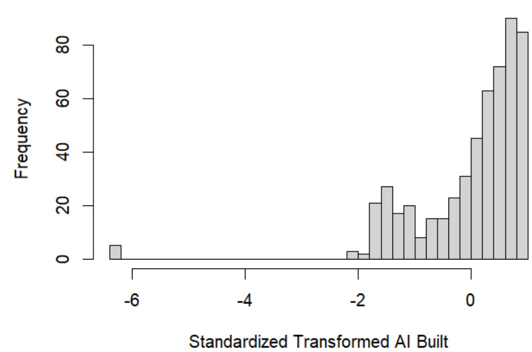
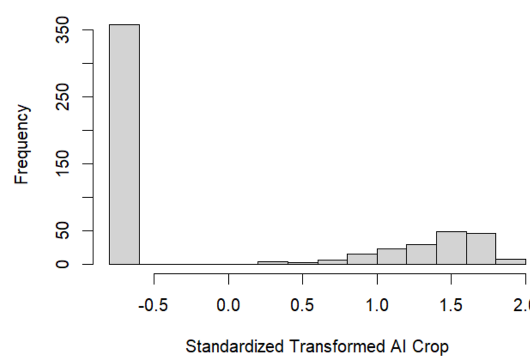
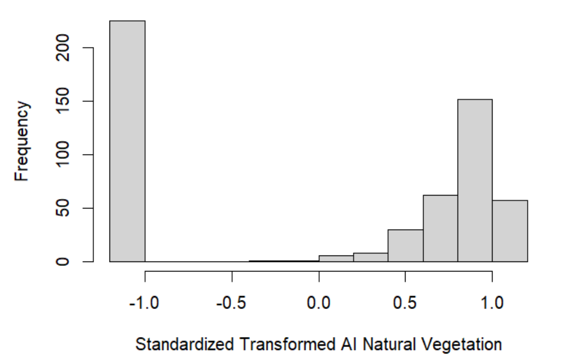
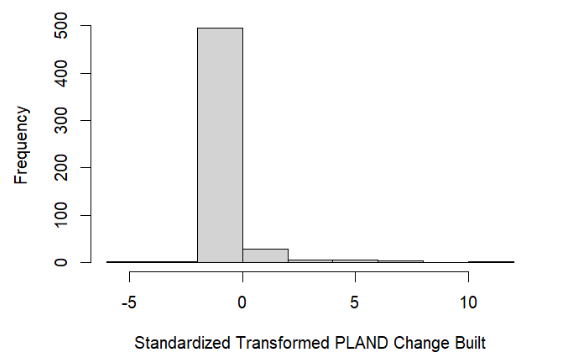
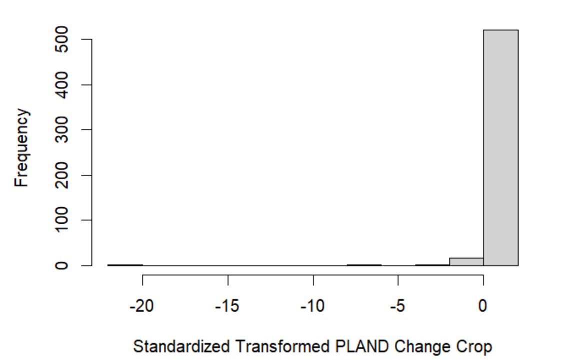
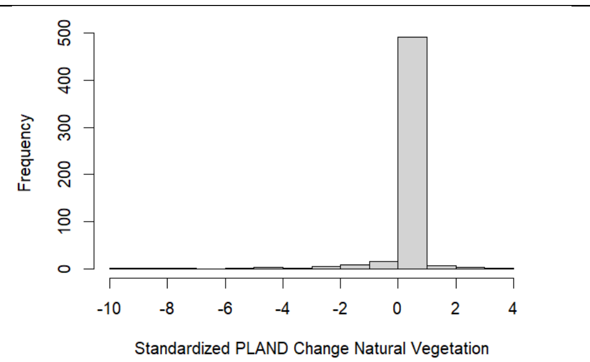


Figure 4.1 Histogram of HCN production.

Table 4.2 Predictors and the transformations applied prior to model generation. All predictors, except for distance, were standardized to a mean of 0 and a standard deviation of 1.

Predictor	Transformation	Transformed and Standardized Distribution
Distance	NA	 *Not standardized by mean and standard deviation, only scaled between 0 and 1

PET	NA	
SHDI	$log(SHDI + 1)$	
AI Built-up	$\sqrt{arcsin(AI\ Built/100)}$	
AI Cropland	$\sqrt[4]{\frac{AI\ Crop}{100} + 0.25}$	

<table>
<tr><td>AI Natural Vegetation</td><td>$\sqrt[4]{\frac{AI\ Nat\,Veg}{100} + 0.25}$</td><td>
</td></tr>
<tr><td>PLAND Change Built-up</td><td>$log(Pch\ Built + 1)$</td><td>
</td></tr>
<tr><td>PLAND Change Cropland</td><td>$0.5 * log\left(\frac{1 + \frac{Pch\ Crop}{100}}{1 - \frac{Pch\ Crop}{100}}\right)$</td><td>
</td></tr>
<tr><td>PLAND Change Natural Vegetation</td><td>NA</td><td>
</td></tr>
</table>

4.1.3 Predictor Correlations

The transformed and standardized versions of the predictors were compared for correlation. No pairs of predictors had VIFs over 2.5, but 5 pairs of predictors had correlation coefficients with absolute values over 0.5 (Hair et al., 2010; Zar, 2010): Distance and AI Built-up, SHDI and AI Cropland, SHDI and AI Natural Vegetation, PLAND change Built-up and PLAND change Natural Vegetation, and PLAND change Built-up and PLAND change Cropland (Table 4.3). These pairs were not included in the same models for model selection.

4.2 Multi-City Models

Model selection was run on 8 combinations of predictors using the *dredge* function in order to evaluate all possible combinations of predictors, while excluding pairs of correlated predictors (Barton, 2019). Twelve models remained after selecting the lowest AICc and highest log-likelihood models from each of the eight runs (Table 4.4). AI Built-up was not included in any of these models. Model 1, the model with Distance as the only fixed predictor, as in Santangelo, Ness, et al. (2022), had an adjusted R^2 of 0.2175, but two other models had higher adjusted R^2 values. Model 5, which included both Distance and AI Cropland, had and adjusted R^2 of 0.2176, and Model 8, which had AI Cropland and AI Natural Vegetation as the fixed predictors, had an adjusted R^2 of 0.2211—the highest of all 12 models. Of the three top models, only Model 1's parameter estimate for distance was significant (Table 4.4), but the p-value of the parameter estimate for AI Cropland in Model 8 was 0.05134.

Table 4.3 Pearsons R correlation coefficients for all transformed and standardized predictors. Bolded values show correlation coefficients with absolute values over 0.5.

	PET	Distance	PLAND Change Natural Vegetation	SHDI	AI Built-up	AI Cropland	AI Natural Vegetation	PLAND Change Built-up	PLAND Change Cropland
PET	1	0.28	0.069	0.11	-0.11	0.14	-0.0040	-0.068	-0.026
Distance	0.28	1	-0.12	0.48	**-0.53**	0.38	0.38	0.098	-0.0090
PLAND Change Natural Vegetation	0.069	-0.12	1	-0.050	0.023	0.061	-0.097	**-0.81**	0.015
SHDI	0.11	0.48	-0.050	1	-0.50	**0.54**	**0.66**	0.077	-0.055
AI Built-up	-0.11	**-0.53**	0.023	-0.50	1	-0.34	-0.48	-0.021	-0.0011
AI Cropland	0.14	0.38	0.061	**0.54**	-0.34	1	0.078	0.011	-0.081
AI Natural Vegetation	-0.0040	0.38	-0.097	**0.66**	-0.48	0.078	1	0.069	0.014
PLAND Change Built-up	-0.068	0.098	**-0.81**	0.077	-0.021	0.011	0.069	1	**-0.58**
PLAND Change Cropland	-0.026	-0.0090	0.015	-0.055	-0.0011	-0.081	0.014	**-0.58**	1

Table 4.4 Parameter estimates for the fixed effects for the selected 12 models, with R^2 and adjusted R^2. A * indicates $p < 0.05$. Models with the highest adjusted R^2 are bolded.

Model	Intercept	Distance	PET	SHDI	AI Cropland	PLAND Change Natural Vegetation	AI Natural Vegetation	PLAND Change Built-up	PLAND Change Cropland	R^2	Adjusted R^2
1	**-1.531 ***	**0.4786 ***								**0.2233**	**0.2175**
2	-1.520 *	0.4553 *				-0.04980				0.2266	0.2150
3	-1.516 *	0.4439 *		0.01628						0.2245	0.2129
4	-1.522 *	0.4539 *				-0.05017			0.09542	0.2304	0.2115
5	**-1.466 ***	**0.3357**			**0.07389**					**0.2292**	**0.2176**
6	-1.330 *				0.1106					0.2133	0.2074
7	-1.335 *				0.1162				0.1642	0.2214	0.2097
8	**-1.293 ***				**0.1075**		**0.05920**			**0.2326**	**0.2211**
9	-1.322 *			0.09124*						0.2098	0.2039
10	-1.327 *			0.09330*					0.1397	0.2146	0.2028
11	-1.328 *		0.03700	0.06269						0.2136	0.2018
12	-1.252 *	0.4658 *						0.02792		0.2280	0.2164

4.3 Single City Models

The fixed effect predictors from Models 1, 5, and 8 were used to generate three sets of binomial regressions of HCN production for each of the 20 selected cities (Table 4.5). Model Set A, which used AI Cropland and AI Natural Vegetation as the only predictors resulted in 16 complete models. Four models were incomplete because the cities had only zeroes as the inputs for one of the predictors. Of the 16 complete models, 6 included at least one significant slope term. The parameter estimate for AI Cropland was significant in 4 models, and the parameter estimate for AI Natural Vegetation was significant in 3 models. All significant terms for both parameters were positive. Model Set B included 17 complete models using AI Cropland and Distance as the predictors (Table 4.5). The parameter estimate for AI Cropland was significant in three models, but all estimates were positive. The parameter estimate for Distance was significant in six models, but the slopes were not consistent in direction, with four positive and two negative slopes. Finally, Model Set C used distance as the only predictor, as in Santangelo, Ness et al. (2022). Nine of the 20 models showed significant slopes, eight of which were positive (Table 4.5).

Table 4.5 Parameter estimates for all single-city binomial models. A * indicates $p < 0.05$.

City	Transect Length (km)	Sample Points	Model Set A		Model Set B		Model Set C
			AI Cropland	AI Natural Vegetation	AI Cropland	Distance	Distance
Fort Collins	4.9	11	0.475	0.5091	0.5803	1.386	1.988 *
La Verne	5.84	11	-0.4824	-0.07998	-0.25	1.156	1.296 *
Bradford	6.31	8	-0.04406	0.2266	-0.08095	-0.4763	-0.7405
Barrie	8.1	13	-1.166	1.108	-0.2059	0.3282	0.06013
Fairbanks	8.68	20	0.1173	-0.0356	0.1138	0.02474	-0.0337
Sudbury	9.21	15	NA	-0.03455	NA	-1.289	-1.289
Sacramento	14.32	27	0.06779	NA	-0.05717	0.9975 *	0.9081 *
Anchorage	15.2	21	NA	-0.241	NA	0.4108	0.4108
Albuquerque	17.27	32	-0.00758	-0.1651	0.006034	-0.7737 *	-0.7739 *
North Bay	17.43	23	0.001647	0.2343	-0.2174	1.026	0.5138
Edmonton	22.68	34	0.2461 *	0.05535	0.4307 *	-0.8526	0.6022
Halifax	23.29	26	NA	0.01227	NA	0.4769	0.4769
Baton Rouge	26.61	36	0.6077 *	0.5663 *	0.1742	2.593	3.143 *
Winnipeg	29.65	37	0.2693 *	-0.1879	0.9364 *	-3.294 *	-0.2308
Calgary	34.29	35	0.0205	-0.05194	-0.1372	1.586 *	1.368 *
Louisville	40.07	39	0.1361	-0.1318	0.1217	-0.197	0.0565
Los Angeles	41.13	28	-0.06714	0.3805 *	-0.149	0.6923 *	0.6705 *
Portland	45.91	39	-0.05499	0.03917	-0.08454	0.275	0.1349
Vancouver	45.99	38	0.17424	0.2065 *	-0.09261	1.496 *	1.326 *
Seattle	52.17	39	0.4187 *	-0.02542	0.3816 *	0.3539	0.7012 *

Chapter 5 Discussion

5.1 Model Interpretation

Two multi-city models (Models 5 and 8) using urban complexity variables had higher adjusted R^2 values than the model including Distance alone (Model 1), as in Santangelo, Ness et al. (2022). This means that the models including urban complexity predictors were able to account for more variability in HCN production than the model with Distance alone. Adding a parameter for AI Cropland to the Distance model slightly improved the explanatory power, which suggests that the Distance model could be enhanced by adding urban complexity variables. The model with parameters for AI Cropland and AI Natural Vegetation only (Model 8) had the most explanatory power (Table 4.4). Ranking the models by adjusted R^2 suggests that urban complexity may be more representative of the drivers of white clover (*Trifolium repens*) evolution than distance from city center. Only the slope of Model 1 was significant, but the slope term for AI Cropland from Model 8 was very close to significant ($p = 0.05134$) and may be significant if the study was repeated with a larger sample size, although this remains uncertain. AI Cropland, representing the connectivity of cropland, was the most promising driver of hydrogen cyanide (HCN) production from the urban complexity framework.

For single city models, 40% of the Distance-alone models (Model Set C) showed a significant positive slope, while 5% showed a significant negative slope (Table 4.5). While adding AI Cropland to the Distance model slightly improved the explanatory power of the multi-city model, it did not enhance the consistency of the strength and direction of the Distance slope term in Model Set B: 23.5% of complete models had a significantly positive Distance slope, while 11.8% were negative. This reduction in the proportion of significant slopes likely occurred because the addition of the AI Cropland term (17.6% significantly positive) took some explanatory power from the Distance term. Similarly, it is difficult to compare Model Set A to Model Set C because of the different numbers of terms: 25% of models had a significant positive slope for AI Cropland and 18.8% had a significant slope for AI Natural Vegetation. All significant slopes for both AI Cropland and AI Natural Vegetation were in cities with transects categorized as Long (Table 4.5), which could be due to differences in city structure or issues with sample size. However, this does not appear to be related to the proportion of zero values for predictors in the dataset for each city—zeroes could lead to bias or reduce sensitivity to trends

(Boulton & Williford, 2018). While four out of the seven complete Short Transect models had datasets for AI Cropland that were over 75% zeroes, AI Cropland was a significant predictor in Seattle, where 76.9% of AI Crop values were zero (Table 5.1).

Table 5.1 Distribution of zeroes in the predictors for Model Set A. Bolded values correspond to significant slopes in Model Set A.

City	Category	Transect Length (km)	Number of Sample Points	% of Points with a value of 0 for AI Crop	% of Points with a value of 0 for AI Natural Vegetation
Fort Collins	Short	4.9	11	54.5	45.5
La Verne	Short	5.84	11	90.9	27.3
Bradford	Short	6.31	8	25.	62.5
Barrie	Short	8.1	13	15.4	15.4
Fairbanks	Short	8.68	20	90.	30.
Sudbury	Short	9.21	15	NA (100.)	33.3
Sacramento	Short	14.32	27	51.9	NA (100.)
Anchorage	Short	15.2	21	NA (100.)	19.0
Albuquerque	Short	17.27	32	81.3	40.6
North Bay	Short	17.43	23	78.3	17.4
Edmonton	Long	22.68	34	**58.8**	29.4
Halifax	Long	23.29	26	NA (100.)	26.9
Baton Rouge	Long	26.61	36	**41.7**	**44.4**
Winnipeg	Long	29.65	37	**54.1**	18.9
Calgary	Long	34.29	35	71.4	62.9
Louisville	Long	40.07	39	38.5	28.2
Los Angeles	Long	41.13	28	85.7	**46.4**
Portland	Long	45.91	39	53.8	64.1
Vancouver	Long	45.99	38	78.9	**39.5**
Seattle	Long	52.17	39	**76.9**	64.1

Cursory post-hoc exploration revealed that the fit of all three top multi-city models improved when applied to Long Transect cities alone, while the explanatory value was minimal when applied to the Short Transect cities alone (Table 5.2). This shows that all three models may be better predictors of HCN production in Long Transect cities than Short Transect cities. The multi-city models may have been biased towards the patterns in Long Transect cities because twice as many sample points came from Long Transect cities (361) compared to Short Transect cities (181). Clines in Short Transect cities may be weaker, directionally inconsistent, and/or driven by different patterns than in long transect cities. Differences in clines between Short and Long Transect cities could be driven by local factors such as compact versus sprawling city structure. Compact and sprawling cities could generate different clines because they are composed of different built typologies and land use patterns (Salingaros, 2006; Sarzynski et al., 2014; Scheer, 2001), which could translate into differences in habitat type, ecological niches, and habitat connectivity (Youngsteadt et al., 2023). This suggests the need to consider overall distance between urban center and nonurban areas in identifying the drivers of urban eco-evolutionary dynamics; however, further investigation is required to determine whether this phenomenon is a figment of the sampling method, such as variation in the number of sample points per city, (Table 5.1) or a property of city structure.

Table 5.2 The top three multi-city models run separately on short and long transect cities. A * indicates $p < 0.05$. Models with the highest adjusted R^2 are bolded.

Model	Intercept	Distance	AI Crop-land	AI Natural Vegetation	R^2	df	Residual df	Adjusted R^2
1	-1.53 *	0.4785 *			0.2233	541	537	0.2175
l-Long	**-1.473 ***	**0.7616 ***			**0.2865**	**360**	**356**	**0.2785**
1-Short	-1.573 *	0.1702			0.1311	180	176	0.1114
5	-1.466 *	0.3357	0.07389		0.2292	541	533	0.2176
5-Long	**-1.357 ***	**0.4206**	**0.1318**		**0.3056**	**360**	**356**	**0.2978**
5-Short	-1.552 *	0.1968	0.0504		0.1321	180	176	0.1124
8	-1.293 *		0.1075	0.0592	0.2326	541	533	0.2211
8-Long	**-1.132 ***		**0.1794***	**0.07998**	**0.2984**	**360**	**356**	**0.2905**
8-Short	-1.503 *		0.05423	0.02116	0.1149	180	176	0.09478

5.2 Implications

This study illuminates how the patterns of urban complexity, extending beyond distance from city center, could be the driving forces behind urban clines in HCN production—most prominently the connectivity of cropland and secondarily the connectivity of naturally vegetated areas. These patterns may vary depending on the distance from urban center to nonurban areas, although further investigation is needed to identify the driver of this pattern.

The use of a multidimensional framework, which accounts for urban complexity and heterogeneity, to understand and measure urban environments was a significant strength of this study, providing a novel perspective in the field of urban eco-evolutionary dynamics. Prior urban ecology and evolution studies often yield conflicting results because the underlying mechanisms governing urban eco-evolutionary dynamics are not well understood (Swan et al., 2021). However, urban studies frequently employ simple urban gradients that fail to capture the variability needed to explain the patterns and interactions at play (Alberti & Wang, 2022). General patterns and mechanistic explanations of urban eco-evolutionary phenomena cannot be developed without accounting for the complexity of urban environments (Alberti & Wang, 2022; Swan et al., 2021). By measuring the heterogeneity, connectivity, and historical contingency of urban landscapes to explain urban-driven evolution across multiple cities (McDonnell & Hahs, 2013), this study investigated a way to capture the complexity of urban landscapes, addressing an important knowledge gap in the field. Results support the value of this framework, which should inform the design of future research into various urban ecological and evolutionary processes. Adopting an urban complexity framework can also better inform the practice of urban planning by emphasizing the importance of landscape patterns and change in shaping both ecological and evolutionary processes on a human timescale.

5.3 Limitations

Several limitations of this study stemmed from the original sampling method used to collect the data for Santangelo, Ness, et al. (2022). For the purposes of the present study, a more ideal method might use randomly selected points around the city, rather than a transect, because of spatial autocorrelation as well as the potential inability to capture the full spectrum of urban forms within each city (Getis, 2007; Goslee, 2006). Additionally, due to the requirement for greater separation between sample points to ensure independence, some samples had to be excluded from the analysis, resulting in cities with few sample points, particularly among Short Transect cities. This could have led to bias in the results and poor model fit in those cities.

Limitations also arose with the urban complexity metrics. This study only tested for the impact of urban complexity at one spatial scale, but landscape heterogeneity, connectivity, and historical contingency may have impacts on ecological processes at multiple scales (Alberti & Wang, 2022). Furthermore, historical contingency was only measured over a five-year period due to data availability. Longer periods of time might reveal different results and additional legacy effects. All urban complexity metrics also had skewed distributions, which may have impacted model fit.

Finally, there is a risk of confounding because there are many possible underlying factors that differ between cities that may not be captured or known, and nonrandom transect sampling could create systemic bias. Similarly, the initial selection of cities and sampling sites is not ideal for this analysis and may not capture true variability of urban form within and between cities, which could impact external validity despite the collection of data from multiple cities.

Chapter 6 Conclusion

This study explores how an urban complexity framework—landscape heterogeneity, connectivity, and historical contingency—may be used to explain urban eco-evolutionary dynamics. Previous studies have shown that global urban-driven evolution is occurring on a human timescale (Santangelo, Ness, et al., 2022), but the mechanisms behind these changes are not understood. Previous studies investigating urban eco-evolutionary dynamics have used broad predictors, such as distance from city center, but cities are highly heterogeneous and dynamic (Alberti et al., 2020; McPhearson et al., 2016; Pickett et al., 2016). By applying the biocomplexity framework proposed by Cadenasso et al. (2006) and applied to urban areas by Alberti and Wang (2022), this study aims to identify how the variation in the human and ecological factors that characterize cities may drive urban eco-evolutionary dynamics. The results of this study can inform urban planning and conservation efforts by clarifying the limits and causes of adaptation (Alberti, 2015; Donihue & Lambert, 2015).

By comparing and integrating predictors from an urban complexity framework with distance from city center, the predictor used in Santangelo, Ness, et al. (2022), I was able to show that multi-city models of urban clines in hydrogen cyanide (HCN) production in white clover (*Trifolim repens*) could be improved by using metrics of urban complexity as predictors. The connectivity of cropland and, secondarily, the connectivity of natural vegetation within 250m of sample points were the most powerful predictors of the frequency of HCN production in white clover. While using these predictors did not improve the significance and consistency of slopes in single-city models, I found that the distance from city center to nonurban areas may impact the explanatory power of these not only these predictors, but distance from city center. Overall, these results emphasize the importance of urban complexity in driving urban eco-evolutionary dynamics.

Future studies should expand on this one by applying the urban complexity framework to understand the drivers of urban HCN production to greater numbers of cities to investigate the consistency of these patterns. The impact of transect length on model fit should also be investigated in order to determine if the multi-city models fit the Long Transect cities better than the Short Transect cities because of the sampling method, such as variation in the number of sample points per city, or because of structural differences between compact and sprawling cities.

Studies should also investigate the role of cropland in clover evolution because the connectivity of cropland was one of the most important predictors identified in this study. Finally, future studies should apply an urban complexity framework to other eco-evo dynamics problems, including exploring the impacts of urban complexity on genetic diversity.

References

Alberti, M. (2015). Eco-evolutionary dynamics in an urbanizing planet. *Trends in Ecology and Evolution*, *30*(2), 114–126. https://doi.org/10.1016/j.tree.2014.11.007

Alberti, M., Correa, C., Marzluff, J. M., Hendry, A. P., Palkovacs, E. P., Gotanda, K. M., Hunt, V. M., Apgar, T. M., & Zhou, Y. (2017). Global urban signatures of phenotypic change in animal and plant populations. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(34), 8951–8956. https://doi.org/10.1073/pnas.1606034114

Alberti, M., Marzluff, J., & Hunt, V. M. (2017). Urban driven phenotypic changes: Empirical observations and theoretical implications for eco-evolutionary feedback. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1712). https://doi.org/10.1098/rstb.2016.0029

Alberti, M., Palkovacs, E. P., Roches, S. Des, De Meester, L., Brans, K. I., Govaert, L., Grimm, N. B., Harris, N. C., Hendry, A. P., Schell, C. J., Szulkin, M., Munshi-South, J., Urban, M. C., & Verrelli, B. C. (2020). The Complexity of Urban Eco-evolutionary Dynamics. *BioScience*, *70*(9), 772–793. https://doi.org/10.1093/biosci/biaa079

Alberti, M., & Wang, T. (2022). Detecting patterns of vertebrate biodiversity across the multidimensional urban landscape. *Ecology Letters*, *25*, 1027–1045. https://doi.org/10.1111/ele.13969

Barton, K. (2019). *MuMIn: Multi-Model Inference* (R package version 1.43.6). https://cran.r-project.org/package=MuMIn

Barton, K. (2023). *Package "MuMIn."*

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Beninde, J., Veith, M., & Hochkirch, A. (2015). Biodiversity in cities needs space: A meta-analysis of factors determining intra-urban biodiversity variation. *Ecology Letters*, *18*(6), 581–592. https://doi.org/10.1111/ele.12427

Boulton, A. J., & Williford, A. (2018). Analyzing skewed continuous outcomes with many zeros: A tutorial for social work and youth prevention science researchers. *Journal of the Society for Social Work and Research*, *9*(4), 721–740. https://doi.org/10.1086/701235

Cadenasso, M. L., Pickett, S. T. A., & Grove, J. M. (2006). Dimensions of ecosystem complexity: Heterogeneity, connectivity, and history. *Ecological Complexity*, *3*(1), 1–12. https://doi.org/10.1016/j.ecocom.2005.07.002

Chen, S., Wong, N. H., Ignatius, M., Zhang, W., He, Y., Yu, Z., & Hii, D. J. C. (2022). ATLAS: Software for analysing the relationship between urban microclimate and urban morphology in a tropical city. *Building and Environment*, *208*. https://doi.org/10.1016/j.buildenv.2021.108591

Commission for Environmental Cooperation. (1997). *Ecological regions of North America: toward a common perspective*. The Commission.

Commission for Environmental Cooperation. (2020). *2015 Land Cover of North America at 30 Meters*.

Coop, I. (1940). Cyanogenesis in white clover (Trifolium repensL.). III. A Study of linamarase, the enzyme which hydrolyses lotaustralin. *N. Z. J. Sci. Technol.*, *22–23*, 71–83.

Corkill, L. (1942). Cyanogenesis in white clover (Trifolium repensL.) V. The inheritance of cyanogeneiss. *NZ J. Sci. Technol.*, *B23*, 178–193.

Donihue, C. M., & Lambert, M. R. (2015). Adaptive evolution in urban ecosystems. *Ambio*, *44*(3), 194–203. https://doi.org/10.1007/s

Erell, E., Pearlmutter, D., & Williamson, T. (2011). *Urban Microclimate: Designing the Spaces Between Buildings*. Earthscan.

Forister, M. L., Pelton, E. M., & Black, S. H. (2019). Declines in insect abundance and diversity: We know enough to act now. *Conservation Science and Practice*, *1*(8). https://doi.org/10.1111/csp2.80

Frame, J., & Newbould, P. (1986). Agronomy of White Clover. *Advances in Agronomy*, *40*, 1–88. https://doi.org/10.1016/S0065-2113(08)60280-1

Getis, A. (2007). Reflections on spatial autocorrelation. *Regional Science and Urban Economics*, *37*(4), 491–496. https://doi.org/10.1016/j.regsciurbeco.2007.04.005

Goslee, S. C. (2006). Behavior of vegetation sampling methods in the presence of spatial autocorrelation. *Plant Ecology*, *187*(2), 203–212. https://doi.org/10.1007/s11258-005-3495-x

Hair, J. F., Black, W. C., Babin, B. J., Anderson, R. E., & Tatham, R. (2010). *Multivariate Data Analysis* (7th ed.). Pearson Education Limited.

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., & Vellend, M. (2008). Ecological consequences of genetic diversity. *Ecology Letters*, *11*(6), 609–623. https://doi.org/10.1111/j.1461-0248.2008.01179.x

Hughes, M. A. (1991). Genetical Society of Great Britain The cyanogenic polymorphism in Trifolium repens L. (white clover). *Heredity*, *66*, 105–115.

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

Johnson, M. T. J., Prashad, C. M., Lavoignat, M., & Saini, H. S. (2018). Contrasting the effects of natural selection, genetic drift and gene flow on urban evolution in white clover (Trifolium repens). *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883). https://doi.org/10.1098/rspb.2018.1019

Jonsen, I. D., & Fahrig, L. (1997). Response of generalist and specialist insect herbivores to landscape spatial structure. *Landscape Ecology*, *12*, 185–197.

Kjærgaard, T. (2003). A plant that changed the world: The rise and fall of clover 1000-2000. *Landscape Research*, *28*(1), 41–49. https://doi.org/10.1080/01426390306531

Kooyers, N. J., Gage, L. R., Al-Lozi, A., & Olsen, K. M. (2014). Aridity shapes cyanogenesis cline evolution in white clover (Trifolium repens L.). *Molecular Ecology*, *23*(5), 1053–1070. https://doi.org/10.1111/mec.12666

Kooyers, N. J., Hartman Bakken, B., Ungerer, M. C., & Olsen, K. M. (2018). Freeze-induced cyanide toxicity does not maintain the cyanogenesis polymorphism in white clover (Trifolium repens). *American Journal of Botany*, *105*(7), 1224–1231. https://doi.org/10.1002/ajb2.1134

Kooyers, N. J., & Olsen, K. M. (2013). Searching for the bull's eye: Agents and targets of selection vary among geographically disparate cyanogenesis clines in white clover (Trifolium repens L.). *Heredity*, *111*(6), 495–504. https://doi.org/10.1038/hdy.2013.71

Kruess, A. (2003). Effects of landscape structure and habitat type on a plant-herbivore-parasitoid community. *Ecography*, *26*(3), 283–290. https://doi.org/10.1034/j.1600-0587.2003.03402.x

McDonnell, M. J., & Hahs, A. K. (2013). The future of urban biodiversity research: Moving beyond the "low-hanging fruit." *Urban Ecosystems*, *16*(3), 397–409. https://doi.org/10.1007/s11252-013-0315-2

McGarigal, K., Cushman, S., & Ene, E. (2023). *FRAGSTATS v4: Spatial Pattern Analysis Program for Categorical Maps.* Computer software program produced by the authors; available at the following web site: https://www.fragstats.org.

McPhearson, T., Pickett, T. A., Grimm, N. B., Niemelä, J., Alberti, M., Elmqvist, T., Weber, C., Haase, D., Breuste, J., & Qureshi, S. (2016). Advancing Urban Ecology toward a Science of Cities. *BioScience*, *66*(3), 198–212. https://doi.org/10.2307/90007565

Miles, L. S., Breitbart, S. T., Wagner, H. H., & Johnson, M. T. J. (2019). Urbanization shapes the ecology and evolution of plant-arthropod herbivore interactions. *Frontiers in Ecology and Evolution*, *7*(AUG). https://doi.org/10.3389/fevo.2019.00310

Nakagawa, S., Johnson, P. C. D., & Schielzeth, H. (2017). The coefficient of determination R Squared and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *J. R. Soc. Interface*, *14*(20170213).

Olsen, K. M., Hsu, S. C., & Small, L. L. (2008). Evidence on the molecular basis of the Ac/ac adaptive cyanogenesis polymorphism in white clover (Trifolium repens L.). *Genetics*, *179*(1), 517–526. https://doi.org/10.1534/genetics.107.080366

Olsen, K. M., & Small, L. L. (2018). Micro- and macroevolutionary adaptation through repeated loss of a complete metabolic pathway. *New Phytologist*, *219*(2), 757–766. https://doi.org/10.1111/nph.15184

Olsen, K. M., Sutherland, B. L., & Small, L. L. (2007). Molecular evolution of the Li/li chemical defence polymorphism in white clover (Trifolium repens L.). *Molecular Ecology*, *16*(19), 4180–4193. https://doi.org/10.1111/j.1365-294X.2007.03506.x

Olsen, K. M., & Ungerer, M. C. (2008). Freezing tolerance and cyanogenesis in white clover (Trifolium repens L. Fabaceae). *International Journal of Plant Sciences*, *169*(9), 1141–1147. https://doi.org/10.1086/591984

Pickett, S. T. A., Cadenasso, M. L., Childers, D. L., Mcdonnell, M. J., & Zhou, W. (2016). Evolution and future of urban ecological science: ecology in, of, and for the city. *Ecosystem Health and Sustainability*, *2*(7). https://doi.org/10.1002/ehs2.1229

Poulton, J. E. (1990). Cyanogenesis in Plants. *Plant Physiol*, *94*, 401–405. https://academic.oup.com/plphys/article/94/2/401/6086196

Ramalho, C. E., & Hobbs, R. J. (2012). Time for a change: Dynamic urban ecology. *Trends in Ecology and Evolution*, *27*(3), 179–188. https://doi.org/10.1016/j.tree.2011.10.008

Rand, T. A., Tylianakis, J. M., & Tscharntke, T. (2006). Spillover edge effects: The dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. *Ecology Letters*, *9*(5), 603–614. https://doi.org/10.1111/j.1461-0248.2006.00911.x

Robitu, M., Musy, M., Inard, C., & Groleau, D. (2006). Modeling the influence of vegetation and water pond on urban microclimate. *Solar Energy*, *80*(4), 435–447. https://doi.org/10.1016/j.solener.2005.06.015

Salingaros, N. A. (2006). Compact City Replaces Sprawl. In A. Graafland & L. Kavanaugh (Eds.), *Crossover: Architecture, Urbanism, Technology* (pp. 100–115). 010 Publishers.

Santangelo, J. S., Johnson, M. T. J., & Ness, R. W. (2018). Modern spandrels: The roles of genetic drift, gene flow and natural selection in the evolution of parallel clines. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1878). https://doi.org/10.1098/rspb.2018.0230

Santangelo, J. S., Ness, R. W., Cohan, B., Fitzpatrick, C. R., Innes, S. G., Koch, S., Miles, L. S., Munim, S., Peres-Neto, P. R., Prashad, C., Tong, A. T., Aguirre, W. E., Akinwole, P. O., Alberti, M., Álvarez, J., Anderson, J. T., Anderson, J. J., Ando, Y., Andrew, N. R., … Johnson, M. T. J. (2022). Global urban environmental change drives adaptation in white clover. *Science*, *375*, 1275–1281. https://www.science.org

Santangelo, J. S., Rivkin, R. L., Advenard, C., & Thompson, K. A. (2020). Multivariate phenotypic divergence along an urbanization gradient. *Biology Letters*, *16*(9). https://doi.org/10.1098/rsbl.2020.0511

Santangelo, J. S., Roux, C., & Johnson, M. T. J. (2022). The effects of environmental heterogeneity within a city on the evolution of clines. *Journal of Ecology*, *110*(12), 2950–2959. https://doi.org/10.1111/1365-2745.14000

Santangelo, J. S., Thompson, K. A., Cohan, B., Syed, J., Ness, R. W., & Johnson, M. T. J. (2020). Predicting the strength of urban-rural clines in a Mendelian polymorphism along a latitudinal gradient. *Evolution Letters*, *4*(3), 212–225. https://doi.org/10.1002/evl3.163

Sarzynski, A., Galster, G., & Stack, L. (2014). Typologies of sprawl: Investigating United States metropolitan land use patterns. *Urban Geography*, *35*(1), 48–70. https://doi.org/10.1080/02723638.2013.826468

Scheer, B. C. (2001). The Anatomy of Sprawl. *Places*, *14*(2), 28–37.

Swan, C. M., Brown, B., Borowy, D., Cavender-Bares, J., Jeliazkov, A., Knapp, S., Lososová, Z., Padullés Cubino, J., Pavoine, S., Ricotta, C., & Sol, D. (2021). A framework for understanding how biodiversity patterns unfold across multiple spatial scales in urban ecosystems. *Ecosphere*, *12*(7), Article e03650. https://doi.org/10.1002/ecs2.3650

Thompson, K. A., Renaudin, M., & Johnson, M. T. J. (2016). Urbanization drives the evolution of parallel clines in plant populations. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1845). https://doi.org/10.1098/rspb.2016.2180

Veres, A., Petit, S., Conord, C., & Lavigne, C. (2013). Does landscape composition affect pest abundance and their control by natural enemies? A review. *Agriculture, Ecosystems and Environment*, *166*, 110–117. https://doi.org/10.1016/j.agee.2011.05.027

Woltz, J. M., Isaacs, R., & Landis, D. A. (2012). Landscape structure and habitat management differentially influence insect natural enemies in an agricultural landscape. *Agriculture, Ecosystems and Environment*, *152*, 40–49. https://doi.org/10.1016/j.agee.2012.02.008

Wu, J., Jenerette, G. D., Buyantuyev, A., & Redman, C. L. (2011). Quantifying spatiotemporal patterns of urbanization: The case of the two fastest growing metropolitan regions in the United States. *Ecological Complexity*, *8*(1), 1–8. https://doi.org/10.1016/j.ecocom.2010.03.002

Youngsteadt, E., Terando, A., Costanza, J., & Vukomanovic, J. (2023). Compact or Sprawling Cities: Has the Sparing-Sharing Framework Yielded an Ecological Verdict? *Current Landscape Ecology Reports*, *8*(1), 11–22. https://doi.org/10.1007/s40823-022-00081-8

Zar, J. H. (2010). *Biostatistical Analysis* (5th ed.). Prentice Hall.