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Perspective

Urban Evolutionary Ecology and the Potential Benefits of Implementing Genomics

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Abstract

Urban habitats are quickly becoming exceptional models to address adaptation under rapid environmental change, given the expansive temporal and spatial scales with which anthropogenic landscape conversion occurs. Urban ecologists in the last 10–15 years have done an extraordinary job of highlighting phenotypic patterns that correspond with urban living, as well as delineating urban population structure using traditional genetic markers. The underpinning genetic mechanisms that govern those phenotypic patterns, however, are less well established. Moreover, the power of traditional molecular studies is constrained by the number of markers being evaluated, which limits the potential to assess fine-scale population structure potentially common in urban areas. With the recent proliferation of low-cost, high-throughput sequencing methods, we can begin to address an emerging question in urban ecology: are species adapted to local optima within cities or are they expressing latent phenotypic plasticity? Here, I provide a comprehensive review of previous urban ecological studies, with special focus on the molecular ecology and phenotypic adjustments documented in urban terrestrial and amphibious fauna. I subsequently pinpoint areas in the literature that could benefit from a genomic investigation and briefly discuss the suitability of specific techniques in addressing eco-evolutionary questions within urban ecology. Though many challenges exist with implementing genomics into urban ecology, such studies provide an exceptional opportunity to advance our understanding of eco-evolutionary processes in metropolitan areas.

Subject area: Genomics and gene mapping, Molecular adaptation and selection
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In cities, the interplay between humans and the environment profoundly shapes eco-evolutionary dynamics that generate wholly novel selective constraints on organisms unlike any experienced in rural or natural environments (Alberti 2015; Donihue and Lambert 2015; McDonnell and Hahs 2015; Johnson and Munshi-South 2017). For instance, heightened road densities in metropolitan areas compel organisms to develop road-crossing strategies that decrease the likelihood of vehicular mortality (Balkenhol and Waits 2009; Riley et al. 2014a), such as changes in sensory mechanisms

that allow individuals to accurately assess vehicular speed (Lima et al. 2015). Acoustic pollution caused by ambient road noise or construction degrades the acoustic environment (Tuomainen and Candolin 2011), which may require species to alter predator alarm calls or song structure to attract mates (Templeton et al. 2016). Case in point, birdsong diversity often decreases (Proppe et al. 2013) and song frequency shifts upward under noisy urban conditions (Slabbekoorn 2013). Human-driven environmental changes can also have community-level effects. For example, food subsidies in cities

are associated with reduced species richness and large-scale community restructuring in which invasive species frequently outcompete natives (Galbraith et al. 2015; Plummer et al. 2015). The timescale with which these examples occur happen on the order of years rather than centuries (McDonnell and Hahs 2015). Consequently, urban environments are quickly being considered natural laboratories to address human-induced, rapid adaptive change (Pickett et al. 2016; Alberti et al. 2017a).

Prior empirical work in urban systems has done an exceptional job detailing the phenotypic consequences of urbanization in a variety of taxa (Table 1). These efforts have enabled researchers to begin describing phenotypic similarities and dissimilarities that exist across urban environments (Alberti 2015; Alberti et al. 2017b). Now given a critical mass of information, we have the opportunity to consider if urban areas across the globe share synonymous ecological dynamics that facilitate instances of parallel evolution (Magle et al. 2012; Johnson and Munshi-South 2017). Much of the work to date, however, is insufficient in completely addressing urban adaptation. This is particularly because assessment of phenotypic patterns alone makes it difficult to ascertain whether expressed traits are simply within the natural range of plasticity or genetic signals of adaptation to local optimum within cities (Miranda et al. 2013; Miranda 2017). Hence, by determining the genetic basis of adaptive change in urban areas we can begin to examine whether previously observed phenotypic changes are locally adapted to metropolitan environments.

The use of traditional genetic markers (i.e., microsatellites and short fragments of mitochondrial DNA) have partially begun to address urban population divergence. For instance, several studies have examined patterns of gene flow and kinship as a function of road ecology and demography (Riley et al. 2003; Balkenhol and Waits 2009; Delaney et al. 2010; Munshi-South and Kharchenko 2010; Munshi-South 2012). A few recent studies have also used a candidate gene approach to observe genotype–phenotype associations correlated with the degree of urbanization (Orsini et al. 2012; Mueller et al. 2013; van Dongen et al. 2015). However, these approaches have several methodological restrictions that limit the types of inferences that can be drawn. Traditional genetic approaches are not as efficient in addressing rates of gene flow specific to adaptive loci or founder-specific inbreeding coefficients because the number of loci surveyed is significantly smaller compared with genomic studies (Allendorf et al. 2010). Traditional methods also provide insufficient coverage of the genome, which may potentially lead to a false assessment of null divergence when populations are separated by small spatiotemporal scales (Ekblom and Galindo 2011; Richardson et al. 2014). Further, if a single identified candidate gene is not associated with the observed trait or that trait is polygenic (i.e., multiple genes for one trait), the potential to accurately describe adaptive genetic variation is reduced (Storz 2005; Flint and Munafò 2013; Shafer et al. 2015).

Various advancements in next-generation sequencing (NGS) methods can overcome limitations inherent in traditional molecular practices by assessing the genome on orders of magnitude greater than previously attainable (Andrews et al. 2016). The number of single nucleotide polymorphisms (SNPs) that can be examined (e.g., 10^3–10^5) dwarf previous benchmarks, which increases the likelihood of detecting loci under positive directional selection (Allendorf et al. 2010; Oyler-McCance et al. 2016). In addition, the reduction in SNP library preparation and sequencing costs makes implementing a genomic framework to ecological studies more tenable (Ekblom and Galindo 2011; Andrews et al. 2016). Growth in the number of reference genomes being described greatly facilitates future genomic

studies for ecologists working on closely related nonmodel organisms (Hoban et al. 2016), and even de novo assembly of genomes is an option when a reference genome is unavailable for use (Kajitani et al. 2014; Mastretta-Yanes et al. 2015). Moreover, the ability to sequence the transcriptome facilitates the development of novel studies examining adaptive and nonadaptive gene expression (Alvarez et al. 2015; MacManes 2016). The parallel surges in both population genomics and urban ecology have built a strong foundation to quantify the genetic consequences of human agency on urban ecoevolutionary processes.

This comprehensive review is partitioned into three overarching sections. First, I survey the literature to assess publication trends in urban molecular studies from 2000 to 2017. Second, I provide a targeted review of studies that intentionally compare urban and rural phenotypes, highlighting the adaptive significance of such studies. Finally, I pinpoint areas that could benefit from a genomics approach by distilling 3 broad questions from previous literature that potentially encapsulate the most impactful areas of study within urban evolutionary ecology over the coming decades (Magle et al. 2012; Alberti 2015; McDonnell and Hahs 2015; McPhearson et al. 2016; Pickett et al. 2016; Johnson and Munshi-South 2017). Those 3 questions are:

(1) What is the finest spatial scale at which anthropogenic structures can reduce gene flow in cities?
(2) How is standing genetic variation related to historical human development in cities?
(3) Are gene–environment associations and expression patterns the product of plasticity or local adaptation?

I close this review by providing considerations and challenges to implementing a genomics approach for urban ecological studies. Throughout the review, I specifically focus on terrestrial and amphibious fauna because they represent the most well-studied taxonomic groups in urban areas (Pickett et al. 2008). This review does not discuss the new and upcoming technologies used to generate and sort genomic data (Storz 2005; Allendorf et al. 2010; Cammen et al. 2016; Hoban et al. 2016; MacManes 2016; Oyler-McCance et al. 2016).

Recent Trends: Urban Molecular Ecology

I surveyed peer-reviewed literature to 1) evaluate trends in publication rates from 2000 through 2017, and 2) map studies by taxon and metropolitan location to visually examine urban research hotspots. Specifically, I performed dual searches of the Web of Science and Google Scholar databases using the terms "urban ecology," "population genetics," and several molecular and NGS terms (e.g., "microsatellite," "mitochondria," "RADseq," "SNPs," "transcriptome," "PCR," "next-generation sequencing," "Illumina"), and paired them with each one of the following terms indicating study taxa—"mammals," "birds," "reptiles," "amphibians," and "invertebrates." Any studies not directly related to the genetics or genomics of terrestrial or amphibious species were excluded, as well as those in which populations were not explicitly sampled within the limits of a designated metropolitan area. Thus, studies that were solely conducted in agricultural or disturbed natural areas were not considered. I also excluded any studies on aquatic and plant species. The remaining 120 articles that were relevant to urbanized terrestrial or amphibious species were further binned by organismal class (i.e., mammals, birds, reptiles, amphibians, and insects), primary methodology (i.e., traditional markers vs. NGS), and metropolitan location.

Table 1. Empirical studies documenting phenotypic differences between urban and rural populations of terrestrial and amphibious fauna

Common name	Scientific name	Region	Trait category	Proposed driver(s)	Citations
Birds					
House sparrows	*Passer domesticus*	EU	B	Population-level habitat differences	Bókony et al. (2012)
		EU	B	Habituation	Vincze et al. (2016)
		EU	M	Increased predator–prey dynamics	Dulisz et al. (2016)
		EU	P, M	Food supplementation	Meillère et al. (2015)
Tree sparrows	*Passer montanus*	AS	P	Population-level habitat differences	Zhang et al. (2011)
Song sparrows	*Melospiza melodia*	NA	P	Social networks and anthropogenic resources	Sewall and Davies (2017)
		NA	B	Food supplementation	Foltz et al. (2015)
Dark-eyed junco	*Junco hyemalis*	NA	B, P	Genetic	Atwell et al. (2012)
Bullfinch	*Loxigilla barbadensis*	CA	B, P	Enhanced immunocompetence	Audet et al. (2016)
European blackbird	*Turdus merula*	EU	M	Latitudinal gradient (Seebohm's rule)	Evans et al. (2009)
		EU	P	Genetic	Partecke et al. (2006)
		EU	B	Genetic	Miranda et al. (2013)
		EU	M	Genetic	Costantini et al. (2014)
		EU	P, M	Genetic	Partecke et al. (2004)
Common mynas	*Acridotheres tristis*	AU	B	Increased food variety in cities	Federspiel et al. (2017)
Eurasian coot	*Fulica atra*	AU	B	Genetic	van Dongen et al. (2015)
Great tits	*Parus major*	EU	B	Food supplementation	Preiszner et al. (2017)
		EU	B	Phenotypic plasticity	Riyahi et al. (2017)
		EU	M	Density of low-carotenoid food items	Biard et al. (2017)
Burrowing owls	*Athene cunicularias*	SA	B	Genetic	Carrete and Tella (2013)
		SA	B, P	Genetic	Rebolo-Ifrán et al. (2015)
		SA	B	Predator-release in urban environments	Carrete and Tella (2017)
Curve-billed thrashers	*Toxostoma curvirostre*	NA	B, P	Water availability	Fokidis and Deviche (2012)
Mammals					
Eurasian red squirrel	*Sciurus vulgaris*	AS	B	Phenotypic plasticity	Uchida et al. (2016)
Fox squirrels	*Sciurus niger*	NA	B	Habituation	Mccleery (2009)
Eastern chipmunk	*Tamias striatus*	NA	B, P, M	Food supplementation	Lyons et al. (2017)
Red fox	*Vulpes vulpes*	EU	P	Urban pollution	Dip et al. (2001)
Coyotes	*Canis latrans*	NA	B	Food supplementation	Murray et al. (2015)
Wild boar	*Sus scrofa*	EU	B	Food supplementation	Stillfried et al. (2017)
Raccoons	*Procyon lotor*	NA	B	Stable food subsidies	Prange et al. (2004)
Reptiles					
Puerto Rican crested anole	*Anolis cristatellus*	NA	M	Percentage of artificial surfaces, genetic	Winchell et al. (2016)
Australian freshwater turtle	*Chelodina longicollis*	AU	B	Artificial manipulation of suburban estuaries	Rees et al. (2009)
Brown anole	*Anolis sagrei*	NA	B	Foraging niche	Lapiedra et al. (2017)
Ornate tree lizard	*Urosaurus ornatus*	NA	P	Down-regulation of hypothalamic-pituitary-adrenal axis	French et al. (2008)
European wall lizard	*Podarcis muralis*	EU	M	Parasite densities	Lazić et al. (2016)
Amphibians					
Marsh frog	*Rana ridibunda*	EU	P	Industrial contamination of downstream waters	Falfushinska et al. (2008)
Insects					
Common field grasshopper	*Chorthippus brunneus*	EU	M	Urban heat-island effect and climate change	San Martin y Gomez and Van Dyck (2012)
Blowfly	*Calliphora vicina*	EU	M	Urban heat-island effect	Hwang and Turner (2009)
Damselfly	*Coenagrion puella*	EU	M	Urban heat-island effect	Tüzün et al. (2017a)
		EU	B, M	Sexual selection	Tüzün et al. (2017b)
Leaf-cutter ants	*Atta sexdens rubropilosa*	EU	B, M	Urban heat-island effect	Angilletta et al. (2007)

Synoptic examples include common and scientific names, region of study (Africa, AF; Asia, AS; Australia, AU; Caribbean, CA; Europe, EU; North America, NA; South America, SA), trait category studied (behavioral, B; physiological, P; morphological, M), control group, and proposed drivers of phenotypic changes. An expanded list with results, specific cities, specific traits measured, control group, and sample size is included in Supplementary Table S2.

The fully annotated reference list of these articles is described in Supplementary Table S1.

From 2000 through 2017, there was a noticeable increase in the overall number of molecular studies performed in urban environments (Figure 1). This growing trend coincides with previously documented increases in publication volume on urban wildlife overall (Magle et al. 2012). Not surprisingly, most studies employed traditional molecular approaches (*n* = 113, ~94%; Figure 1). Of the 7 studies that did employ an NGS approach, 5 focused on Rodentia species (i.e., white-footed mice, *Peromyscus leucopus*, Harris et al. 2013, 2015; Harris and Munshi-South 2016; Munshi-South et al. 2016; the brown rat, *Rattus norvegicus*, Puckett et al. 2016), one on great tits (*Parus major*, Watson et al. 2017), and one on African mosquitos (*Anopheles gambiae* and *Anopheles coluzzii*, Cassone et al. 2014). Most publications focused on mammalian species (*n* = 52, 43.7%), with both birds and insects tied for second-most represented in the literature (*n* = 26 per clade, 21.8% each). Of all studies surveyed in this analysis, only 33 studies of the 120 explicitly compared urban populations to a natural, forested, or rural counterpart. Further, existent urban molecular studies span a wide geographic distribution, but most studies are in temperate locations (Figure 2).

These general findings highlight the depth and breadth of accomplished work performed in urban habitats worldwide, but similarly emphasize areas of future research need. First, mammals are overrepresented in the number of urban molecular studies performed (Figure 1). Second, only 26.9% of previous studies intentionally assess genetic structure of both urban and rural populations. While within-urban molecular studies are valuable for assessing genetic structure as a function of extreme anthropogenic habitat fragmentation, a suitable control population is necessary to determine if local adaptation is occurring in urban environments (Richardson et al. 2014; Hoban et al. 2016). Third, most studies reviewed were conducted in North America and Europe, with temperate zones and wealthier countries being overrepresented in the literature (Figure 2). Cities in tropical and desert biomes are undoubtedly going to have varying climatic conditions that may interact with anthropogenic features much differently than conditions in temperate cities. Hence, there is tremendous opportunity to expand urban molecular work into climatic regions and biomes not well represented here. Finally, there is a lack of species diversity throughout the NGS publications that currently exist (Figure 1). However, that is expected to change rapidly in the next few years with the increasingly low-cost
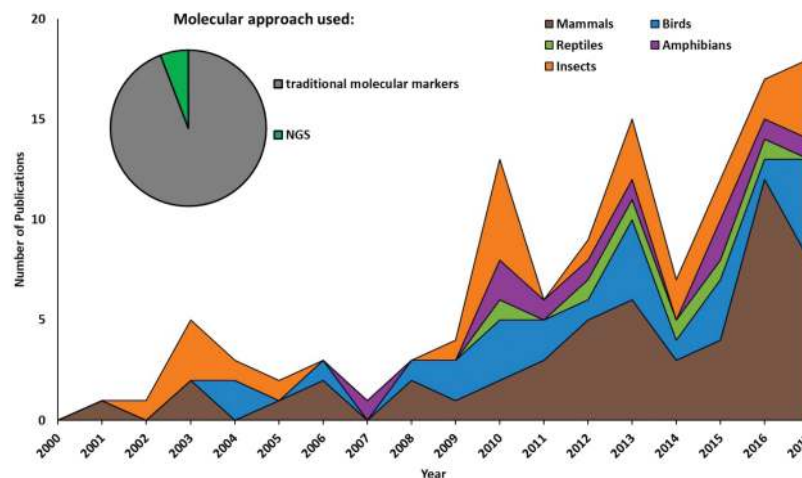

Figure 1. Number of publications on the molecular ecology of terrestrial and amphibious fauna in urban environments, as well as the proportion of studies (pie chart) that used traditional molecular techniques (i.e., microsatellite and/or mitochondrial analyses) versus next-generation sequencing (i.e., genomic approaches), from 2000 to 2017. See online version for full colors.

Figure 2. Map of study locations on the molecular ecology of mammals (brown), birds (blue), reptiles (green), amphibians (purple), and insects (orange) in cities across the globe. Citations can be found in Supplementary Table S1. See online version for full colors.

of high-throughput sequencing and parallel growth in the number of reference genomes for non-model organisms (Cammen et al. 2016; Oyler-McCance et al. 2016).

Human-Driven Phenotypes: Urban Versus Rural Comparisons

Within the last few years, urban ecologists, designers, and environmental planning professionals have worked together to articulate the links among specific anthropogenic features and individual phenotypic outcomes (Clucas and Marzluff 2011; Alberti 2015; Donihue and Lambert 2015; McDonnell and Hahs 2015; Pickett et al. 2016). In so doing, a concerted effort to describe the adaptive significance of human-driven changes to functional traits has been made (Sol et al. 2013; Alberti et al. 2017b). Here, I review and synthesize recent work across terrestrial and amphibious fauna that examine phenotypic modifications (e.g., behavior, physiology, and/or morphology) as a function of urban living. I specifically focus on studies that explicitly compare urban versus nonurban populations for several reasons. Landscape-level approaches are certainly invaluable in describing correlational patterns among anthropogenic features and observed traits, as seen in a broad range of previous urban studies (Tigas et al. 2002; Prange et al. 2004; Gehrt et al. 2013; Riley et al. 2014b; Poessel et al. 2017). However, such studies by themselves are insufficient to examine the mechanistic basis underlying observed phenotypic patterns (Shochat et al. 2006; Rees et al. 2009). Comparison of urban populations to an adequate control provides an experimental design better equipped to determine the fitness benefits of an urban phenotype and identify the drivers that induce such adaptations (Donihue and Lambert 2015). Several common garden studies comparing offspring from urban and rural habitats have provided supporting information for this claim (Table 1, Supplementary Table S2). Further, comparison of geographically and ecologically distinct habitats in follow-up gene-phenotype analyses is less likely to encounter problems of spatial autocorrelation that would significantly limit a study's power to interpret local adaptation in urban systems (Hoban et al. 2016).

By no means is this review complete: previous reviews have comprehensively described phenotypic adaptations exhibited toward human drivers (Tuomainen and Candolin 2011; Bateman and Fleming 2012; Sol et al. 2013; Donihue and Lambert 2015; Šálek et al. 2015; McDonnell and Hahs 2015; Alberti et al. 2017a). Whereas prior reviews have pooled studies conducted in urban and natural environments (i.e., anthropogenic fragmentation of natural green space), and included landscape-level analyses within urban areas, this review is unique in that it is solely focused on urban versus rural comparisons (Table 1).

Behavioral Adjustments

Initial adaptive responses toward anthropogenic pressures are often behavioral (Ditchkoff et al. 2006; Tuomainen and Candolin 2011; Lowry et al. 2013). Proper behavioral modifications to urban contexts by founder individuals in the short term are expected to provide fitness benefits that ultimately have cascading influences on future population dynamics and distribution (Tuomainen and Candolin 2011). Over the relative long-term, if we assume that such behavioral traits covary with underlying genotypes, then we may expect such heritable behaviors to proliferate and result in phenotypic differentiation among urban and nonurban populations (Lowry et al. 2013; Sol et al. 2013; McDonnell and Hahs 2015).

There are several examples in the literature that support this hypothesis, particularly in avian systems (Table 1; fully annotated information in Supplementary Table S2). Flight initiation distance (FID: a measure of distance at which an organism flees from an encroaching human) is most frequently used to assess human-associated boldness in urban taxa (Sol et al. 2013). However, it is by no means the only observed behavioral modification, with several studies demonstrating reduced alarm calling (Møller and Ibáñez-Álamo 2012), reduced neophobia (Audet et al. 2016; Greggor et al. 2016; Ducatez et al. 2017; Riyahi et al. 2017), and greater problem-solving performance (Papp et al. 2015; Audet et al. 2016; Preiszner et al. 2017) in urban organisms. The unifying theme amongst most of these studies is the central role food subsidies in cities play in driving selection for reduced proximity to humans. Individuals that maintain close proximity to humans are consequently able to withstand adverse environmental conditions by exploiting abundant food resources provided by humans (Møller et al. 2013). This, paired with an often-dramatic reduction in mortality from relaxed predation pressure, disadvantages individuals that are either less plastic or more timid in human-contexts (Møller and Ibáñez-Álamo 2012; Lowry et al. 2013).

Whether these apparent urban–rural differences in behavior are primarily explained by plasticity, locally adapted phenotypes, or both is still uncertain. However, a repeated measures approach across contexts and time may begin to help disentangle environmentally dependent and genetically associated behaviors (Miranda et al. 2013; Miranda 2017). Recent developments in the study of animal personality provide an established rubric for quantifying behavior that is consistent across multiple time points and contexts (Dingemanse and Réale 2005; Dingemanse et al. 2010). In addition, personality is heritable and is frequently associated with underlying genetic components (van Oers et al. 2005; Dochtermann et al. 2014). Hence, animal personality research may be well-situated to determine if behaviors linked to anthropogenic drivers are also genetically linked.

Several recent studies have already provided data that successfully integrate personality research and urban ecology (Bókony et al. 2012; Miranda et al. 2013; Carrete and Tella 2013, 2017; Arroyo et al. 2017; Lapiedra et al. 2017). Others have also demonstrated that personality variation is linked with specific gene regions (e.g., DRD4 exon 3) that are selected for in urban avian systems (Mueller et al. 2013; Riyahi et al. 2015; van Dongen et al. 2015), supporting the hypothesis that selection acts on specific personality types in urban environments. Despite the potential benefits of a personality approach, several drawbacks still exist to implementing such methods in wild settings. Quantifying personality necessitates multiple measures from a single individual, which may require a mark-recapture study. If relocating an individual is particularly challenging either due to difficulty in capture, low abundance, or low survivorship, quantifying personality may be improbable. In addition, recapture for all studied individuals may occur in different contexts and at different developmental time points, which may add more undue variance. For these reasons, the ease and feasibility of recapture will determine the potential success of quantifying personality in urban studies.

Physiological Adjustments

A multitude of human-driven biotic and abiotic stressors in cities pressure organisms to develop effective coping mechanisms that maintain homeostasis (Isaksson 2015). Human disturbances such as light pollution (Dominoni et al. 2014; Swaddle et al. 2015) and artificial elevation of noise levels (Proppe et al. 2013; Slabbekoorn 2013; Templeton et al. 2016) disorient the circadian rhythms and

auditory signals of organisms, whereas urban heat-island effects disrupt physiological systems sensitive to temperature (Angilletta et al. 2007; Hwang and Turner 2009; Tüzün et al. 2017a). Fluctuations in resource availability, novel predators, and conspecific densities reorient trophic dynamics that characteristically alter competition regimes within already novel environments (Fischer et al. 2012; Giraudeau et al. 2015; Plummer et al. 2015). In sum, these human-induced stressors present in cities are not represented in other habitats, and should differentially affect endocrine mechanisms of urban and rural individuals (Shochat et al. 2006; Clucas and Marzluff 2011; Alberti et al. 2017a; Lyons et al. 2017).

Indeed, recent research focus has sought to examine potential population differences in stress physiology as a function of urbanization (Table 1). Justifiably so, as stress hormones (i.e., glucocorticoids) govern the fight-or-flight response in all organisms (Partecke et al. 2006; Isaksson 2015). Moreover, stress hormones often mediate the expression of behavioral traits (Carere et al. 2010; Taff and Vitousek 2016) and strongly covary with personality variance in a population (Korte et al. 2005; Carere et al. 2010). It makes intuitive sense, then, that baseline stress profiles may be a direct or indirect target of selection in urban environments. For instance, individuals able to quickly return to baseline glucocorticoid concentrations post-stressor may fair better in an anthropogenic environment with constant stressors (i.e., direct target of selection; Bonier 2012). Comparatively, a behavior under positive selection (e.g., human tolerance) may be mediated by a specific glucocorticoid profile (i.e., indirect target of selection). In both instances, initial colonizers of cities may experience novel urban stressors (i.e., vehicles, disease, competition) that alter stress profiles of parents, subsequently preparing the endocrine profiles of their offspring (Watson et al. 2017). This may result in the parental transmission of locally adapted stress phenotypes, suited for urban living, within a small number of generations.

Previous work in several taxa has assessed glucocorticoid variance in relation to urbanization, but no uniform patterns emerge across studies (Table 1). A priori predictions suggest that urban organisms should express higher baseline stress because of more frequent experience with anthropogenic stressors (Isaksson 2015). However, several studies observed lower corticosterone concentrations for urban compared to rural individuals (Table 1). The broad differences in results across studies may be explained by the inherently complicated nature of quantifying individual hormonal baselines. Functional or upstream differences in endocrine systems may contribute to the observed variation in serum or fecal concentrations. For instance, to account for greater concentrations of free-floating glucocorticoids, urban individuals may develop endocrine profiles with greater stress hormone receptor densities and sizes (Koolhaas et al. 1999). Urban individuals may also exhibit greater sensitivity to glucocorticoids (Bonier 2012); hence, urban and rural individuals may exhibit synonymous glucocorticoid concentrations, yet show marked differences in behavioral profiles. Functional modifications to the hippocampus due to chronic stress can also occur (Magarinos et al. 1997). Such structural changes may affect the upstream production of, and sensitivity to, hormones (Fokidis and Deviche 2012; Sewall and Davies 2017). Alterations to products of other endocrine systems (e.g., testosterone, thyroxine) can interact with stress physiology to either mask or accentuate urban–rural differences (Mastorakos et al. 2006). Further, several pollutants and chemicals in cities (e.g., organochlorine, DDT, phthalates) disrupt proper endocrine function and negatively impact reproductive health and behavior (Falfushinska et al. 2008; Tuomainen and Candolin 2011); thus, variation in behavior may be explained more

by environmental contaminants rather than intrinsic differences in hormonal production.

Given these hypotheses, a cautious optimism may be necessary to interpret single-sample concentration differences as a signal of local adaptation. Disentangling the multitude of potential changes to functional endocrine responses (i.e., sensitivity, receptor density, receptor number), and how they correspond to adaptive processes, may be intractable with single concentrations from multiple individuals. Genomics and epigenetic mechanisms may provide a workable solution. It is known that environmental stressors have the potential to methylate DNA, and subsequently affect how gene expression is regulated (Riyahi et al. 2015; Watson et al. 2017). Use of RNAseq techniques would help to disentangle the complex endocrine responses that could occur with urban stressors, and prove valuable to determining how rapid epigenetic changes to endocrine function are related to anthropogenic drivers.

Morphological Adjustments

Morphological modifications observed in urban habitats are less well documented compared with endocrine or behavioral measures, yet arguably have as much functional significance in persistence throughout the urban matrix. Of the three broad phenotypic categories documented here, we may predict that the distribution of morphological forms is more selectively constrained within urban habitats because small changes in limb or wing structure can significantly alter an organism's ability to locomote within the urbanized landscape. Moreover, residential or commercial development in cities alter landscape features that fundamentally affect how organisms traverse an urban environment (Hoban et al. 2016; Lowry et al. 2017a). For these reasons, morphological investigations can provide solid inferences of local adaptation in urbanized landscapes.

To date, morphology–environment relationships in urban ecological studies have most often been observed in smaller organisms with short life-histories and dispersal distances (Table 1). For instance, several reptile and insect species demonstrate variation in limb length and appendage properties that increase the organism's ability to traverse smooth manmade structures in cities (San Martin y Gomez and Van Dyck 2012; Donihue 2016; Winchell et al. 2016). Whether similar variation exists in limb morphology of mammals, birds, or amphibians remains unclear. However, prior work has demonstrated morphological differences in cranial capacity, with urban populations of multiple taxa exhibiting larger craniums compared to rural populations (Snell-Rood and Wick 2013). Other studies have focused on body size and condition, with mixed results across taxa (Table 1). Several authors suggest that urban–rural differences in morphology are related to the abundance of anthropogenic food subsidies in cities (Table 1), not unlike studies on increased human tolerance. Hence, there may indeed be a strong likelihood that food subsidies are driving changes to coordinated suites of traits (i.e., behavior, hormones, and morphology), and a genomic investigation may be best equipped to identify such associations.

Synthesizing Urban Ecology with Genomics

The swell of urban ecology studies in recent decades has certainly provided convincing evidence that urban populations are locally adapted to anthropogenic environments. Such studies have converged on the importance of socio-ecological factors in affecting evolutionary change of urban populations (Alberti 2015; Donihue and Lambert 2015; McDonnell and Hahs 2015; McPherson et al. 2016). Still, determining whether selection acts on standing

variation or novel mutations, describing synonymous genetic solutions across landscapes, or assessing the scale at which microgeographic adaptation occurs requires knowledge of the genetic loci underpinning urban phenotypes (Richardson et al. 2014; Tiffin and Ross-Ibarra 2014; Hoban et al. 2016). These questions are central to evolutionary biology, and encompass emerging themes in urban evolutionary ecology. Previous use of fragment-based markers (i.e., microsatellites or mitochondria) to describe genetic differentiation is necessarily limited by the number of loci surveyed, and therefore provides a limited resolution to assess adaptive divergence (Tiffin and Ross-Ibarra 2014). We now have an array of genomic tools to provide even further evidence for microgeographic adaptation to cities. Determining which genomic technique is appropriate for the emerging urban ecological questions of interest, however, is not a trivial endeavor. Here, I revisit the 3 broad questions that will arguably define the field of urban evolutionary ecology within future decades (see Introduction section), and position the role of genomics in facilitating that forward march. In so doing, I lean on recent impactful studies in urban systems that have successfully employed a genomics approach as a proof of concept for other urban systems. Further, I attempt to highlight how specific genomic techniques may be appropriate for the question at hand, with the understanding that several methodological options exist to address a single question.

Previous reviews have done an exceptional job of meticulously describing the sequencing and computational minutiae of approaches such as restriction site-associated DNA sequencing or RADseq (Andrews et al. 2016; Benestan et al. 2016; Lowry et al. 2017b; Mckinney et al. 2017), SNP arrays and target sequence capture (Cammen et al. 2016), whole genome sequencing (WGS; Kajitani et al. 2014; vonHoldt et al. 2016), genotyping-by-sequencing (GBS; Narum et al. 2013; He et al. 2014), population-level genome re-sequencing (Ekblom and Galindo 2011; Oyler-McCance et al. 2016), and transcriptomic sequencing (Wang et al. 2009; Alvarez et al. 2015). For a more comprehensive reading of techniques addressed here, interested urban ecologists should delve further into these reviews.

What Is the Finest Spatial Scale at Which Anthropogenic Structures Can Reduce Gene Flow in Cities?

This question is fundamental to evolutionary biology, and is certainly not restricted to urban systems. Such an inquiry is difficult to generalize across various taxa and habitats. These concerns aside, myriad cities do exhibit similar spatial arrangements of green spaces, roadways, and buildings that establish a densely-populated urban core surrounded by decreasing human densities in suburban space that radiates outward (Johnson and Munshi-South 2017). This repeated pattern among multiple cities suggests that shared socioecological processes across urban areas may similarly select for traits of organisms regardless of specific city or taxon (Hendry et al. 2017). Hence, urban ecological properties may converge on a spatial threshold of geographic distance, that by which movement and gene flow is inhibited.

Consequently, principles from island biogeography and metapopulation theory may be useful paradigms with which to view genetic connectivity and isolation in urban systems (Magle et al. 2010). This is particularly because the tenets of both theories demonstrate that species persistence, whether across oceanic islands or terrestrial habitat fragments, is directly linked to patch connectivity and size (Losos et al. 1967; Ricklefs et al. 1967; Moilanen and Hanski 2006).

These key drivers ultimately have genetic consequences, with larger more connected patches exhibiting greater genetic diversity and admixture (Lowe and Allendorf 2010). Dispersal and gene flow are similarly dictated by an intervening matrix (e.g., roads, buildings, etc.) in urban fragments as they are in oceanic islands or terrestrial habitat fragments. However, as Magle et al. (2010) have previously articulated, the intervening matrix in cities is comprised of multiple elements (i.e., not just open ocean or habitat) that vary over contemporary timescales. These variable elements may strengthen or dampen matrix permeability for dispersing organisms despite geographical distance between urban fragments (Magle et al. 2010). Consequently, anthropogenic elements within the urban matrix may be as equally important as matrix size in genetically isolating populations. Indeed, the molecular studies surveyed here have observed genetic differentiation within cities at geographic distances under 1 km (Dronnet et al. 2005; Noël et al. 2007; Hemme et al. 2010; Mikulíček and Pišút 2012; Jha and Kremen 2013; Lourenço et al. 2017).

An emerging question is whether genomic tools are better equipped to detect divergence at small spatial scales in cities. The chosen solution may be determined according to sample size and dispersal capability of the study species. One of the benefits of using, say, a RADseq approach compared with microsatellites or mitochondrial DNA is an expansion in genomic coverage (Oyler-McCance et al. 2016), increasing the likelihood of detecting divergence at fine spatial scales (Figure 3). Certainly, RADseq-generated SNPs have extended inferences made from previous microsatellite explorations in heterozygosity estimates (Hoffman and Nichols 2011) and population structure (Malenfant et al. 2015) of several nonmodel organisms, so it may be reasonable to expect similar resolution improvements in determining the spatial scale of divergence (which is typified in the conceptual diagram, Figure 3). In addition, RADseq, double-digest RADseq (ddRADseq), or other RAD methods can be performed without a reference genome from close relatives (Andrews et al. 2016; Benestan et al. 2016), though any available reference genome greatly improves interpretive power (Mcmahon et al. 2014; Shafer et al. 2015). This effectively opens the door to genomic investigations for urban ecologists working on nonmodel systems. Still, at some zenith SNPs generated from RADseq may be equally able to detect divergence as traditional markers (Figure 3), so the scale of study, the organism's dispersal ecology, sampling design, and the specific research question should dictate which technique is most appropriate.

SNP array and target sequence capture (TSC) approaches may be viable alternatives to RADseq if a reference genome is made available (Grueber 2015). Both techniques involve the design of custom arrays that target specific regions of the genome (Cammen et al. 2016). The caveat is that arrays contain biased sets of pre-ascertained SNPs (i.e., ascertainment bias) from specific populations that may portray a dramatically different picture of demographic history and natural selection than a whole genome sequencing approach (Helyar et al. 2011; Lachance and Tishkoff 2013). For instance, if founding populations were isolated by historical urban development, and the alleles targeted by SNP arrays had randomly drifted out of the observed population, then SNP arrays may incorrectly suggest strong natural selection. In this instance, a WGS approach would correctly identify historical demographic patterns as the primary explanation behind observed genetic structure (Hoban et al. 2016). Using SNP arrays and WGS in tandem could reduce the effects of ascertainment bias if careful filtering is done based on SNP genomic context before array development (Cammen et al. 2016; Humble et al. 2016). Intentional
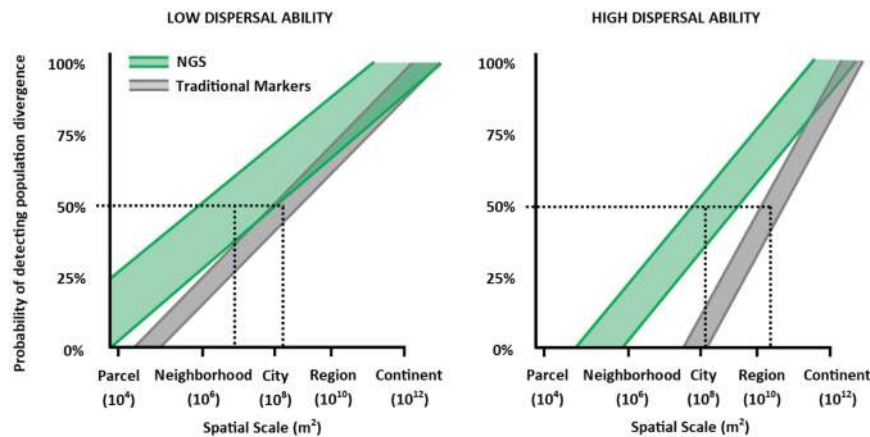
Figure 3. A conceptual model of the relationship between next generation sequencing (NGS; >1000 loci surveyed) and traditional molecular approaches (i.e., 5–50 loci surveyed) in detecting population divergence over varying spatial scales and species' dispersal ability. In this conceptual figure, whole-genome sequencing (WGS) should provide substantially more coverage of the genome as compared with reduced representation methods (i.e., RADseq). This inherent variation is reflected in the shaded regions, with WGS considered at the zenith of detection. In both cases of heightened or reduce dispersal ability, NGS methods are expected to detect divergence at smaller spatial scales. See online version for full colors.

sampling across the geographic range of the target species can also limit ascertainment bias (Malenfant et al. 2015; Cammen et al. 2016).

Though an improvement over traditional molecular approaches, reduced representation libraries may also be too sparse to survey adaptive loci because of the random nature with which SNPs are generated. Short fragments are chosen near restriction cut sites and randomly distributed across (mostly) noncoding regions, at best only covering ~5% of the genome (Ekblom and Galindo 2011). This is before filtering processes and SNP quality checks, so the final volume of available SNPs to use in genetic analyses is often less than 5%. Given these considerations, gathering sufficient knowledge of the species' dispersal ecology and the relative importance of anthropogenic barriers (e.g., road density, temperature gradients, degree of impervious surface) to the study organism is suggested before making the genomics leap. For reference, a single representative study on 23 populations of white-footed mice (*n* = 191 individuals) in New York City, NY used a ddRADseq protocol to demonstrate both strong relationships between resistance distance (i.e., the degree of impervious surface) and genetic structuring, as well as an overall negative relationship between urbanization and genomewide variation (Munshi-South et al. 2016).

How Is Standing Genetic Variation Related to Historical Human Development in Cities?

When graded on geologic or ecological timescales, cities have relatively short yet dramatic developmental histories. Punctuated and often sudden human developments can radically alter or eradicate entire habitats on the order of years or decades (Gardner-Santana et al. 2009; Magle et al. 2010). Thus, historical dispersal or migration patterns across a less-developed urban landscape in 1900s may be fundamentally different from patterns in the 21st century (Munshi-South and Nagy 2014). For instance, anthropogenic development around urban fragments may isolate once highly connected habitat patches. This may have led to genetic bottlenecks with high levels of drift in the past that predict genetic structure in the modern era. Historical development may have also established novel niches or corridors that become increasingly permeable for new invaders, creating novel trophic interactions. Hence, modeling historical urban development in cities is invaluable to understanding observed

patterns of genetic diversity and structure in contemporary urban populations (Harris and Munshi-South 2016; Hoban et al. 2016; Lourenço et al. 2017). Yet, demography is often considered a confounding factor (i.e., background noise in a null or neutral model) in identifying signals of selection, so few studies (traditional markers or NGS) focus primarily on historical demographic patterns to understand standing genetic variation (Oyler-McCance et al. 2016; Lowry et al. 2017a).

Using an RNAseq protocol in a study population of 191 white-footed mice (*P. leucopus*), Harris and Munshi-South (2016) demonstrated that urban populations experienced strong bottlenecks with the conversion of 97% of green space into small urban parks over a 400-year period in New York City, NY. Comparatively, Lourenço et al. (2017) used microsatellite markers to demonstrate that declines in effective population sizes of fire salamanders (*Salamandra salamandra*) were concomitant with increasing urbanization in Oviedo, Spain. Both studies suggest functional connectivity is lost with historical rises in urbanization that have longstanding consequences for contemporary standing genetic variation, a hypothesis supported by other authors (Vandergast et al. 2009; Beninde et al. 2016; Krtinić et al. 2016). However, NGS techniques considerably improve the accuracy of estimating some demographic parameters (Shafer et al. 2015). Moreover, single population deviations may weight more heavily in statistical analyses of microsatellite markers, impeding adequate assessment of the role that long-term isolation and demographic history play in genetic variation (Lourenço et al. 2017). If it is reasonable to assume that focal populations exhibited minimal gene flow, then use of a reduced representation sequencing approach may be sufficient (Funk et al. 2016). Investigations into deeper historical time or in relation to specific urbanization events may require greater genomic resolution, thus whole-genome sequencing and differentiation outlier methods using genome-wide SNPs may be most suitable (Liu and Fu 2015; Hoban et al. 2016).

Are Gene–Environment Associations and Expression Patterns the Product of Plasticity or Local Adaptation?

Alberti et al. (2017a) recently conducted an elegant meta-analysis demonstrating that global rates of phenotypic change are greater

in urban versus in natural and nonurban systems. In addition, the authors were able to link specific urban disturbances to more than 1600 examples of animal and plant phenotype modifications (Alberti et al. 2017a). It may be reasonable to predict that these urban signatures, connected to human-associated selective drivers, are underpinned by specific genetic loci or patterns of expression if we consider these phenotypic modifications adaptive (Figure 4). With overwhelming evidence that anthropogenic pressures do elicit phenotypic changes, the next critical step is to establish the mechanistic basis behind these urban signatures. Keen investigation of DNA methylation processes, for instance, may provide insight into how urban and rural habitat differences are transduced into variant phenotypic outcomes (Isaksson 2015; Riyahi et al. 2015). Methylation can profoundly influence gene expression patterns according to environmental conditions (Watson et al. 2017). Hence, this epigenetic mechanism is important for creating phenotypic variation, and may help answer whether observed urban signatures are a product of plasticity or local adaptation (Isaksson 2015; McDonnell and Hahs 2015).

Previous single candidate gene approaches in black swans (*Cygnus atratus*; Payne et al. 2012; van Dongen et al. 2015) and great tits (*P. major*; Riyahi et al. 2017) have proved valuable in discovering specific genotypic markers underlying adaptive behavior in urban birds. In these examples, prior knowledge of the link between a specific suite of candidate genes and behavior (Fidler et al. 2007; Korsten et al. 2010) made a single candidate gene approach sufficient. This process necessarily limits study scope to information on previously identified traits and is unable to provide an exploratory perspective that may discover the multitude of traits and loci that contribute to adaptation (Funk et al. 2012). In addition, traditional candidate gene analyses are ill-equipped to account for polygenic traits or pleiotropy (i.e., a single gene affecting multiple traits; Flint and Munafò, 2013). For these reasons, a transcriptomic or whole-genome approach could prove valuable to addressing genotype–phenotype associations.

Perhaps the most cost-effective sequencing means of establishing gene-phenotype and environment associations may be SNP arrays. Arrays developed within several candidate genome regions could be designed to compare multiple urban individuals and/or population, assuming ascertainment bias is accounted for. Alternatively, a genotyping-by-sequencing (GBS) approach generates a large number of SNPs for use in genotyping with no reference sequence limits

and easy scalability to large populations (Narum et al. 2013; He et al. 2014). SNPs developed from GBS can subsequently be used in genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping to investigate candidate loci related to phenotypic traits (Narum et al. 2013). This is particularly attractive for traits with highly polygenic architectures (Hoban et al. 2016). Moreover, GBS is substantially less complicated than original RAD methods because there are fewer DNA purification steps, and fragments are not size-selected allowing for the assessment of variable fragment lengths across the genome (He et al. 2014).

A third and characteristically different option may be transcriptomic sequencing (i.e., RNAseq), which provides a large number of potentially differentially expressed genes (Wang et al. 2009; Alvarez et al. 2015; Oyler-McCance et al. 2016). For example, urban white-footed mice populations in New York City, NY demonstrate higher levels of expression for candidate genes involved in lipid metabolism, innate immune response, and detoxification (Harris and Munshi-South 2016). The authors posit that expression variation among rural and urban populations is a direct result of variation in individual diets, as urban mice eat more anthropogenic foods rich in fat and carbohydrates. Similarly, compared with rural populations, urban great tits in Malmö, Sweden demonstrated increased expression in genes related to immune and inflammatory responses, detoxification, and genes linked to endocrine stress responses (Watson et al. 2017). The authors suggested that differences in gene expression between urban and rural individuals reflected differential stress-exposure in the 2 contrasting environments (Watson et al. 2017). Both examples strongly underscore the process by which urban–rural differences in the degree of human drivers (i.e., food subsidies, increased disease prevalence in cities) can induce habitat-specific variance in individual expression patterns and phenotypic traits.

There may be several aspects to consider before endorsing an RNAseq protocol. First, minimal degradation to tissue samples and RNA can result in severe loss of technical power in sequencing the transcriptome. Samples must therefore be immediately stored in RNAlater (Ambion, Inc., Austin, TX) solution, liquid nitrogen, Trizol, ethanol, or salt-saturated DMSO to avoid transcriptome degradation (Cammen et al. 2016; Harris and Munshi-South 2016; Oyler-McCance et al. 2016). Second, positively selected genes might be missed if coverage is too low, as they are often expressed in a more tissue-specific manner than evolutionarily conserved genes (Ekblom and Galindo 2011). Finally, a single or multilocus quantitative PCR
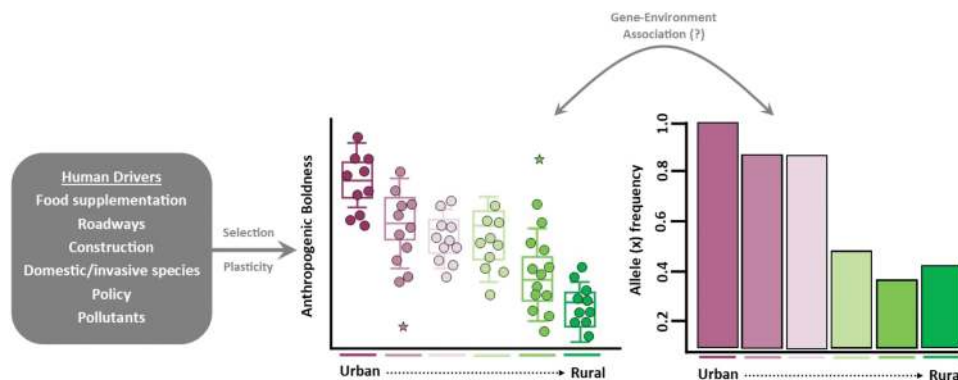

Figure 4. A conceptual model on the eco-evolutionary feedbacks of human drivers on urban phenotypes and underlying genotypes. Anthropogenic drivers may affect the distribution of labile behavioral traits (e.g., boldness) that are often genetically associated. Resultant phenotypes may either fall within the range of trait plasticity or signify gene–environment associations. Disentangling whether such associations are due to demographic history, genetic drift, or positive selection is now more feasible with high-throughput sequencing methods. See online version for full colors.

(i.e., qPCR) could provide as much meaningful information with a fraction of the cost (Alvarez et al. 2015). The qPCR procedure is already used to perform quality checks on tissue samples before RNAseq, and subsequently validates expression postsequencing (Wang et al. 2009), so using a qPCR approach may be less costly and intensive than genomewide RNAseq. However, this is only true if the specific candidate genes are known from previous transcriptomic studies on the taxa of interest. Consequently, for taxa lacking any prior information, a comparative transcriptomics approach may be the most beneficial.

Conclusions and Considerations

Evidence in recent decades has been mounting to suggest microgeographic adaptation is occurring within cities across the globe (Figures 1 and 2). In addition, a number of recent articles have demonstrated convergent phenotypic changes to anthropogenic stressors within cities (Table 1; Supplementary Table S2), underscoring the power of cities in affecting population change (Alberti et al. 2017b; Johnson and Munshi-South 2017). At this moment, we have a unique opportunity as ecologists and evolutionary biologists to decipher the genetic mechanisms that underscore these urban signatures (Figure 4). Genomic tools and inference may provide that additional link to solidifying urban environments and their role as agents of change. In so doing, we can address fundamental questions in both urban ecology and evolutionary biology that seek to understand the processes that facilitate rapid adaptation.

Still, caution is warranted when deciding whether a genomic approach is most appropriate for the study system and question at hand. Careful consideration should be taken in designing a study to maximize statistical power. Moreover, the question should necessarily guide sampling protocol decisions. For instance, if the aim is to determine whether urban and rural populations are genetically divergent, sampling effort uniformly along an urban–rural gradient may not be optimal because admixture among intermediate populations may impede statistical power to detect gene–environment associations (Hoban et al. 2016). However, if the aim is to describe relatedness in relation to the degree of urbanization, uniform sampling along an urban–rural gradient is necessary. Sampling scale is also important, but determining a sufficient sample size is dependent on several complex factors, including the number and type of markers used, the study taxa, habitat structure, and the question of interest (Hoban et al. 2016). Moreover, though not discussed here in great detail, computational requirements, bioinformatics tools, and statistical programs to properly filter and identify SNP variants vary greatly (Helyar et al. 2011; Andrews et al. 2016; Oyler-McCance et al. 2016). A steep learning curve may exist for urban ecologists unfamiliar with the many postsequencing analysis programs that exist (Ekblom and Galindo 2011; Shafer et al. 2015; Andrews et al. 2016; Cammen et al. 2016), which may weigh strongly as a deciding factor in whether implementing genomics is worthwhile. There are no clear-cut techniques that are significantly better than others in addressing the questions posed in this review; thorough consideration of the pros and cons should be properly weighted with constraints of the biological system(s) of interest. Finally, even though having a reference genome helps, repetitive regions or paralogs may be missed in postfiltering and quality control steps because the reference genome is not representative of the study population (Hoban et al. 2016).

These considerations aside, the hope is that this perspective will stimulate future research endeavors implementing genomic approaches to addressing relevant eco-evolutionary questions in urban fauna. Urban expansion will undoubtedly increase throughout the 21st century, and genomic tools will be crucial to determining the evolutionary consequences urban wildlife will face in response.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

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