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Morphological Differentiation in White-footed Mouse (Mammalia: Rodentia: Cricetidae: *Peromyscus leucopus*) Populations from the New York City Metropolitan Area

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ABSTRACT

Genetic studies have shown that New York City white-footed mouse (*Peromyscus leucopus*) populations exhibit substantial genetic structure and high levels of allelic diversity and heterozygosity. These studies have also identified mutations and genes involved in the divergence of urban and rural *P. leucopus* populations. To investigate whether morphological change mirrors the genetic differentiation observed in New York City *P. leucopus* populations, we conducted univariate and multivariate analyses on 4 external and 14 skull variables to compare urban, suburban and rural *P. leucopus* populations from in and around New York City. The only significant morphological differences among the three populations were in upper and lower toothrow lengths, both of which had high loadings in our principal components analyses. In general, rural individuals were found to have longer upper and lower toothrows than urban ones. This difference is likely due to the relationship between food quality and size of dental occlusal surfaces. Generally, lower-quality food requires more chewing and its consumption is facilitated by larger occlusal surfaces. Our results suggest that urban mice consume a higher-quality diet or food that requires less chewing than their rural counterparts by making use of the availability of natural food sources in rich, vegetative understories characteristic of urban forest fragments. Our cluster analysis of the skull variables revealed that urban and suburban populations are more similar to one another than to the rural population.

Introduction

Through industry, pollution and the introduction of invasive species, humans are responsible for rapid and pervasive alteration of the global landscape. Nowhere are humans more influential than in urban contexts. Humans sculpt the urban landscape and oversee many of its ecological processes, directly influencing the ways organisms interact with each other and the environment. More than half of the world's population now occupies urban areas and two-thirds of the world population is predicted to reside in urban areas by 2050 (United Nations 2015). Understanding how natural populations respond to human-dominated landscapes is thus increasingly important. Urban systems deviate from rural systems and are characterized by distinct chemical and physical milieus, dynamic processes and biotic communities. Multiple studies have shown that cities are viable ecosystems in which many organisms flourish (Faeth et al. 2005; McKinney 2008). Ecologists and evolutionary biologists are interested in the ability of species

to adapt to these human-dominated ecosystems (Donihue and Lambert 2015).

In the face of substantial ecological change, organisms can adapt (through evolution or phenotypic plasticity), migrate or go extinct. Urbanization typically results in a considerable loss of biodiversity through habitat loss and fragmentation and the introduction of nonnative species. The destruction and transformation of large continuous natural habitats into small isolated fragmented patches of urban habitats (e.g., city parks) surrounded by barriers to dispersal (e.g., roads and buildings) reduces the number of "urban avoiders," which require large undisturbed contiguous habitats to sustain stable populations (Blair 2001; McKinney 2002, 2006). At the same time that urban avoiders disappear, "urban exploiters" and "urban adapters" rise in numbers. Urban exploiters are primarily nonnative species that take advantage of ecological subsidies from humans (Johnston 2001). Commensal organisms such as pigeons and rats are examples of urban exploiters. Urban adapters are native species that can adapt to urban habitats and use both natural and urban resources (McKinney 2002, 2006). The pattern in which native biotas in urban areas are replaced by nonnative commensals and locally expanding species is observed all over the world and is referred to as biotic homogenization (McKinney 2006).

Urban adapters are of great interest for studying the evolutionary consequences of urbanization in local populations. In particular, the white-footed mouse, *Peromyscus leucopus,* is an emerging model organism for such studies. This North American species is broadly distributed with a range covering the eastern two-thirds of the United States and abutting portions of southern Canada, extending into southern Mexico (Linzey et al. 2008). However, it is absent from the coastal plain areas of the southeastern states and Florida (Linzey et al. 2008). It occupies a variety of habitats throughout its sizeable range, from semideserts to high-elevation forests, achieving highest densities in warm, dry forests and brushy areas at low to middle elevations (Linzey et al. 2008). At present, it is the only *Peromyscus* species in the New York City (NYC) metropolitan area (J. Munshi-South, unpubl. data).

New York City, among the oldest and most developed cities in North America, has the largest population size and density in the United States. Despite the large concentration of people, urban forests and vegetation make up a fifth of the city's land cover (Lu et al. 2009; US Census Bureau 2015). *P. leucopus* inhabits most surveyed, forested areas in NYC, but is replaced by house mice (*Mus musculus*) and Norway rats in urban matrix dominated by impervious surfaces (J. Munshi-South, unpubl. data). In contrast to previous results from nonurban *Peromyscus* populations, *P. leucopus* populations in NYC's forest fragments have high levels of allelic diversity and heterozygosity at neutral loci within populations (Munshi-South and Kharchenko 2010; Munshi-South et al. 2016). In a related study, Munshi-South (2012) found that gene flow among populations occurred through vegetated corridors such as cemetery perimeters, unmowed fencerows and parkway medians. Munshi-South and Nagy (2014) reported that even small (as determined by park area) and very isolated (as determined by the number of years since each park was founded and the number of years since major infrastructure projects were completed around park perimeters) patches of green spaces are adequate to maintain genetic variation because the observed heterozygosity of populations in larger urban parks, or parks that have been isolated for shorter periods, was not greater than that of populations in smaller more isolated urban parks. Harris et al. (2015) identified mutations and genes involved in the divergence of urban and rural *P. leucopus* populations by sequencing pooled mRNA. These genes play roles in xenobiotic metabolism and immunological function.

In response to environmental stresses associated with urbanization, white-footed mouse populations evolved adaptations that better enabled them to deal with new predators, competitors, pathogens, parasites, toxins and pollutants. Munshi-South et al. (2016) found that genomic variation is inversely related to urbanization (as measured by the percentage of impervious surface and human population density; see Materials and Methods) and that urbanization increases genetic differentiation among city populations. Using population genomic modeling, Harris et al. (2016) were able to distinguish recent anthropogenic changes from older climatic events. They also found that the estimated divergence times for several recently isolated *P. leucopus* populations reflect the history of urbanization in NYC. Altogether,

these studies suggest that urbanization drove genetic differentiation in NYC *P. leucopus* through genetic drift and local adaptation in fragmented urban populations with very limited gene flow.

New York City is not the only city where local white-footed mouse populations have experienced genetic change in response to anthropogenic effects. Pergams et al. (2003) reported the replacement of the A haplotype by the M haplotype in the mitochondrial DNA of Chicago white-footed mice between 1855 and 2000. Given that this rapid change in the mitochondrial genotype coincided with urban development in the region, they concluded that the M haplotype likely confers an adaptive advantage in the transformed environment or is unconditionally beneficial and was introduced by human-mediated migration of mice from populations outside the region.

For studying evolution in urban contexts, Donihue and Lambert (2015) proposed a three-part approach: (1) identify phenotypic traits that change with ecological context, (2) distinguish between phenotypic plasticity and natural selection through common garden or reciprocal transplant experiments and (3) identify drivers of adaptation. No study, to the best of our knowledge, has implemented this approach, but several studies have reported both rapid genetic and morphological change in mouse populations. Pergams and Lacy (2008) found sequential directional genotype replacement in Chicago white-footed mice and that contemporary mice had longer external morphological traits and shallower skulls, indicating that the local population was replaced by neighboring populations, likely promoted by ecological changes occurring during the 20th century. More specifically, the contemporary mice were longer in total length, with longer, wider rostra and longer, shallower skulls (Pergams and Lacy 2008).

Among studies that have documented rapid morphological change in mammals, insular rodent studies preponderate, but more recently, there has been increased investigation into morphological change in response to anthropogenic environments (Pergams and Ashley 1999, 2001; Pergams et al. 2015). Pergams and Lawler (2009) reported frequent, rapid, morphological change in rodents driven by expanding human popula-

tion size and climate change. Snell-Rood and Wick (2013) found that for 2 out of 10 small mammal species in their study—the white-footed mouse and the meadow vole (*Microtus pennsylvanicus*)—urban populations had larger cranial capacities (after controlling for body size) than rural populations of these species. However, they did not find increases in cranial capacity over time in urban environments for any species, suggesting that there is initial benefit in behavioral plasticity provided by larger brains; yet after initial colonization of a new but predictable environment, the costs of plasticity cause integration of behavior facilitating city living and a decrease in brain size over time in isolated urban populations.

Tomassini et al. (2014) reported that human population growth in urban Italy, which led to the spread of artificial illumination, concentrated moths and provided *Pipistrellus kuhlii* with a more convenient source of food. The shift in diet from soft-bodied dipterans to hard-bodied moths selected for an increase in brain size without an accompanying increase in general body size. One explanation is that a longer skull facilitates controlling larger prey like moths and a wider cranium provides greater area for the origin of masticatory muscles (Evin et al. 2011; Tomassini et al. 2014). A second explanation is that streetlights presented bats with new challenges, such as developing new foraging approaches to target different forms of prey; these pressures could have selected for larger brains in this species (Tomassini et al. 2014).

In this study, we use categorical comparisons (i.e., urban, suburban and rural; see below) to investigate whether morphological change accompanied genetic differentiation observed in NYC *P. leucopus* populations. In the expectation of selection for increased cognition in urban contexts, we predict that urban populations will have increased cranial capacity relative to suburban and rural populations (Snell-Rood and Wick 2013). Given that urban forests have very dense vegetation compared with suburban and rural forests with high deer densities, and that urban adapters make use of the availability of natural food sources in rich vegetative understories, we also predict that urban populations will exhibit larger body size than suburban and rural populations (McKinney 2006).
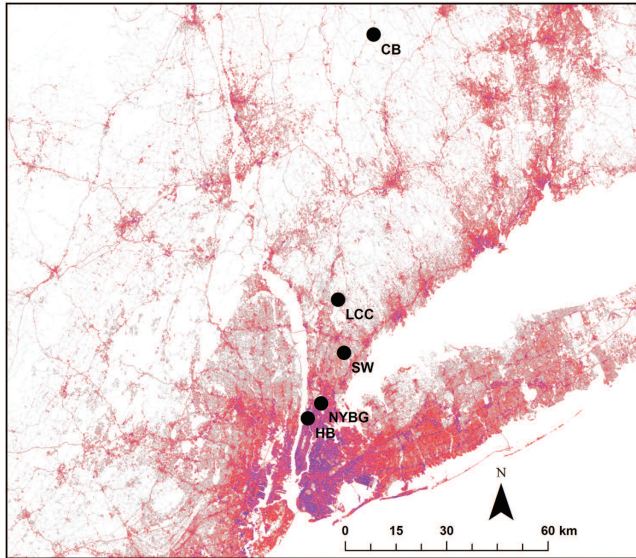
FIGURE 1. Geographic locations of the five sites in the New York City metropolitan area where white-footed mice were collected for this study. Red to purple shading represents increasing percentages of impervious surface cover at 30 m resolution from the 2011 National Land Cover Database (see Munshi-South et al. 2016), from 0 (no shading) to 100% (purple shading). *Abbreviations, from south to north*: Urban: HB, Highbridge Park (New York, NY); NYBG, New York Botanical Garden (Bronx, NY). Suburban: SW, Saxon Woods; LCC, Louis Calder Center (Westchester County, NY). Rural: CB, Cornwall Bridge (Litchfield County, CT).

Materials and Methods

Data Collection

We examined the skeletons and skins of 47 (18 urban, 17 suburban and 12 rural) adult *P. leucopus* specimens collected from five sites in rural Connecticut and parks in and around NYC (Figure 1, Appendix). Sampling sites were classified as urban, suburban or rural using percentage of impervious surface cover and human population density criteria (see Munshi-South et al. 2016). Urban sites consisted of secondary forest typically dominated by oaks, hickories, maples and sometimes tulip trees, with very thick, largely invasive understories. Suburban and rural sites contained similar canopies, but the understories were largely stripped of thick vegetation by deer herbivory. To ensure that the specimens studied were adults, each skeleton was examined to confirm that all teeth had fully erupted, the spheno-occipital (basilar) suture had fused and long bone epiphyses had fused. Of the 18 urban specimens, 11 were collected in the New York Botanical Garden in the Bronx and 7 were collected in Highbridge

Park in Manhattan. For the 17 suburban specimens, 10 were collected in the Louis Calder Center and 7 are from Saxon Woods Park, both in Westchester County, New York. All 12 rural specimens were collected in Cornwall Bridge in Litchfield County, Connecticut. All 47 specimens were collected by Munshi-South between 2014 and 2015, and are now in the mammal collection in the Peabody Museum of Natural History's Division of Vertebrate Zoology at Yale University (see Appendix). This is the first paper to report findings on these specimens. Previous NYC *P. leucopus* studies used tissue samples collected before 2014.

To compare body size, we analyzed the four standard external measurements taken with rulers by Munshi-South and recorded on museum tags: (1) total length (TL), (2) tail length (TAIL), (3) hind foot length (HF) and (4) ear length (EAR). External skull measurements (braincase length, breadth and height) reflect relative cranial capacity, an oft-used proxy for cognitive functioning and intelligence (Finarelli 2006; Cairó 2011; Snell-Rood and Wick 2013). To compare cranial

capacity and other aspects of the skull, a total of 14 cranial and mandibular measurements were recorded by Yu with digital calipers. Eleven of the measurements were taken following Collins and George (1990) (see Pergams and Lacy 2008, fig. 1; Pergams and Lawler 2009, fig. 1), unless otherwise indicated; these measurements include: (5) greatest length of skull (GL), (6) length of braincase (LBC), "measured from the midline of the nasal-frontal suture to the furthest point of the occipital bone" (Finarelli 2006:1028), (7) depth of braincase (DBC), (8) breadth of braincase (BB), (9) zygomatic breadth (ZB), (10) interorbital breadth (IB), (11) breadth of rostrum (BR), (12) length of palate to nasals (LPN), "measured as the greatest distance from the end of the nasals to the mesopterygoid fossa" (Pergams and Lawler 2009:2), (13) upper toothrow length or alimentary toothrow length (AL), (14) length from supraorbitals to nasals (LSN), "measured as the least distance from the supraorbital notch to the tip of the nasals" (Pergams and Lacy 2008:453) and (15) length of incisive foramen (LIF). The remaining three measurements from Sargis et al. (2014) include: (16) mandibular condylo-incisive length (MCIL), (17) mandibular condyle height (MCH) and (18) lower toothrow length (LTL). For AL, LSN, LIF, MCIL, MCH and LTL, the measurements were recorded on the right side. Following Snell-Rood and Wick (2013), all skull measurements were taken to the nearest 0.01 mm. Because of either damage to the skeletons or lack of external measurements recorded by the collector, some measurements were not available for certain specimens. The complete dataset is included in the Supplementary Materials.

Data Analysis

We visually inspected normal probability plots and used the Shapiro-Wilk *W* statistic to determine normality of distribution (Pergams et al. 2015). Holm-Bonferroni sequential corrections were applied to address the issue of running multiple tests. To assess sexual dimorphism, two-sample *t*-tests were done, followed by Holm-Bonferroni sequential corrections.

To determine whether the urban, suburban and rural populations are morphologically distinct from one another, one-way analysis of variance, with the Tukey honest significant difference post-hoc test ($P < 0.05$), was performed on each

variable. We used the Levene statistic to test the assumption of equal variance.

To examine size and shape differences among the three populations, unrotated principal components analysis (PCA) using a correlation matrix was performed on the raw data from the 14 skull variables. To maximize the number of features used in the PCA, two suburban specimens (YPM MAM 013718 and YPM MAM 013723) with many missing measurements from breakage were removed from the analysis; the number of skull variables that could be included increased from 7 to all 14. To assess the degree of similarity among the populations, a cluster analysis (unweighted pair-group average) was conducted on population means.

SPSS, v. 22.0 (IBM Corporation, Armonk, NY) was used for univariate analyses. For all univariate tests, pairwise deletion was used to minimize losses of samples due to missing variables. JMP, v. 12.0 (SAS Institute, Inc., Cary, NC) and STATISTICA, v. 6.0 (StatSoft, Inc., Tulsa, OK) were used for PCA and cluster analysis, respectively.

Results

Univariate

All 18 variables in each of the three populations are normally distributed. There are no significant differences between the sexes for any of the 18 features in the urban, suburban or rural populations. This is consistent with other studies of *Peromyscus* (Rich et al. 1996; Sternburg and Feldhamer 1997; Pergams and Lacy 2008) in which little or no size dimorphism was reported.

In one-way analysis of variance of the 18 morphological variables in the three populations, significant differences ($P < 0.05$) were present in only two skull features: upper toothrow length and lower toothrow length. Tukey honest significant difference post hoc test results show that differences in upper toothrow length ($P = 0.032$) and lower toothrow length ($P < 0.001$) exist between the urban and rural populations. In general, rural individuals have longer upper and lower toothrows than urban ones (Table 1).

Multivariate

In the PCA of 14 skull variables in just the urban and rural populations, PC1 explains 51.4% of the

TABLE 1. Summary statistics for morphological (4 external and 14 skull) measurements. *Abbreviations:* AL, upper toothrow length or alimentary toothrow length; BB, breadth of braincase; BR, breadth of rostrum; DBC, depth of braincase; EAR, ear length; GL, greatest length of skull; HF, hind foot length; IB, interorbital breadth; LBC, length of braincase; LIF, length of incisive foramen; LPN, length of palate to nasals; LSN, length from supraorbitals to nasals; LTL, lower toothrow length; MCH, mandibular condyle height; MCIL, mandibular condylo-incisive length; SD, standard deviation; TAIL, tail length; TL, total length; ZB, zygomatic breadth.

| | Population designation | | | | | | | | |
| | Urban | | | Suburban | | | Rural | | |
Measurements	Mean (mm) ± SD	Range (mm)	n	Mean (mm) ± SD	Range (mm)	n	Mean (mm) ± SD	Range (mm)	n
1) TL	173.47±12.51	149–193	17	169.24±14.05	148–190	17	166.33±13.71	132–179	12
2) TAIL	77.94±5.71	69–89	17	75.47±6.49	62–84	17	78.42±6.11	65–86	12
3) HF	20.79±1.02	19–23	17	20.53±1.01	18–22	17	20.33±0.89	19–22	12
4) EAR	15.74±1.08	14–18	17	15.44±2.08	12–19	17	16.17±1.03	14–18	12
5) GL	26.13±0.64	25.27–27.28	18	25.95±0.94	23.93–27.36	16	25.81±1.13	22.66–26.78	12
6) LBC	17.15±0.34	16.51–17.77	18	16.99±0.59	15.46–17.68	16	16.91±0.69	15.13–17.74	12
7) DBC	7.87±0.25	7.31–8.23	18	7.71±0.30	7.12–8.26	17	7.83±0.28	7.34–8.26	12
8) BB	11.65±0.27	10.93–12.02	18	11.57±0.32	10.83–12.12	17	11.74±0.25	11.17–12.13	12
9) ZB	13.30±0.43	12.71–14.30	18	13.28±0.66	11.63–14.35	17	13.47±0.53	12.05–13.93	12
10) IB	4.14±0.13	3.85–4.32	18	4.11±0.21	3.71–4.49	17	4.07±0.12	3.90–4.38	11
11) BR	4.62±0.16	4.31–4.84	18	4.64±0.23	4.30–4.99	17	4.76±0.27	4.17–5.15	12
12) LPN	14.25±0.47	13.52–15.03	18	14.09±0.56	13.09–14.97	16	14.00±0.64	12.21–14.54	12
13) AL	3.55±0.09	3.42–3.74	18	3.57±0.10	3.42–3.76	17	3.65±0.10	3.52–3.80	12
14) LSN	10.52±0.53	9.73–11.25	18	10.64±0.56	9.82–11.59	16	10.23±0.64	8.54–10.91	12
15) LIF	5.11±0.25	4.73–5.56	18	4.98±0.28	4.26–5.41	17	5.15±0.26	4.49–5.42	12
16) MCIL	16.40±0.60	15.54–17.21	18	16.26±0.72	14.94–17.35	16	16.29±0.79	14.45–17.02	12
17) MCH	6.06±0.25	5.63–6.50	18	5.95±0.38	5.27–6.51	16	6.09±0.39	5.36–6.50	12
18) LTL	3.63±0.11	3.46–3.85	18	3.70±0.09	3.56–3.90	16	3.79±0.10	3.57–3.91	12

TABLE 2. Unrotated factor loadings from a principal components analysis (PCA) of 14 skull variables in only urban and rural samples. Values greater than 0.4 are in bold.

Variable	PC 1 (51.4%)	PC 2 (14.1%)	PC 3 (9.8%)	PC 4 (6.8%)
Skull length (GL)	**0.951878**	–0.018238	–0.095252	–0.215210
Braincase length (LBC)	**0.816617**	–0.072761	0.076511	–0.348349
Braincase depth (DBC)	**0.690859**	–0.274301	0.315149	0.076836
Braincase breadth (BB)	**0.639826**	0.094926	**0.505863**	–0.117074
Zygomatic breadth (ZB)	**0.852653**	0.191476	0.202844	–0.055651
Interorbital breadth (IB)	0.322976	–0.251408	**0.743317**	0.080263
Rostrum breadth (BR)	**0.621794**	0.186955	0.123056	**0.580044**
Palatonasal length (LPN)	**0.932986**	–0.072822	–0.195966	–0.118689
Upper toothrow length (AL)	0.021319	**0.882767**	0.149817	–0.352088
Supraorbital-nasal length (LSN)	**0.828244**	–0.141705	**–0.423871**	–0.208602
Incisive foramen length (LIF)	**0.761550**	0.243271	–0.089236	0.349864
Mandibular condylo-incisive length (MCIL)	**0.909220**	0.078862	–0.164091	–0.035263
Mandibular condyle height (MCH)	**0.742093**	–0.014197	–0.344532	0.321531
Lower toothrow length (LTL)	–0.077648	**0.936393**	–0.010193	0.128315

variance and represents a size axis, with nearly all measures loading positively, except the negatively weighted lower toothrow length (Table 2). Eleven of these 14 measures have loadings >0.6. PC2 explains 14.1% of the variance and is influenced by upper and lower toothrow lengths, both of which have very high positive loadings (>0.88). The third component explains 9.8% of the variance and represents braincase breadth, interorbital breadth and negatively weighted supraorbital-nasal length. The fourth component explains 6.8% of the variance and has the greatest contribution from rostrum breadth. The plot of the first two principal components shows some overlap between the two populations (Figure 2), but along PC2, most rural specimens plot in the upper quadrants and most urban individuals plot in the lower ones, which indicates that the rural population averages longer upper and lower toothrows than the urban one.

In the PCA of 14 skull variables in urban, suburban and rural populations, the first principal component explains 55.3% of the variance and represents a size vector, with all 14 measures loading positively and nearly all loading strongly (Table 3); 12 of these 14 measures have loadings >0.5. The second component explains 13.5% of the variance and is influenced by upper and lower toothrow lengths, both of which have very high positive loadings (>0.9). PC3 explains 8.5% of

the variance and represents braincase breadth, interorbital breadth and negatively weighted supraorbital-nasal length. PC4 explains 5.5% of the variance and has the greatest contributions from interorbital breadth and negatively weighted mandibular condyle height. In the plot of the first two principal components, there is considerable overlap among the three populations (Figure 3), particularly along PC1, indicating similar body size variation. However, along PC2, most rural specimens (10 of 12) plot in positive morphospace, whereas most urban individuals (15 of 18) plot in negative morphospace, indicating longer upper and lower toothrows in the rural population.

In the cluster analysis of population means of the 14 skull variables, the urban and suburban populations are much more similar to one another than to the rural population (Figure 4).

Discussion

Suburbs are difficult to define, as there is no clear agreement on what constitutes a suburban landscape. They have been defined according to many different criteria from population density to location and physical appearance (Forsyth 2012). In this study, suburban areas were defined by the percentage of impervious cover and human population density, but Wittig (2009) argued that a
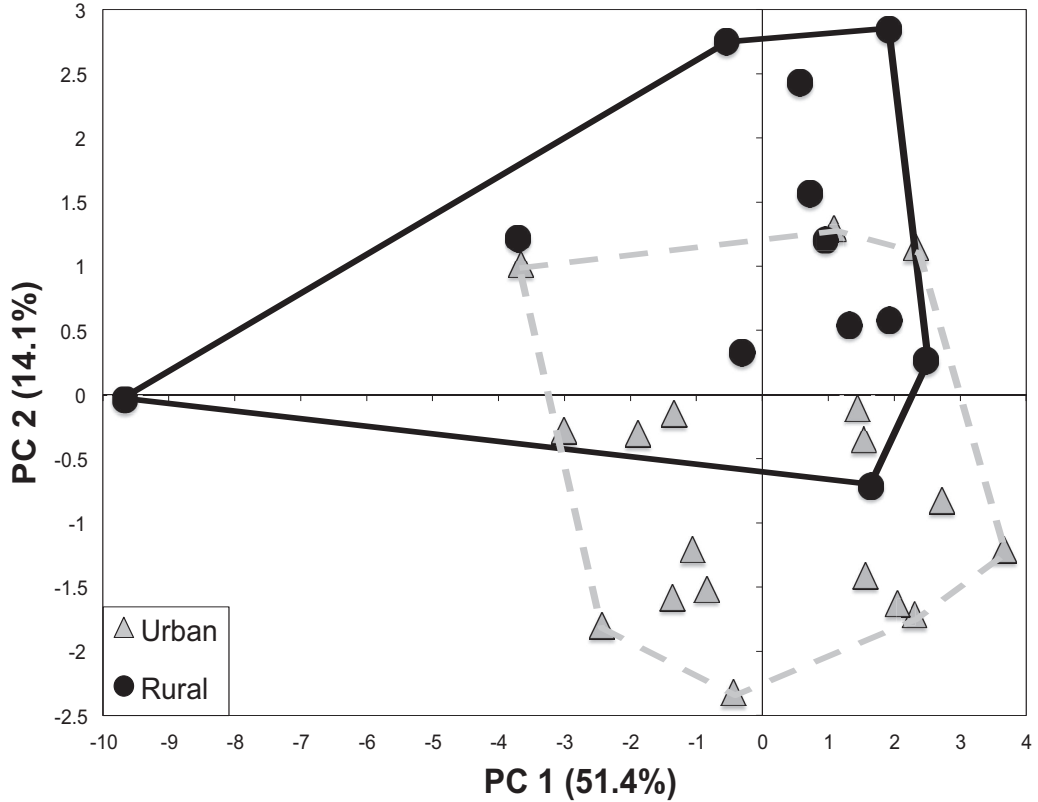
FIGURE 2. Plot of factor scores on the first two axes from a principal components analysis (PCA) of 14 skull variables in only urban and rural samples.

TABLE 3. Unrotated factor loadings from a principal components analysis (PCA) of 14 skull variables in urban, suburban and rural samples. Values greater than 0.4 are in bold.

| Variable	PC 1 (55.3%)	PC 2 (13.5%)	PC 3 (8.5%)	PC 4 (5.5%)
Skull length (GL)	**0.940150**	0.058116	−0.253696	0.128761
Braincase length (LBC)	**0.822270**	0.018502	−0.150470	0.365913
Braincase depth (DBC)	**0.703964**	−0.355670	0.323787	−0.213622
Braincase breadth (BB)	**0.718218**	−0.120864	**0.419451**	−0.041755
Zygomatic breadth (ZB)	**0.913513**	0.096079	0.131401	0.056728
Interorbital breadth (IB)	**0.514965**	−0.200888	**0.558832**	**0.449783**
Rostrum breadth (BR)	**0.669914**	0.176413	0.292250	0.013101
Palatonasal length (LPN)	**0.922526**	−0.024904	−0.301655	0.034328
Upper toothrow length (AL)	0.132872	**0.909419**	0.064461	0.177863
Supraorbital-nasal length (LSN)	**0.809553**	−0.029555	**−0.459399**	0.064771
Incisive foramen length (LIF)	**0.787281**	0.061706	0.118748	−0.360012
Mandibular condylo-incisive length (MCIL)	**0.910242**	0.045852	−0.231384	−0.020964
Mandibular condyle height (MCH)	**0.802975**	−0.069170	−0.027735	**−0.407138**
Lower toothrow length (LTL)	0.061967	**0.905695**	0.169061	−0.195046
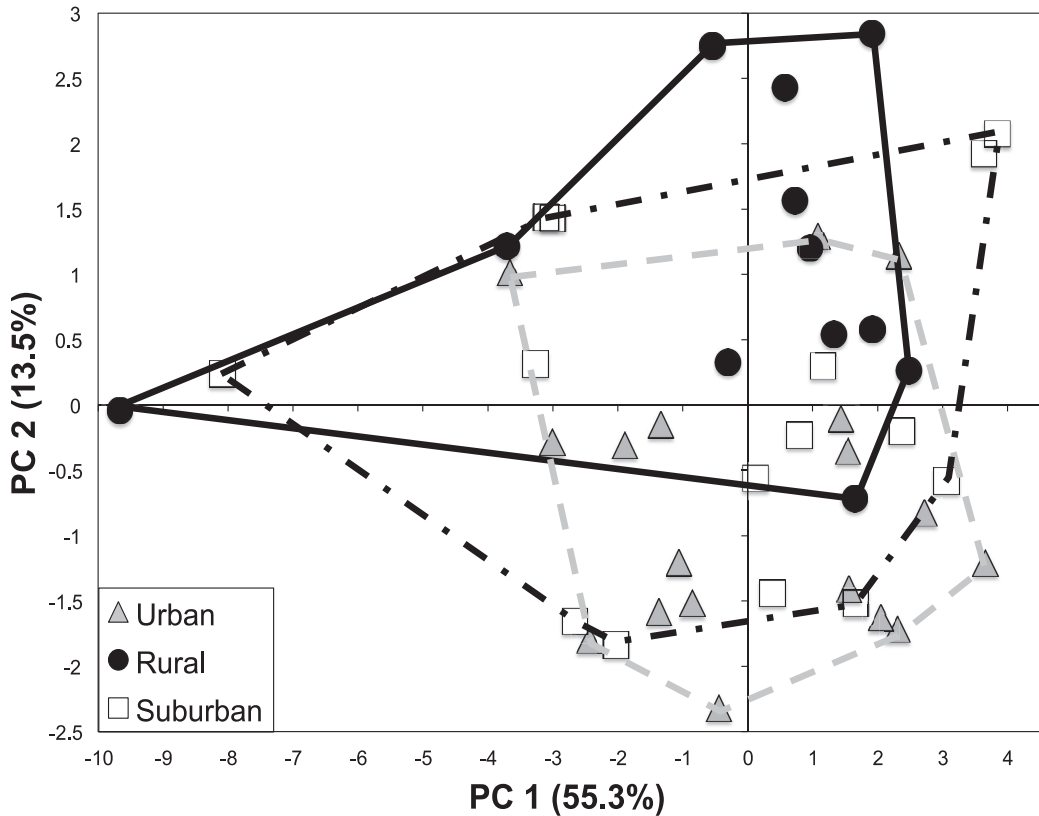
FIGURE 3. Plot of factor scores on the first two axes from a principal components analysis (PCA) of 14 skull variables in urban, suburban and rural samples.

comprehensive understanding of urban ecology must also encompass less densely populated areas in order to see the full spectrum of urban effects. Towns in Westchester County are generally considered suburbs of NYC, but much of the county has a higher human population density than many cities (US Census Bureau 2011). Therefore, urban ecosystems comprise not just city centers, but also suburban areas. In this view, there is no clear demarcation between urban and suburban, so it is not surprising that the urban and suburban *P. leucopus* populations in this study were most similar in their skull morphology.

Urban areas are often characterized by the heat island effect, prevalence of invasive plants as food sources, dense understories in forests and extensive fragmentation (Akbari et al. 2001; White et al. 2002; Zhang et al. 2004; Gaston et al. 2005; Shustack et al. 2009; Munshi-South 2012). Spring seems to appear earlier in cities than in the surrounding rural regions in a phenomenon referred

to as early greenup (Shustack et al. 2009). Urban heat islands are metropolitan areas that are significantly warmer than their neighboring rural areas due to lower vegetation cover, darker surface materials in the urban landscape and human activities (Akbari et al. 2001). The plant configuration of urban and rural areas frequently differs because of horticultural practices (Gaston et al. 2005; McKinney 2006). As a result, urban areas are regularly dominated by exotic and invasive flora that contribute to early greenup and heat islands. Invasive exotic honeysuckle species, including *Lonicera maackii* and *Lonicera japonica,* are principally responsible for earlier greenup of the arboraceous community in urban forests (Schierenbeck 2004; Shustack et al. 2009). They are among the earliest species to produce leaves and are prevalent in the understory of many American urban forests, including NYC parklands (Yost et al. 1991; Kostel-Hughes et al. 1998; Schierenbeck 2004; Shustack et al. 2009). The pre-
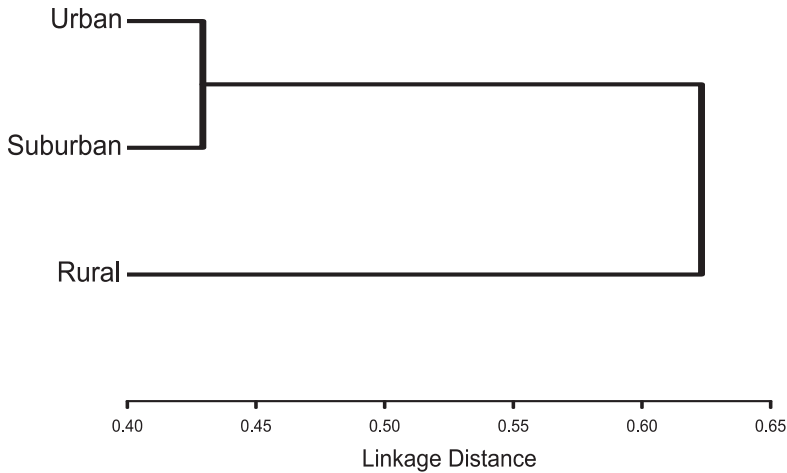
FIGURE 4. Phenogram from a cluster analysis of 14 skull variables, presented with Euclidean distances.

ponderance of these species in cities is likely due to the fact that they were once popularized in North America as ornamental species (Luken and Thieret 1996; Schierenbeck 2004).

Yet another feature of city habitats is extensive fragmentation with a low degree of connectivity between habitat patches. Given the ubiquitous barriers to dispersal (roads and buildings) and few urban greenways (cemetery perimeters, unmowed fencerows and parkway medians), small mammals living within city parklands tend to be somewhat dispersal-limited (Baker et al. 2003; Angold et al. 2006; Munshi-South 2012). Hence, urban adapters such as *P. leucopus* can experience dietary shifts and show changes in foraging behavior to persist within urban parks.

White-footed mice are generalist consumers that feed on a wide range of food items from plant matter (fruits, nuts and green vegetation) to animal foods (insects and eggs) (Wolff et al. 1985). When given the chance to choose among foods with differing nutritional content, *P. leucopus* and other small mammals have been found to distinguish high-quality from low-quality foods and adjust their diet to favor certain foods (Lewis et al. 2001; Muñoz and Bonal 2008; Rose et al. 2014). In particular, although carbohydrates and proteins are complementary nutrients, *Peromyscus* species have been shown to prefer foods higher in protein value than in carbohydrate value (Vickery et al. 1994; Lewis et al. 2001; Shaner et al. 2007). However, white-footed mice have also been found to

feed on less desirable foods even when their protein and energy needs have been satisfied by other resources (Lewis et al. 2001). Furthermore, they consume larger quantities of food on a mixed diet of millet seeds (higher energy content) and mealworms (higher protein content) than when only one type of food is available (Shaner et al. 2007). These studies indicate that for an omnivore such as *P. leucopus*, relative availability of certain foods outweighs overall food availability in deciding foraging behavior.

In this study we found no significant differences in features other than upper and lower toothrow lengths among urban, suburban and rural populations, which departs from previous studies of *P. leucopus* morphology in urban environments (Pergams et al. 2003; Pergams and Lacy 2008; Snell-Rood and Wick 2013). However, it should be noted that Pergams and Lacy (2008) performed their morphological comparisons as time series, whereas all the specimens that we analyzed are contemporary. The differences in upper and lower toothrow lengths between urban and rural *P. leucopus* populations are likely related to the relationship between food quality and size of dental occlusal surfaces. Generally, lower-quality food requires more chewing and its consumption is facilitated by larger occlusal surfaces (Ungar 2010). Taken together, and assuming that there is no simultaneous reduction in tooth width, urban mice seem to consume a higher-quality diet or food that

requires less chewing compared to that consumed by rural mice.

Although unexpected, this is not necessarily difficult to explain. As human population density increases so do the quality and abundance of food sources. Urban mice can choose from natural food sources available in rich, vegetative understories. These thick understories are made up of native and exotic plants that are home to arthropods and can produce novel seeds and fruits (Leston and Rodewald 2006). In contrast, deer populations in rural areas reduce the vegetative understory and inhibit regeneration of many plants, resulting in reduced resources for rural mice (Stewart 2001). Using multiple genome scan and genotype–environment association approaches, Harris and Munshi-South ("Signatures of positive selection and local adaptation to urbanization in white-footed mice (*Peromyscus leucopus*)," preprint, posted 15 September 2016, http://biorxiv.org/content/early/2016/09/15/038141) observed patterns of divergent positive selection between urban and rural populations of *P. leucopus* in the NYC metropolitan area, and discovered that most candidate genes were involved with metabolism, specifically dietary specialization on lipids and carbohydrates. They hypothesized that urban *P. leucopus* consume more fat in their diets from increased seed or invertebrate availability, and that local adaptation facilitates metabolism of increased lipid and carbohydrate proportions. Our results corroborate their hypothesis, but further research on dietary differences and dental specializations among the three populations, and common garden experiments in which individuals from contrasting habitats are raised in controlled captive conditions that assess metabolic traits when mice from different habitats are fed a consistent diet, would both shed more light on this issue.

Phenotypic plasticity is a potential alternative explanation for upper and lower toothrow length differentiation between urban and rural populations. Rapid morphological change potentially explained by phenotypic plasticity has been reported for rodents and bats (Pergams and Lawler 2009; Snell-Rood and Wick 2013; Tomassini et al. 2014). However, it is difficult to distinguish plasticity from adaptive evolution and plasticity itself is subject to evolution (Via et al. 1995; Agrawal 2001; Ghalambor et al. 2007). More information is needed on the heritability of traits examined in morphological comparisons to tease apart plasticity versus adaptive evolution as explanations for morphological change.

The phenomenon of rapid evolutionary change in human-dominated environments is likely widespread and expected to accelerate in the future because of increasing urban settlement, climate change and modification of the environment (Steffen et al. 2015; Venter et al. 2016). Species that are able to respond quickly to human-driven landscape changes will have a higher chance of persisting within these anthropogenic contexts. *P. leucopus* is found in many human-dominated landscapes and is thus a useful model of anthropogenic selection on wildlife. By studying contemporary evolution in cities, biologists and ecologists can gain insights into which species and populations can adapt to extraordinarily novel environments, and how species and populations can be managed to optimize their adaptive potential. The conservation of biodiversity is evermore critical in this age of ubiquitous environmental change.

Acknowledgments

Appendix
Specimens Examined

Specimens examined, by locality. *Abbreviation*: YPM MAM, Division of Vertebrate Zoology Mammal Collection, Peabody Museum of Natural History, Yale University.

Urban

(n = 18)

Bronx, NY (Bronx County): New York Botanical Garden, 40.87163 N, 73.874079 W (YPM MAM 013698, 013699, 013700, 013701, 013702, 013703, 013704, 013705, 013706, 013707, 013708); Manhattan, NY (New York County): Highbridge Park, 40.839182 N, 73.933781 W (YPM MAM 013727, 013728, 013729, 013730, 013731, 016253, 016254).

Rural

(n = 12)

Cornwall, CT (Litchfield County): Cornwall Bridge, 41.787584 N, 73.385292 W (YPM MAM 016255, 016256, 016257, 016258, 016259, 016260, 016261, 016262, 016263, 016264, 016265, 016266).

Suburban

(n = 17)

Armonk, NY (Westchester County): Louis Calder Center, 41.128624 N, 73.73042 W (YPM MAM 013709, 013710, 013711, 013712, 013713, 013714, 013715, 013716, 013717, 013718); White Plains, NY (Westchester County): Saxon Woods Park, 40.988344 N, 73.753852 W (YPM MAM 013719, 013721, 013722, 013723, 013724, 013725, 013726).

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