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Evolution in Cities

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Abstract

Although research performed in cities will not uncover new evolutionary mechanisms, it could provide unprecedented opportunities to examine the interplay of evolutionary forces in new ways and new avenues to address classic questions. However, while the variation within and among cities affords many opportunities to advance evolutionary biology research, careful alignment between how cities are used and the research questions being asked is necessary to maximize the insights that can be gained. In this review, we develop a framework to help guide alignment between urban evolution research approaches and questions. Using this framework, we highlight what has been accomplished to date in the field of urban evolution and identify several up-and-coming research directions for further expansion. We conclude that urban environments can be used as evolutionary test beds to tackle both new and long-standing questions in evolutionary biology.

INTRODUCTION

Cities have existed in some form for thousands of years. The development of evolutionary theory and the modern synthesis occurred more recently, within the last couple of centuries, paving the way for the study of evolution in cities (Johnson & Munshi-South 2017). Even so, while early explorations of urbanization-adjacent effects of industrial pollution on the evolution of peppered moth coloration foreshadowed the field of urban evolutionary research (Kettlewell 1955), it is only within the last decade that it has really taken off. The paradigm shift from contemporary evolution as rare to commonplace (Reznick et al. 2019) potentially bolstered the exploration of urban evolution. And it seems likely that urban evolutionary biologists have benefited greatly from the dedicated work of urban ecologists to elevate urban habitats from an artificial environment to be avoided for research into a legitimate realm of study (reviewed in Rivkin et al. 2019).

Regardless, in this very short time window, the field of urban evolution has expanded on a number of different fronts from fundamental questions of evolutionary ecology (Rivkin et al. 2019) to applied research questions (Lambert & Donihue 2020) and, most recently, to the interplay of these questions with societal dimensions (Des Roches et al. 2021). While this expansion does not entail the discovery of new evolutionary forces, it has provided a wealth of data on how the evolutionary mechanisms of natural selection, gene flow, mutation, and genetic drift operate and interact in the novel environmental landscapes of cities. To this end, there are already several excellent reviews and syntheses of the field of urban evolution (Alberti et al. 2015, Des Roches et al. 2021, Johnson & Munshi-South 2017, Donihue & Lambert 2015, Lambert et al. 2020, Miles et al. 2019, Rivkin et al. 2019, Schell 2018, Schell et al. 2020, Szulkin et al. 2020b). Each of these works has contributed important, unique information to the field, and we do not wish to duplicate these efforts. Emerging from these works, however, is a dialogue about the extent to which cities can be used to isolate specific urban drivers and evolutionary consequences versus broader examinations of pan-urban effects on evolution (e.g., for discussions of the different viewpoints, see Szulkin et al. 2020b). In the first part of our review, we seek to address these exchanges by developing a framework that considers the different types of evolutionary research questions that can be explored in cities and aligns these questions with specific approaches for how cities can be used.

A FRAMEWORK FOR HOW CITIES CAN BE USED IN EVOLUTIONARY RESEARCH

As evolution simply involves heritable changes in populations of organisms across generations, it is not difficult to imagine the great diversity of evolutionary research that can be performed in cities. Generally, such research questions can be divided into three major research axes: investigating mechanisms (selection, gene flow, mutation, and drift), answering basic versus applied research questions, and using cities to isolate particular variables (e.g., heat, aridity) versus examining pan-urban effects. In some cases, the distinctions between categories are overlapping. For example, using cities as climate-change proxies to explore how thermally sensitive traits evolve in response to warming can serve both basic and applied research goals by uncovering how thermal performance traits evolve over contemporary timescales while also estimating the potential for species to adapt to global change (Diamond & Martin 2021b).

The multifarious nature of changes within and between cities likewise contributes to the diversity of evolutionary questions that can be addressed. While the high dimensionality of urban changes might superficially appear to render cities difficult environments for evolutionary research, in fact, this variation can be harnessed to serve many research agendas. Field biologists have long contended with environmental variation. However, form follows function here, and the way in which this environmental variation is harnessed follows the question being asked (Gillespie et al. 2017).

Urban environments, with their attendant variation, are simply another ecotype or biome in this regard (Niemelä 1999). Cities can be used to isolate specific drivers; for example, urban heat island effects can be examined by selecting sites where variables other than surrounding imperviousness are similar. Or cities can be used to explore evolution in response to multiple stressors; for example, responses to heat island effects and pollutants can be examined by selecting sites with elevated imperviousness and proximity to chemical runoff. Cities can also be used to study unmeasured pan-urban effects on organisms. Importantly, none of these approaches are mutually exclusive: Single-stressor studies can build to multi-stressor research, and pan-urban effects can be refined to tease apart major drivers (e.g., Gorton et al. 2018). Of course, it is important to recognize the crucial role of site selection, and recent work reinforces how improper site selection can lead to incorrect interpretations of shifts in biodiversity under global change (Mentges et al. 2021). Similarly, evolutionary biologists working on patterns of phenotypic selection in the wild are well acquainted with the possibility of environmental covariance leading to incorrect interpretations of patterns of selection (Mitchell-Olds & Shaw 1987). While cities do not solve the site-selection problem, they also do not necessarily exacerbate it by default. As ever, biologists need to be vigilant regarding the natural history of their system; key environmental axes of variation; and their interactions, i.e., how organisms experience urban environments (Shultz et al. 2021). **Figure 1** summarizes these considerations and lays out a framework for guiding alignment between study designs that focus on different attributes of cities, from pan-urban effects to specific factors, and the types of inferences that can be made from each approach (see also **Supplemental Table 1**).

Supplemental Material >

WHAT HAPPENS TO ORGANISMS AS THEY EXPERIENCE THE URBAN ENVIRONMENT?

Evolutionary trajectories of city-dwelling species are likely to depend on properties of the species themselves and the specific nature of how species interact with the urban environment. For example, consider the following questions regarding species evolutionary history and interaction with urban agents that might drive evolutionary change. How did organisms come to be in the city—were they resident within the city footprint during the urbanization process, or were they excluded from the urban environment during development and have since recolonized? What was the temporal nature of urban development over these periods—rapid or slow? How do organisms experience the spatial variation associated with urbanization—as a very steep transition or more gradual compared with a typical undeveloped habitat? Do species have continuous tracts of suitable habitat as they move into or within the city footprint, or are they fragmented, potentially across meta-populations? If species were initially excluded and attempted to recolonize (or in the case of range-shifting species, attempted an initial colonization), were all species equally successful? Or does the urban environment now harbor a new mixture of species and their attendant traits, with potential downstream effects on evolutionary change? Did some urban species have no previous association with the particular geographic area? Were they instead introduced into the city? Do some species use urban environments opportunistically while being resident elsewhere or vice versa? Each of these considerations has the potential to alter evolutionary trajectories. Evolutionary biologists have recently called for greater focus on these sorts of questions, with more attention given to species' natural history, evolutionary history, and microenvironmental variation when studying evolution in the wild (Travis 2020). Studying evolution in urban contexts brings these considerations into sharp focus given the lack of deep-time historical antecedents for urban environments and the recency of the urbanization process in many locations (Güneralp et al. 2020).

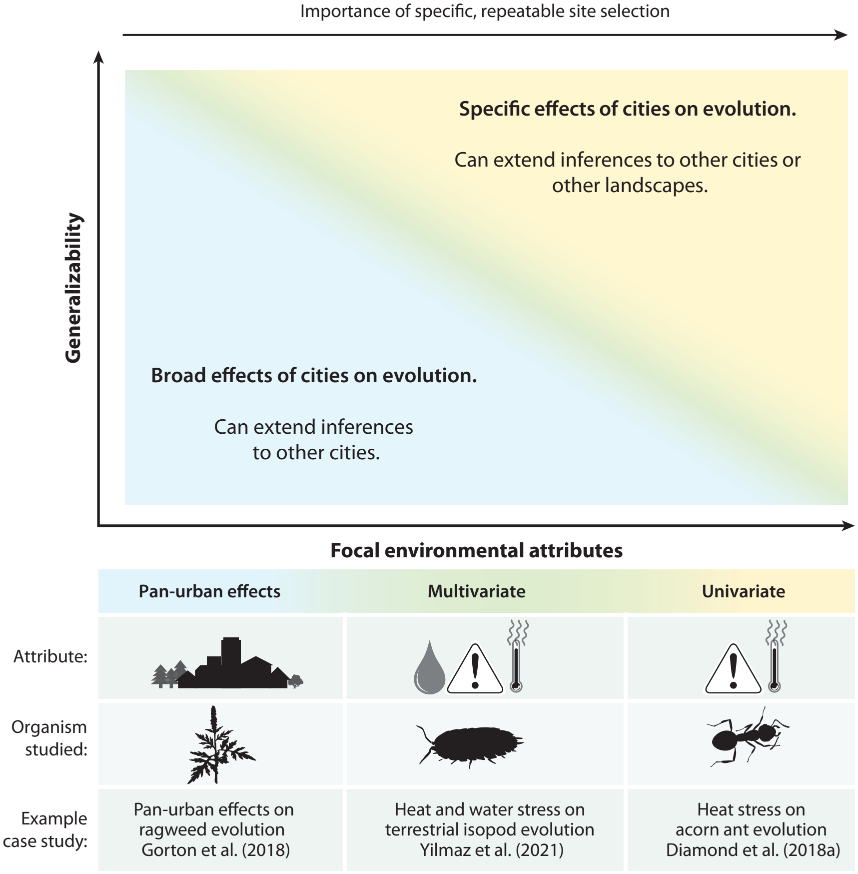

Figure 1

Graphical representation of the different ways cities can be used to address evolutionary questions. As the specificity of the particular sites selected for study increases, cities can be used to isolate individual variables, for example, heat and aridity. With this design, the results can be generalized to other cities and other landscapes. By contrast, pan-urban studies capture a broad range of multifarious urban changes. Results from these types of studies can be used to make inferences regarding evolution in response to multifarious changes across other cities. As exemplars (from left to right on the x-axis), Gorton et al. (2018) examine pan-urban effects on ragweed evolution across diverse urban habitats, Yilmaz et al. (2021) examine heat and water stress on terrestrial isopod evolution at particular urban sites, and Diamond et al. (2018a) examine heat stress on acorn ant evolution at particular urban sites.

The question of what qualifies as an urban environment or effect has been hotly contested for decades (reviewed in Niemelä et al. 2011). We embrace a broad definition of urban. This definition includes everything from suburban development to heavily industrialized areas. We further consider peri-urban effects, i.e., areas adjacent to the city footprint, as well as urban-induced effects that act at a distance beyond the city footprint. We recognize that this definition potentially includes small settlements, megacities, and even nonurban areas that are nonetheless affected by urbanization within the general category of urban. However, as long as authors provide appropriate georeferencing and relevant environmental variable data (e.g., land-use classification, impervious surface area, temperature, humidity, heavy metal concentrations), we see no need to argue the definition of urban, as it depends on the particular study system. The field is moving in this direction, and recent work has outlined a framework for quantifying urban environmental variables for addressing evolutionary questions (Szulkin et al. 2020a). One item we wish to highlight in this context is the degree to which peri-urban effects and urban evolution studies in modestly urbanized habitats might not self-report as urban evolution studies. For example, consider the work of Weldon (1899) who examined selection on green crab carapace size in response to rapid pollution of the waters of Plymouth Sound; the classic peppered moth work of Kettlewell (1955, 1956), who examined the evolution of light and dark color morph frequencies as a consequence of industrial pollution effects on the visibility of moths to predators; and recent work on the evolution of the metabolic rate of fish in response to elevated water temperature warmed by runoff from nearby geothermal heating for homes (Pilakouta et al. 2020). None of these studies were billed as urban evolution, although they could plausibly be considered as such. This becomes especially important for quantifying the overall frequency and strength of urban evolution, as it potentially leads to underrepresentation of studies of peri-urban or modest urban changes.

In addition to these higher-level considerations regarding the tempo and mode of urbanization over space and time, it is also important to consider the particular agents that might drive evolutionary change in cities. Urban environments are often warmer, drier, and more polluted in terms of sound, light, and chemical toxicants, with altered hydrology due to impervious surfaces and soil compaction; altered food resources, including supplementation with human food and shifts in typical prey items; and a suite of other changes (Szulkin et al. 2020a). There are of course exceptions to these broad patterns, such as localized remediation of dryness via irrigation and inverted urban temperature effects leading to urban cool islands in desert cities (Imhoff et al. 2010). Structural changes imposed by buildings and other types of development can cause substantial habitat fragmentation in addition to serving as agents of selection (Winchell et al. 2016, 2018). Evolutionary responses then unfold from these changes with high potential for interaction among evolutionary mechanisms. For example, cities might filter individuals of a particular species, leading to shifts in the genetic composition of populations, or entire species might be excluded based on trait characteristics, leading to shifts in biotic interactions and selection landscapes (Aronson et al. 2016). Under habitat fragmentation, population sizes might be reduced, leading to a more prominent role for genetic drift and limits on adaptive evolution due to limited additive genetic variance. Such limits could be counterbalanced by potentially elevated mutation rates (e.g., Somers et al. 2002), although deleterious mutations could be problematic in this regard, especially in small populations.

DETECTION AND ATTRIBUTION OF URBAN EVOLUTION

The question then concerns how signatures of urban evolution can be assessed, classified, and attributed to particular forces and mechanisms. Again, there are no urban-specific tools here, but rather, standard tools of the trade for evolutionary biologists are applied in urban contexts. Indeed, the novelty of these approaches stems from the specifics of sampling urban environments and the particular comparisons being drawn (**Figure 1**, **Supplemental Table 1**). Overwhelmingly, urban evolution studies focus on neutral processes, using molecular tools to examine population genetic structure and make inferences regarding how gene flow and genetic drift operate in urban settings (**Figure 2**). Often, these studies examine pan-urban effects on population structure, although occasionally specific urban-associated features can be disentangled (e.g., Beninde et al. 2016, Munshi-South et al. 2016). By contrast, there are only a handful of studies focused on the effects of urbanization on mutation. In mice and European herring gulls, mutation rates were higher in heavily industrialized areas of cities (Somers et al. 2002, Yauk & Quinn 1996). Similarly, bank voles exhibited higher mutation rates in radiation-contaminated areas of Chernobyl, a (dramatic)

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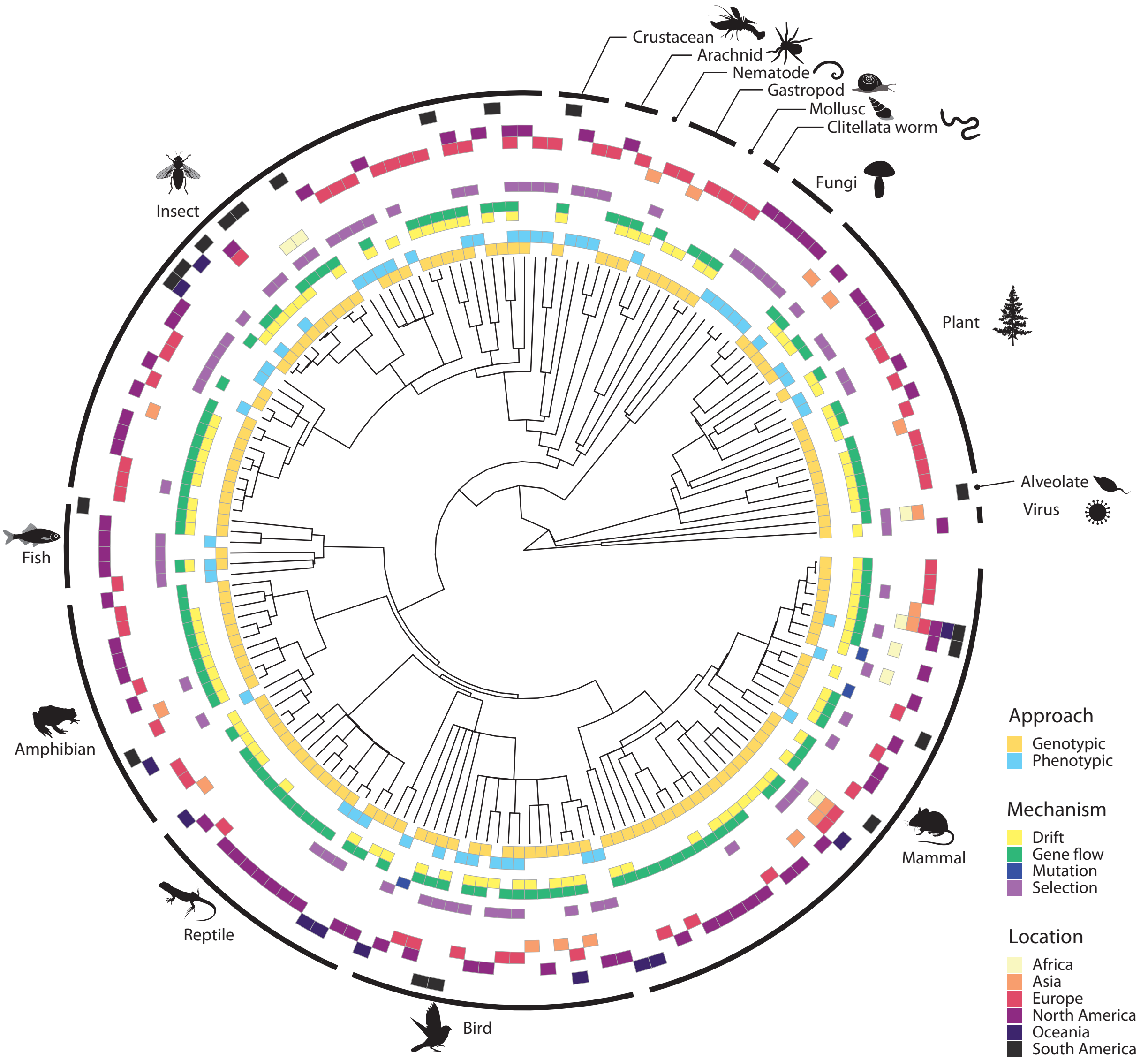

Figure 2

Evolutionary relationships based on the NCBI taxonomy among the species that have been studied for urban evolution. Color-coding of the blocks corresponds with three main categories: approach (genotypic or phenotypic), mechanism (drift, gene flow, mutation, or selection), and location (Africa, Asia, Europe, North America, Oceania, or South America). Species can take on multiple attributes within each category. Results from multiple studies for a given species are combined. A total of 169 species are summarized in the plot. Our study selection criteria included the studies reviewed in Johnson & Munshi-South (2017) as well as a forward search from studies that cited Alberti (2015), Donihue & Lambert (2015), Johnson & Munshi-South (2017), Miles et al. (2019), and Rivkin et al. (2019). We also supplemented this list with studies that predate the Johnson & Munshi-South (2017) review and studies from Szulkin et al. (2020b) that did not cite the above-listed reviews and synthesis articles. We follow the methods of Johnson & Munshi-South (2017) and only report studies with positive evidence for at least one mechanism of urban evolution. The data summarized in this figure can be found in **Supplemental Table 4**. Abbreviation: NCBI, National Center for Biotechnology Information.

Supplemental Material >

urbanization-induced change, with the effects extending beyond the city footprint, resulting in an adjacent effect rather than an intracity effect (Baker et al. 2017).

Related to the studies on urban-driven shifts in mutation rate, there have also been a small number of studies focused on shifts in methylation patterns in cities (McNew et al. 2017, Riyahi et al. 2015, Thorson et al. 2019, Watson et al. 2021). Although we still have much to learn about the environmental induction, heritability, and functional significance of epigenetic variation, it is potentially an important source of variation for adaptive evolution (Verhoeven et al. 2016). In this light, evidence of divergence between urban and rural populations in DNA methylation across multiple species is intriguing and opens an exciting area for future research.

Phenotypic and molecular studies of adaptive urban evolution are perhaps the most rapidly growing area of the field (Lambert et al. 2020). Assuming organisms are reasonably well adapted to their ancestral environments, populations are likely to be displaced from this fitness peak when confronted with novel urban environments. Whether and how populations are able to recover from this urban-associated maladaptation is thus a central question (Diamond & Martin 2020a). The potential for compensatory adaptive evolution to allow populations to track a new fitness optimum is thus highly relevant for exploring the potential of species to remain within city footprints and to respond to ongoing changes in cities and globally, e.g., under global climate change.

In practice, phenotypic and molecular approaches likely represent different starting points for the assessment of adaptation. Molecular signatures of adaptation can be assessed using genomic tools to determine whether particular loci are under selection and to make inferences regarding the particular urban stressors that might be acting (e.g., if genes involved in chemical detoxification are under selection). In addition, such studies can provide information on nonadaptive evolutionary forces including gene flow and drift (Perrier et al. 2020) (**Supplemental Table 2**). Molecular evolution studies provide insights into a broad range of urban changes and their effects on adaptive evolution (Schell 2018). As a sample, we consider the molecular evolution studies published since the comprehensive urban evolution review by Johnson & Munshi-South (2017). These studies have documented urban–rural evolutionary divergence and have identified loci under selection relating to heat (Campbell-Staton et al. 2020), pollution (Andrew et al. 2019), metabolism (Harris & Munshi-South 2017, Konorov 2018, Ravinet et al. 2018), immunity (DeCandia et al. 2019a, DeCandia et al. 2019b), and cognition (Mueller et al. 2020) in urban populations. Furthermore, a study on viral evolution revealed an elevated nucleotide substitution rate, purifying selection, and recent recombination in urban environments (Su et al. 2019). In some cases, evolutionary divergence was evident among highly structured populations with little evidence of gene flow between urban and rural populations (e.g., Homola et al. 2019, Perrier et al. 2018). In others, there was evidence of divergence despite the presence of gene flow (e.g., Khimoun et al. 2020, Theodorou et al. 2018). A number of studies pointed to the highly polygenic nature of urban adaptation. For example, in the great tit, gene–environment association tests revealed a high number of single nucleotide polymorphisms distributed across the genome with small association scores for urbanization, consistent with a polygenic response to urbanization (Perrier et al. 2018).

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Phenotypic evidence of evolution can be found through common garden experiments, particularly those that rear urban and nonurban organisms for one or multiple generations under a range of different urban-relevant treatments and assess key traits and fitness. This experimental design allows for the assessment of genetic changes between populations and any fitness advantages conferred by those trait changes (e.g., Brans et al. 2017, Martin et al. 2019, Santangelo 2020b). Quantitative genetic breeding designs can likewise uncover the potential for evolutionary divergence between urban and nonurban populations, with attribution to additive genetic variance and other sources of variance, such as maternal effects, under some designs. As above, such trait data need to be linked to fitness to evaluate the evidence supporting such changes being adaptive.

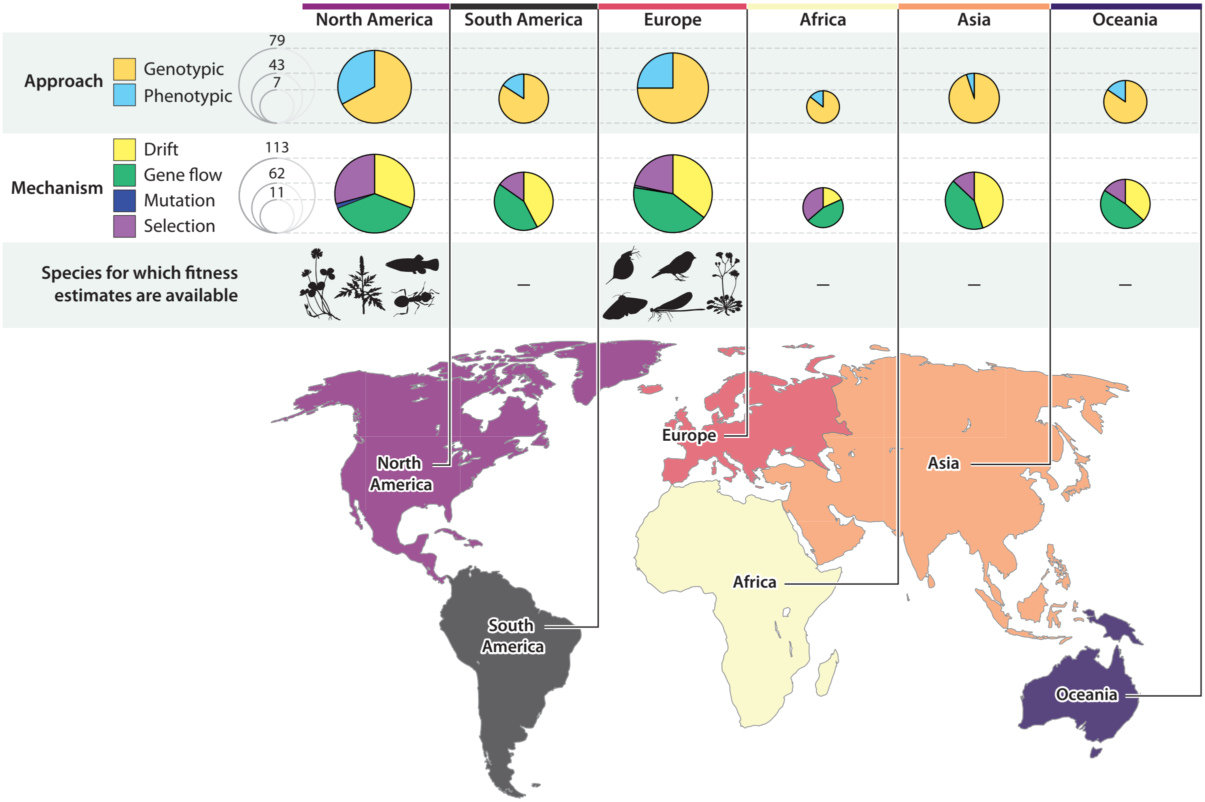

Figure 3

Geographic summary of the studies that explore one or more of the four evolutionary mechanisms and use phenotypic and/or genotypic approaches. The sections of the pie charts represent the proportion of studies that addresses each mechanism or approach for a given continent. The overall size of each pie chart corresponds to the total number of individual records available for each continent (note that the scale key is on a natural-log scale). Across all continents, 177 combinations of species and geographic location were summarized (some of the 169 species summarized in **Figure 2** had responses across multiple continents); for a given species-location combination, there can be multiple records for either (or both) the evolutionary mechanism being examined or the approach used, e.g., a study that examines selection, gene flow, and genetic drift using phenotypic and genotypic approaches. Organism silhouettes indicate species for which fitness estimates across urban and rural environments were obtained (e.g., to test for local adaptation). Dashes indicate continents for which there are no species with fitness estimates available (see **Supplemental Table 3** for study details).

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Reciprocal transplant experiments performed in the field are the most direct way of assessing local adaptation to cities; however, these experiments are still rare in urban settings (Lambert et al. 2020). Indeed, there are only nine study systems that have examined potential local adaptation to cities [higher fitness of urban and rural populations in their home versus away environment and/or that of local versus foreign populations within a given environment sensu Kawecki & Ebert (2004)] using measurements of total fitness or fitness proxies (e.g., survival, fecundity) (**Figure 3**, **Supplemental Table 3**). Further, several of these studies were performed in the laboratory rather than field settings. While the majority of these studies show clear support for local adaptation, including in acorn ants (Diamond et al. 2018a, Martin et al. 2021), holy hawksbeard plants (Cheptou et al. 2008), killifish (Reid et al. 2016), peppered moths (Kettlewell 1955, 1956), and water fleas (Brans & De Meester 2018, Brans et al. 2017), others find no evidence for local adaptation (Capilla-Lasheras et al. 2017). Still others paint a more complex picture of local adaptation to cities. For example, a reciprocal transplant study of ragweed found that rural population plants had higher fitness in all environments, which is inconsistent with local adaptation. However, countergradient variation in flowering phenology and stronger net selection in the away transplant treatment (either rural-origin transplanted to urban environments or vice versa) compared with the home treatment (rural-origin transplanted to the rural environment or urban-origin transplanted to the urban environment) nonetheless suggested the presence of local adaptation (Gorton et al. 2018). As another multifaceted example, in white clover, the formation of urban cool islands during the winter (via reduced snow cover and exposure to cooler air temperatures) may select for decreased cyanogenesis—while high levels provide defense against herbivores, cyanogenesis carries a cost of making plants more susceptible to cold stress. Equal numbers of cyanogenic and acyanogenic genotypes were transplanted into urban and rural sites during summer to rule out differential herbivory as an alternative explanation for urban temperature-driven patterns of decreased cyanogenesis. No evidence of selection for reduced cyanogenesis in urban habitats was detected via seed set (i.e., the relative fitness of cyanogenic and acyanogenic genotypes was comparable across urban and rural sites), and selection via plant biomass actually indicated the opposite pattern of selection for increased cyanogenesis of plants in the urban habitat during the growing season (Thompson 2016). Cyanogenic genotypes did receive less herbivory damage across all sites; however, the amount of herbivory was not associated with urbanization. While these results suggest that differential herbivory does not explain patterns of reduced cyanogenesis of urban plants, fitness would need to be measured over winter to directly test whether urban cool islands select for reduced cyanogenesis. It is also useful to point out that there can be evidence of urban adaptation without evidence of local adaptation. For example, a study of urban damselflies found that urban populations have higher survival at all laboratory rearing temperatures, demonstrating urban adaptation but suggesting a lack of local adaptation (Tüzün et al. 2017a).

Mendelian traits (those under the control of a few loci with dominant and recessive alleles) can be used to assess adaptive genetic changes in populations in response to urbanization. For example, evolutionary divergence (changes in allele frequency) has been observed in the Mendelian traits of cyanogenesis in white clover and coloration in snail shells (Johnson et al. 2018, Kerstes et al. 2019). Repeated shifts in allele frequency associated with these traits and loci that are putatively under selection relative to neutral loci can be used to make adaptive inferences. Such results can be followed up with whole-organism manipulations such as common garden experiments or reciprocal transplant studies to examine the fitness consequences and further evaluate the adaptive nature of such shifts in Mendelian traits. Similarly, measures of quantitative genetic differentiation among populations from common garden experiments relative to differentiation at neutral loci, i.e., $Q_{\mathrm{ST}}/F_{\mathrm{ST}}$ ratios, can also be used to make comparable inferences regarding adaptive evolution (Whitlock 2008).

We have concerned ourselves here and in the remainder of this review with microevolutionary responses to urbanization. At broader scales, evolutionary inferences can be made using phylogenetic comparative techniques (e.g., Winchell et al. 2020). Their application might point to relative differences in the rate and magnitude of evolutionary change of urbanization-relevant traits or the potential importance of biotic interactions and niche conservatism. However, in the context of our review, while these analyses can provide critical groundwork for laying out hypotheses regarding the forces that shape urban evolution, they would need to be followed up with experimental work to uncover the underlying mechanisms. Indeed, a running theme of our review is that components of individual systems can be pieced together to understand a broader view of evolution in cities. This integration can take many forms, including uniting molecular and phenotypic approaches and applying well-established frameworks such as the morphology-performance-fitness framework (Arnold 1983) to understand the agents of selection and evolutionary responses in cities.

For example, populations of creek chub inhabiting urban streams have evolved stiffer bodies, allowing for more energy efficient swimming, potentially as an adaptation to increased water velocities caused by stormwater runoff (Kern & Langerhans 2019; for another example integrating morphology and locomotor performance, see Winchell et al. 2018).

CITIES CAN BE USED TO ADDRESS OUTSTANDING QUESTIONS IN EVOLUTIONARY BIOLOGY

Studying urban evolution in cities can inform the degree to which organisms might cope with urbanization effects and thus inform urban conservation and management plans (Lambert & Donihue 2020, Shaffer 2018). This body of research has also considered interactions between human societal dimensions and urban evolution of nonhuman organisms (Des Roches et al. 2021, Schell et al. 2020). Because applied urban evolution questions and societal feedbacks have been treated in-depth elsewhere, we use the rest of this review to explore the use of cities to address more basic research questions in evolutionary biology. In this section, we provide a broad overview of evolutionary research questions that could benefit from research in urban environments, as well as research questions that might be possible to answer only in cities. While this is not a comprehensive set of questions, it ideally highlights some ways in which urban evolutionary research might be extended in new directions.

Time Course and Contingency of Evolution

Although human settlements vary considerably in the length of time since their initial construction and their rate of expansion, the trend of accelerating conversion of natural to urbanized land means that there are many opportunities to explore relatively recent exposure to urban effects (Niemelä et al. 2011). Of course, the timescales must all be interpreted relative to the generation time of individual species. For many organisms exposed to urbanization changes over the last century or so, this means they have been exposed to the urbanization process for dozens to hundreds of generations. This temporal variation provides an opportunity to examine the forces and constraints acting on organisms over time as they experience urban effects.

For example, in many organisms, genetic correlations between key performance and life-history traits, such as body size, growth rate, and development time, change significantly in magnitude, or even sign, when exposed to novel environmental conditions, including the types of novelty seen in cities, such as exposure to pollutants, changes in thermal regime, or changes in food resources (Sgrò & Hoffmann 2004). The strong trade-offs (negative genetic correlations, e.g., large adult body size at the expense of slower juvenile growth rate) characteristic of well-adapted populations in their typical environments tend to be weakened or become positive in novel environments (Service & Rose 1985). Over time, selection may refine phenotypes in the new environment, with the return of trade-offs as adaptation unfolds (Sgrò & Hoffmann 2004).

Yet neither environment-dependent expression of genetic correlations nor the time course of these changes as organisms experience novel environments are well established for many types of environmental change, including urbanization (Wood & Brodie 2015). Because negative genetic correlations are generally thought to constrain evolution, the effects of environmental change, such as urbanization, on genetic correlations are important to consider, as they may shape the rate, magnitude, and direction of evolutionary responses. Furthermore, novel environments such as cities might change not only the expression of genetic correlations but also the expression of genetic variance in the individual traits themselves and thus their evolutionary potential (i.e., trait heritability) (Sgrò & Hoffmann 2004). Cities could be leveraged to address these questions regarding the nature and time course of changes in trait genetic variances and genetic correlations in response to urbanization. In particular, the time course of urban evolution could be accomplished by examining populations across neighborhoods of different ages or even cities of different ages. While there have been calls to examine the effects of city age on urban evolutionary responses (Alberti 2015)—and there is considerable intra- and interspecific variation in urbanized habitats of different ages to work with (Szulkin et al. 2020b)—the field has not yet fully embraced this research direction.

A related time-scale question focuses on the potential costs of urban adaptation; i.e., does contemporary adaptive evolution to the city come at a cost to adaptation to the ancestral rural environment? These costs might be difficult or impossible to attribute to contemporary adaptation in long-diverged populations. Recent work suggests that costs of contemporary urban adaptation might be important. For example, in acorn ants, the adaptive evolution of higher heat tolerance and concomitant losses in cold tolerance have led to the loss of adaptation to the ancestral rural environment (Diamond et al. 2018a, Martin et al. 2021).

Because cities effectively harbor living collections of different time points across populations' evolutionary trajectories, they can be used to address questions regarding the contingency and reversibility of evolution. For example, reversion of once-urbanized areas to more natural states (regreening, as is common in the midwestern United States) could be used to explore this question (Turo & Gardiner 2020). Specifically, whether it is possible and how long it takes to revert to phenotypes comparable to non- or less urbanized phenotypes could be examined in this context. Intraurban variation in the age of neighborhoods and intercity variation in overall city age could be harnessed to further explore whether the length of time different areas have been exposed to the novel urban environment before reversal of the urban effects influences phenotypic reversion. Whether future evolution is contingent upon prior evolutionary change is a major open question in the field of evolutionary biology (Blount et al. 2018), and cities could be uniquely leveraged to address it.

Multifarious Environmental Changes and Trait Responses

Given the multifarious nature of changes to urban environments, including biophysical factors and biotic interactions, cities can be excellent venues for examining the evolutionary responses to different combinations of environmental novelty. In some cases, environments might be altered by degree rather than kind, for example, increased mean temperature (Diamond & Martin 2020b). Or they might represent truly novel conditions, for example, the presence of chemical pollutants. Where environments lie on the novelty spectrum is an important question in and of itself, as such distinctions can have important effects on trait evolution (Snell-Rood et al. 2018). Additionally, the combination of different novel environmental attributes and their impact on evolution is an important related question. For example, while cities are often hotter and drier than nearby undisturbed habitat, the patchwork mosaic of the urbanization process often yields microsites with differing environmental trait combinations (Ouyang et al. 2018). Thus, it is possible to have warm, dry urban sites but also warm, wet urban sites because of supplemental irrigation. This type of environmental variation would allow individual drivers of evolutionary change to be disentangled rather than simply observing the net effects of multiple environmental changes on evolution. While these types of landscape mosaic patterns are documented in cities (Stabler et al. 2005), they have not been used to address this particular question.

Especially in the case of adaptive urban evolution, a major goal is to tease apart specific drivers and traits under selection to uncover the nature of adaptive urban evolution. In some cases, such as for generalized stress-response pathways, evolution to one urban stressor, such as high temperature, could also lead to cross-tolerance to other stressors, such as aridity and pollutants

(Sinclair et al. 2013). Here, it may be difficult to tease apart which factor or factors are driving the evolutionary response. Likewise, negative relationships among traits can arise from resource allocation trade-offs across different urban stressors, and such trade-offs can modify evolutionary trajectories (Sgrò & Hoffmann 2004).

The strongest forward momentum on multifarious environment changes and evolutionary trait responses in cities comes from pace-of-life syndrome evolution. These studies routinely focus on integrated trait responses to multiple axes of environmental change (Brans et al. 2018, Charmantier et al. 2017, Sepp et al. 2018). For example, Brans and colleagues (2018) found evidence of evolutionary divergence in pace-of-life syndromes of water fleas in response to urbanization. Whereas urban populations had highly structured syndromes across multiple stress physiology, energy metabolism, and life-history traits to cope with multifarious urban stressors (e.g., heat, pollution), rural populations exhibited no such structuring. Specifically, urban populations evolved higher energy reserves and more efficient stress coping mechanisms that, in turn, contributed to the evolution of a faster pace of life in cities.

Repeatability of Evolution

We include both parallel and convergent evolution under the umbrella concept of the repeatability of evolution in response to shared selection pressures across different cities, acknowledging that while these are related concepts, they are not necessarily the same (Bolnick et al. 2018, Losos 2011). The meanings of these terms and their distinctions have changed over time and between disciplines, with some definitions placing an emphasis on phylogenetic relatedness and others on the underlying developmental pathways or genetic loci producing similar traits. However, because closely related species can evolve similar phenotypes using different genes and distantly related species from shared ancestral genes, neither of these definitions are unambiguous (Bolnick et al. 2018). For our purposes, parallel evolution refers to the same species or very closely related lineages that evolve similarly across multiple urbanized landscapes. In the case of convergent evolution, we refer to different species, not closely related, that evolve similarly. Whether different populations evolve similarly in response to shared selection pressures is an area of intense study in evolutionary biology as it speaks directly to the predictability of evolution. Likely owing to the centrality of this question, there have already been a number of urban evolution studies focused on parallel evolution (summarized in Santangelo et al. 2020a). These studies have been conducted at both the phenotypic and genotypic levels, though few studies test for parallelism at both levels within the same system (but see Reid et al. 2016). More tests are needed, particularly within the same study system, to eliminate interspecific confounding variables and evaluate hypotheses regarding the prevalence of parallel evolution at different levels of biological organization. The expectation is that parallelism decreases from phenotypic to genotypic levels of organization, owing to the relatively higher number of molecular pathways that are able to achieve comparable phenotypes (Losos 2011).

As a first synthesis of the overall evidence for parallel evolution (that is, at the phenotypic and genotypic levels), a recent vote-counting meta-analysis found that 14 out of 18 species showed positive evidence for parallel evolution in cities (Santangelo et al. 2020a). Further, a surprisingly large percentage of species (44%) showed evidence of parallelism across all cities tested. Although the number of species for which parallel evolution was tested is still relatively low ($n = 18$), the taxonomic breadth was large, encompassing arthropods, birds, fishes, mammals, plants, and reptiles. Likewise, all four evolutionary mechanisms (gene flow, drift, mutation, and selection) were tested, though the mechanism biases we highlighted earlier still remain (**Figure 3**), with most studies focusing on gene flow and drift, fewer on selection, and only three on mutation. As the evidentiary basis for parallel evolution grows, potentially important moderators can be examined in a quantitative fashion, including (*a*) genotypic versus phenotypic data; (*b*) generation time and time since urbanization of the city and/or site as proxies of the length of time populations had to evolve to urbanization; (*c*) dispersal ability as a proxy for gene flow to oppose and potentially overwhelm adaptive evolution; (*d*) degree of habitat fragmentation within the city as a measure of relative differences in the potential to alter gene flow and drift; (*e*) whether species are native, introduced, or human commensals, as the provenance of species might predictably alter the nature of their repeated evolutionary responses, e.g., a single introduction that spread versus repeated introductions of independent populations and lineages; (*f*) the type of trait being measured, e.g., physiological traits, which are potentially more repeatable compared with behavioral traits, which generally show greater variance; and (*g*) specificity of site selection, with the isolation of specific urban factors being more likely to show evidence of repeatability than focusing on pan-urban effects. Such broadscale tests of the repeatability of evolution might give more variable results when looking at pan-urban effects, owing to the specific changes associated with individual cities (e.g., cities with a high level of exotic species versus more intact native communities or cities with high levels of chemical pollution versus less polluted cities), which would ideally be controlled for in studies where specific urban factors were isolated. This could be a powerful way to test for repeatability because it makes a testable hypothesis about the multifarious nature of urban changes and the strength of parallel evolution.

Owing to the fact that many tests of the repeatability of evolution are tests of the same species across different cities, rather than tests of the same gene or trait evolutionary response to urbanization across multiple species, there is a limited number of tests of urban convergent evolution. Using whole-organism physiological trait phenotypes across a range of terrestrial and aquatic ectothermic species, Diamond & Martin (2021b) found evidence of convergent evolution of gains in heat-tolerance traits and losses of cold-tolerance traits in response to urban heat islands. Likewise, using genomic approaches, Homola et al. (2019) found evidence of convergent evolution between wood frog and American bullfrog responses to urbanization. Further, Ravinet et al. (2018) did something similar in very distantly related human commensals, finding comparable shifts among house sparrows and domesticated dogs in genes associated with craniofacial and skull development genes and starch digestion.

Sexual Selection

Despite the strong potential for the development, transmission, and reception of signals involved in animal communication to be altered by urbanization, there are relatively few studies that have addressed the evolutionary consequences of cities for sexually selected traits. The majority of studies have focused on the consequences of urbanization for signal transmission (Sepp et al. 2020). From these studies, we have many examples of signal divergence between urban and rural environments: Urban dark-eyed junco populations have less white in their tail, a sexually selected trait, than rural populations (Yeh 2004); males of many bird species alter their songs in habitats with more noise pollution (e.g., Luther et al. 2016); and light pollution leads to reduced and altered pheromones in moths (Van Geffen et al. 2015). Yet, for the large majority of these cases, it is unknown whether they represent plastic or evolved responses to urbanization and whether these changes are adaptive in the urban environment (Sepp et al. 2020). Notably, a handful of recent studies have measured the fitness consequences of altered male signals in urban environments. Urban male túngara frogs, for example, produce more conspicuous calls than males from rural populations and consequently attract more females (Halfwerk et al. 2019). Moreover, this appears to be an example of adaptive phenotypic plasticity, as a translocation experiment revealed that urban males are able to produce less conspicuous calls in nonurban habitats, but forest males moved to the city cannot shift to producing conspicuous calls (Halfwerk et al. 2019). Consequently, urban males produce more attractive calls in the urban environment, where the risks of predation and parasitism are low, but avoid the risks of the more conspicuous call in the forest environment.

The transmission of signals for communication is just one aspect of sexual selection. The altered environment of cities has the potential to affect many other aspects such as intrasexual competition (e.g., Tüzün et al. 2017b), mate choice preferences (e.g., Des Aunay et al. 2014), mating systems, physiological links between individual condition and traits (Hasegawa et al. 2014), and the strength of sexual selection itself (Sepp et al. 2018). A recent comprehensive review of sexual selection in urban environments emphasized the need to investigate aspects of sexual selection beyond male signaling in urban environments (Sepp et al. 2020). As one example, Tüzün and colleagues (2017b) found that the fragmented nature of urban habitats resulted in greater flight endurance for urban populations of damselflies, likely through spatial sorting (only individuals with high endurance can colonize these isolated habitats). As males engage in scramble competition for access to females at ponds, the increased mean endurance in urban ponds consequently resulted in stronger sexual selection for male flight endurance in urban populations.

Mechanism Interactions

Supplemental Material >

Relatively few studies have explored multiple evolutionary mechanisms within the same study system. In addition, most studies that have explored multiple mechanisms tend to examine gene flow and drift (**Figure 2**) rather than the relative importance and/or interplay of other mechanisms. Long-standing questions in evolutionary biology concern the potential for gene flow to swamp the effects of selection across gradients; however, this topic has rarely been broached at the phenotypic level in the context of urbanization (but see **Supplemental Table 2** for genomic examples). Indeed, this is perhaps one of the questions that can uniquely be addressed in an urban context, given the often steep spatial gradients from urban to nonurban environments. The closest analog is perhaps a study of anuran development rate across a steep elevational gradient versus a more gradual latitudinal gradient. Significant countergradient variation in development rate was found in both cases, but it was much weaker in the steep elevational gradient, providing evidence that gene flow was swamping selection (Bachmann et al. 2020). On the other hand, gene flow, along with selection, has the potential to also promote adaptation. In Gulf killifish, adaptation to industrial toxins has occurred via this mechanism. Interspecific gene flow, through hybridization, enabled adaptive introgression from Atlantic killifish to Gulf killifish (Oziolor et al. 2019). Other potential mechanism interactions include the effects of urban fragmentation on population size and subsequent adaptive evolutionary responses to urban stressors, e.g., whether small effective population sizes constrain adaptive evolution (Kinnison & Hairston 2007). Additionally, the interplay between selection, which winnows variation, and elevated mutation rate, which generates variation, could also be an important interaction in urban contexts. Regardless of the specific mechanisms and interactions under consideration, comparative studies of intra- and interspecific variation across urban steepness gradients (some urban gradients taper more gradually while others have sharper boundaries against nonurban habitat) and urban patchiness and fragmentation could be used to make broader inferences about the nature of evolutionary mechanism interactions.

Species Interactions

Not only can cities alter abiotic landscapes and agents of selection, they can also shift the biotic landscape, potentially leading to shifts in species interactions and biotic selection pressures.

These shifts can occur in both mutualistic and antagonistic species interactions (reviewed in Irwin et al. 2020). For example, near-complete turnover of pollinator communities across urban and rural habitats was associated with evolutionary divergence of floral morphology, phenology, and life-history traits in white clover (Santangelo et al. 2020b). However, altered biotic selection landscapes need not be due to community turnover. In bumble bees, shifts in body size that result from urban fragmentation and elevated temperatures lead to shifts in pollination services (Theodorou et al. 2021). On the antagonistic interaction side, evolution of larger body size in brown anoles was mediated by the variation in the abundance of predatory curly-tail lizards across the city, with larger anoles being less vulnerable to predation (Chejanovski & Kolbe 2019). Similarly, tri-trophic interactions in the goldenrod gall fly system have been altered by urbanization. Birds that preferentially prey on gallmakers that induce large plant galls (and are opposed by parasitoid wasps that preferentially prey on gallmakers that induce small plant galls) are less abundant in urbanized habitats, leading to stronger directional selection rather than the typically observed stabilizing selection on gall size (Start et al. 2018). Yet despite this strong directional selection in urban habitats, gall size phenotypes were similar across urban and nonurban habitats. The lack of phenotypic divergence could suggest potentially important roles for gene flow, genetic constraints (e.g., low heritability, genetic covariances), or environmental effects (plasticity). The gall fly study reinforces an important point: In general, there are relatively few studies of urban evolution driven by altered species interactions. Certainly there are a number of studies that show evidence of altered biotic selection regimes in cities (e.g., see Irwin et al. 2020), and data syntheses show that the magnitude of phenotypic divergence in cities when driven by altered species interactions is much greater than when driven by abiotic factors (Alberti et al. 2017). However, there are still few studies that show evolutionary divergence in cities being driven by altered species interactions.

Eco-evolutionary Feedbacks

The previous discussion of species interactions raises the issue of potential feedbacks of urban-driven evolution to ecology. As urban environments alter abiotic and biotic landscapes, these shifts can drive evolutionary change with the potential to then alter those abiotic and biotic landscapes. The potential for such feedbacks is high, although empirical demonstrations of feedbacks are nascent. Given the multi-layered nature of eco-evolutionary feedbacks, it is likely prudent to begin with demonstrated examples of urban evolution and work backward to the potential consequences for altered ecologies. Des Roches and colleagues (2021) consider several systems for such explorations including brown rats, mosquitos, water fleas, and white clover. For illustration purposes, the water flea urban evolution of higher heat tolerance and smaller body size (Brans et al. 2017) could feedback to alter pond community structure through changes to water flea population dynamics and impacts on toxic algal blooms. We emphasize, however, that any of the work summarized in **Figure 2** and other reviews and syntheses of urban evolution could realistically be examined in the context of eco-evolutionary feedbacks. In particular, whether cities are sources of ecological opportunity (Wellborn & Langerhans 2015, Borden & Flory 2021), for example, with the generation of new niches or more fine-scale differentiation of microhabitats, is key in this regard. Questions regarding the effects of eco-evolutionary feedbacks in cities on the speciation process (see the sidebar titled Speciation in Urban Habitats), overall patterns of biodiversity (i.e., whether cities are sources or sinks of biodiversity), and ecosystem function are important not only for basic questions in evolutionary biology but also for applied urban ecology and evolution.

SPECIATION IN URBAN HABITATS

With variable environmental gradients, microhabitats, and dispersal barriers, urban environments are an exciting venue for speciation research. At the simplest level, city environments could create barriers to gene flow and eventually cause populations to undergo reproductive isolation, for example, via Dobzhansky–Muller incompatibilities caused by mutation-order speciation or drift. By contrast, cities could also cause ecological speciation, as divergent selection between urban and rural populations could lead to reproductive isolation. Although this aspect of urban evolution is quite new, with few examples, several recent syntheses have identified a number of candidates to explore potential urban-driven speciation (Halfwerk 2021, Thompson et al. 2018). The divergence of the London Underground mosquito (*Culex molestus*) from the surface-dwelling *Culex pipiens* currently represents our best potential example of urban-driven speciation. Matings between *C. pipiens* and *C. molestus* generally result in inviable or infertile offspring, while host shifts and changes in diapause and reproductive physiology in *C. molestus* suggest reproductive isolation may be in part due to ecological speciation (Byrne & Nichols 1999). As more examples of urban speciation are found, estimating the frequency and speed of urban speciation may shed light on whether urbanization will result in net gains or losses of biodiversity in the future.

Role of Plasticity

A key component of urban evolution studies is disentangling evolved responses from plasticity (Diamond & Martin 2016). However, one should bear in mind that plasticity can interact with evolution in a number of ways. One of plasticity's most important roles in urban evolution is simply to buy time for species to evolve to the urban environment. Plastic responses are generally expressed within generations and so can operate more quickly than evolutionary changes, which occur across generations. Thus, plasticity can act as an initial buffer against urban stressors, allowing time for adaptive evolutionary responses to occur. Whether more plastic species are more successful at entering and persisting in the city compared with less plastic species, and subsequently, whether those species evolve in response to urbanization (i.e., plasticity-led evolution), has not yet been tested (Diamond & Martin 2021a). Alternatively, if plasticity is, on its own, sufficient to buffer organisms against urban stressors, such variation might be shielded from selection, dampening evolutionary responses to urbanization (Muñoz & Losos 2017). Notably, plasticity can also act across generations. Such transgenerational plasticity could be adaptive in an urban context provided that organisms moving between urban and rural habitats are able to anticipate their future conditions (Donelson et al. 2018). Additionally, plasticity might itself be the target of selection. For example, for species that move between urban and rural environments, it is possible that the trait mean might not change but that plasticity evolves instead. Or for urban-resident populations, urbanization with its alterations to environmental variance (e.g., night-time-biased warming that reduces diurnal temperature variance) could select for dampened or enhanced plasticity depending on the specific trait and environmental factor (e.g., see Diamond et al. 2018b for an example of evolved plasticity in response to urban temperature variance). And the plasticity need not be adaptive to elicit an evolutionary response: In cases of strongly maladaptive plasticity, as might be expected when organisms initially encounter urban environments to which they are ill-suited, selection can favor the loss of plasticity (Ghalambor et al. 2015).

CONCLUSION

Throughout this review, we have emphasized the critical role that cities play in driving contemporary evolution and the high potential for cities to address outstanding questions in evolutionary biology. Doing so requires a careful alignment between how cities are used and the research questions being asked. We developed a framework to help guide this alignment, focusing on the specificity of site selection and the extent to which conclusions from urban evolution studies could be generalized to other contexts. With this in mind, we summarize emerging horizons in urban evolutionary biology (see Future Issues).

FUTURE ISSUES

1. The spatio-temporal variation within and among cities can be used to examine critical early stages of evolutionary responses to environmental change, including changes in additive genetic variance, alterations to genetic correlations, and costs of adaptation that might no longer be detectable in long-diverged populations. Doing so requires careful attention to species' natural history and the selection of sites within cities for study.
2. The multifarious changes in cities that alter selective landscapes and their effects on natural and sexual selection provide unique opportunities to explore how novel combinations of environmental factors and multiple traits of organisms respond to environmental change.
3. Cities provide incomparable venues to examine long-standing questions in evolutionary biology regarding the repeatability of evolution in response to shared pressures. Research so far suggests positive support overall for common evolutionary responses to shared pressures.
4. The field of urban evolution is moving beyond disentangling plastic from evolved responses to examining how urbanization can capitalize on interactions between these mechanisms (e.g., how plasticity might facilitate or forestall evolution in cities), as well as interactions between adaptive evolutionary responses and neutral processes, including drift, gene flow, and mutation.
5. While contemporary evolution in cities is increasingly documented, the study of potential feedbacks of urban evolution on urban ecology is nascent. Eco-evolutionary feedbacks can have important consequences not only for shaping future evolutionary trajectories but also for applied research questions regarding the maintenance and enhancement of ecosystem function in urban environments.

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