MOLECULAR ECOLOGY

Molecular Ecology (2010) **19**, 4242–4254 doi: 10.1111/j.1365-294X.2010.04816.x

Rapid, pervasive genetic differentiation of urban white-footed mouse (*Peromyscus leucopus*) populations in New York City

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Abstract

We investigated genetic diversity and structure of urban white-footed mouse, *Peromyscus leucopus*, populations in New York City (NYC) using variation at 18 microsatellite loci. White-footed mice are 'urban adapters' that occur at higher population densities as habitat fragments are reduced in area but have a limited ability to disperse through urbanized areas. We hypothesized that this combination of traits has produced substantial genetic structure but minimal loss of genetic variation over the last century in NYC. Allelic diversity and heterozygosity in 14 NYC populations were high, and nearly all of our NYC study sites contained genetically distinct populations of white-footed mice as measured by pairwise F_{ST}, assignment tests, and Bayesian clustering analyses performed by Structure and BAPS. Analysis of molecular variance revealed that genetic differences between populations separated by a few kilometres are more significant than differences between prehistorically isolated landmasses (i.e. Bronx, Queens, and Manhattan). Allele size permutation tests and lack of isolation by distance indicated that mutation and migration are less important than drift as explanations for structure in urban, fragmented *P. leucopus* populations. *Peromyscus* often exhibit little genetic structure over even regional scales, prompting us to conclude that urbanization is a particularly potent driver of genetic differentiation compared to natural fragmentation.

Keywords: environmental history, habitat fragmentation, microsatellites, New York City, *Peromyscus leucopus*, population genetics, population structure, urbanization

Received 14 May 2010; revision received 30 July 2010; revision accepted 30 July 2010

Introduction

Urbanization represents one of the most pervasive forces of anthropogenic change over the last century. More than 50% of the human population now occupies urban areas, and most ecosystems will experience urbanization in the near future (Crane & Kinzig 2005; Palmer *et al.* 2005). Severe habitat fragmentation is a frequent outcome of urbanization, and remnant urban habitat patches are typically managed as patches with discrete, high-contrast edges (e.g. city parks). These urban patches are likely to be small in size, dominated by invasive species, and surrounded by barriers to dispersal (Mahan & O'Connell 2005), but do contain the necessary features to support native species with small home range requirements. However, small urban patches often contain extremely high population densities of just a few urban 'adapters' (Shochat 2004), leading to biological homogenization in urban landscapes (McKinney 2006). Few studies have examined the molecular ecology of urban wildlife, despite urban ecosystems providing ready-made 'experiments' of the evolutionary effects of fragmentation. Here, we examine population genetic differentiation of an archetypal urban adapter, the white-footed mouse (*Peromyscus leucopus*), inhabiting small, isolated forest fragments in New York City (NYC).

Species differences influence the genetic consequences of urbanization, but only a single species that is a poor

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disperser through the urban matrix has been examined to date; Noel *et al.* (2007) found that eastern red-backed salamanders in isolated urban fragments become genetically structured and lose genetic variation. Population structure among urban red foxes (Wandeler *et al.* 2003), great tits (Bjorklund *et al.* 2010), and grassland butterflies (Wood & Pullin 2002) has also been reported, in all cases owing to increased isolation in urban patches. However, the isolating mechanisms vary widely, including founder effects (foxes), source-sink dynamics (tits), and lack of suitable habitat for dispersers (butterflies) after urbanization. Human commensals such as the Norway rat exhibit moderate gene flow through the urban landscape, preventing substantial genetic structure from developing (Gardner-Santana *et al.* 2009). Additional studies are needed to understand the genetic implications of urbanization for terrestrial vertebrates with limited urban dispersal capabilities; such species probably constitute the majority of nonvolant taxa in regional species pools.

Urban adapters achieve unusually high population densities as a result of artificially high primary productivity (Pickett *et al.* 2001; Shochat *et al.* 2006), less severe temperature fluctuations as a result of the 'heat island' effect (McKinney 2002), a more stable and abundant food supply from invasive plants and other human supplementation (Shustack *et al.* 2009), and release from trophic pressures in urban environments (Faeth *et al.* 2005). These populations may also become crowded owing to an inability of residents to disperse across urban landscapes (Grear & Burns 2007), resulting in a breakdown of connectivity in urban metapopulations. Genetic structure and loss of heterozygosity among populations of urban adapters will depend on genetic drift and the amount of migration through the landscape, if any, and whether effective population sizes remain sufficiently large to maintain genetic diversity.

Studies from multiple regions have reported a *negative* relationship between habitat patch area and population density of white-footed mice (Nupp & Swihart 1996; Bowers & Matter 1997; Krohne & Hoch 1999; Anderson *et al.* 2003; Wilder & Meikle 2005). White-footed mouse populations respond positively to road density (Rytwinski & Fahrig 2007), and high-density populations in small patches exhibit a higher genetic emigration rate than lower-density populations in large patches (Anderson & Meikle 2010). A positive response to roads is at odds with the vast majority of studies that find avoidance or population decline of mammals around transportation infrastructure (Benítez-López *et al.* 2010). When the intervening matrix is permeable, such as in forest-agriculture mosaics, white-footed mouse populations retain genetic variation despite habitat fragmentation (Mossman & Waser 2001). The closely related deer mouse, *P. maniculatus*, does not exhibit substantial genetic structure over even broad geographic regions because contemporary gene flow breaks down differentiation at hypervariable loci (Great Lakes region, Taylor & Hoffmann 2010; west coast of North America, Yang & Kenagy 2009). However, changes in relative frequencies of particular mitochondrial haplotypes over the last century may be correlated with urbanization, indicating possible adaptations to anthropogenic pressures (Pergams *et al.* 2003; Pergams & Lacy 2008).

NYC is one of the most highly developed regions in the world, but natural areas comprise 20% of NYC's land cover (Lu *et al.* 2010). NYC is also comprised of four separate landmasses (the Bronx, Queens and Brooklyn on western Long Island, Manhattan Island, and Staten Island) each containing multiple white-footed mouse populations, facilitating comparisons of genetic differentiation among populations on an individual landmass to differentiation between landmasses. In this study, we examine white-footed mice from 15 populations on three landmasses in NYC to understand the genetic impacts of severe fragmentation on an urban adapter with limited dispersal ability. To this end, we use genotypes of 18 microsatellite loci, traditional summary statistics of genetic variation and population differentiation, and recently developed evolutionary clustering analyses. We predict that white-footed mice in NYC, in contrast to previous results from nonurban *Peromyscus* populations, will exhibit considerable levels of genetic diversity and structure over microgeographic scales owing to high-contrast ecological boundaries between habitat patches and the impermeable urban matrix, high population densities in small patches that promote the persistence of *de novo* private mutations, and enhanced genetic drift owing to rapid onset of fragmentation from urban infrastructure.

Methods

Field sampling

We conducted trapping surveys for white-footed mice from June to October 2008 and May to October 2009 at 14 sites in NYC and one rural area (site 1) 67 km north of Central Park (site 6, Fig. 1) in Manhattan. All trapping sites consisted of a largely invasive vegetative understory and Appalachian oak-hickory or successional northern hardwoods canopy as defined by Edinger *et al.* (2002), except for sites 10 (successional oldfield), 12 (successional shrubland), and 13 (salt marsh edge). Mice were trapped at each site over three nights using one to four arrays of 49 Sherman live traps

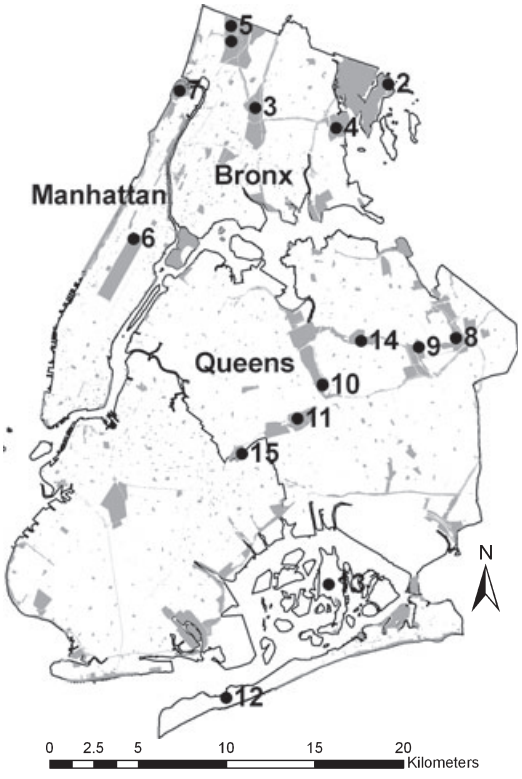

Fig. 1 Location of trapping sites in New York City (NYC). Not pictured is site no. 1, Black Rock Forest, located ~67 km north of no. 6, Central Park. Site 5 contained two sampling locations on either side of a major roadway and is thus represented by two points. Grey areas represent NYC parklands.

(aluminium, 3″ × 3″ × 9″) placed 15 m apart in 7 × 7 grids. Additional trapping surveys were conducted at some sites to achieve a target sample size of 20, although we did not trap this many individuals from a few low-density sites even after two to three surveys (Table 1). Traps were internally baited with birdseed a few hours before sunset and checked and closed each morning. Captured animals were removed from the traps to be sexed and weighed, and then, the terminal 1 cm of the tail was snipped and stored in 90% ethanol for genetic analyses. Recaptured individuals were identified by the tail snipping and released without further handling. All animal handling procedures were approved by the Animal Care and Use Committee at the authors' institution.

DNA extraction, microsatellite genotyping, and genetic variation

We extracted DNA from 312 individual tail snips using the protocol for rodent tails provided with the Qiagen DNEasy tissue kit. Eighteen previously described microsatellite loci (Table 2) were PCR-amplified in 25 μL volumes using PuReTaq Ready-to-Go PCR beads (GE Healthcare, UK) and published thermal cycling profiles (Table 2). PCR of loci from Mullen *et al.* (2006) included one primer with a CAG or M13R tail and an associated probe with one of three fluorescent dyes (WellRED D2, D3, or D4), whereas other loci (Schmidt 1999; Chirhart & Honeycutt 2000) were amplified using a fluorescently labelled forward primer. PCR products were pool-plexed in panels of two to three loci distinguished by fluorescent dye and/or allele size ranges, and fragments were separated and sized alongside a Wellred-labelled size standard on a Beckman Coulter CEQ8000 automated capillary sequencer. Alleles were scored using automatic binning procedures followed by visual inspection using the Beckman Coulter fragment analysis software. Genotypes were then imported into GenAlex 6.2 (Peakall & Smouse 2006) for subsequent analysis and conversion to other file types.

Each locus was tested for departures from Hardy–Weinberg equilibrium over the entire sample of 312 genotypes and within each of the 15 trapping sites using Genepop 4.0 (Raymond & Rousset 1995; Rousset 2008) with a Bonferroni correction for multiple tests. Linkage disequilibrium over the entire sample and within each population was examined for each locus pair using 10 000 permutations and a correction for multiple tests in FSTAT 2.9 (Goudet 2002). To characterize the genetic diversity of white-footed mice in NYC, we calculated the number of alleles, observed and expected heterozygosity, and F_{ST} at each locus, and the mean number of alleles, effective alleles, private alleles, observed and expected heterozygosity, and F_{ST} for all loci at each site, using GenAlex.

Population structure

As a preliminary evaluation of population structure, we calculated pairwise F_{ST} values in GenAlex using 10 000 random permutations of the data and Bonferroni correction to assess significance. Microsatellite allele sizes are potentially more informative than allelic identity for measuring genetic differentiation (e.g. as measured by R_{ST}), assuming that microsatellites follow a stepwise mutation model and the mutation rate is higher than the migration rate or time since divergence between populations (Hardy *et al.* 2003). To examine this possibility, we used the allele size permutation test in SPAGeDi 1.1 (Hardy & Vekemans 2002). This procedure tests the null hypothesis of no difference between F_{ST} and R_{ST}, and simulations show that it finds significant differences when one statistic has a lower mean square error than the other (Hardy *et al.* 2003). Given that genetic structure is likely to be influenced by the extent

Table 1 Study sites, sample sizes, and genetic characteristics of white-footed mice at each site estimated using genotypes at 18 microsatellite loci. All locus pairs were in linkage equilibrium

Site no.	Site	Km from Central Park	N	N_A	N_E	N_P	H_O	H_E	F_{ST}	HWD
1	Black Rock Forest	67.2	18	12.6	9.1	11	0.793	0.874	0.091	Bw4-13, Bw4-93, Bw4-249, Pml04
2	Hunters Island	16.9	27	8.9	5.3	6	0.770	0.800	0.039	Bw4-249, Pml06
3	NY Botanical Garden	10.2	33	10.5	5.9	4	0.775	0.816	0.046	Bw4-93, Bw4-249
4	S. Pelham Bay	13.1	14	7.8	5.1	1	0.689	0.772	0.111	Bw4-13, Bw4-129
5	Van Cortlandt Park	13.3	24	11.3	7.3	10	0.763	0.853	0.102	Bw4-129, Bw4-249
6	Central Park	0	15	7.3	5.1	3	0.752	0.785	0.032	Bw4-249
7	Inwood Hill Park	8.9	20	10.1	6.0	9	0.819	0.830	0.012	Bw2-1
8	Alley Pond Park	19	9	7.1	5.2	2	0.736	0.789	0.063	Bw4-13
9	Cunningham Park	17.1	25	10.9	6.6	6	0.769	0.829	0.067	Bw4-13, Bw4-93
10	Flushing Meadows	13.4	30	9.3	5.8	4	0.734	0.809	0.090	Bw4-13, Bw4-249
11	Forest Park	13.6	11	8.5	5.9	3	0.766	0.807	0.054	Bw4-249
12	Fort Tilden	26.3	18	7.0	4.5	1	0.727	0.746	0.023	
13	Jamaica Bay	22.4	11	6.4	4.5	5	0.625	0.761	0.177	Bw4-13, Bw4-93
14	Kissena Park	14.1	24	9.8	5.6	8	0.730	0.783	0.070	Bw4-249
15	Ridgewood Reservoir	13.5	33	9.9	5.7	6	0.736	0.810	0.087	Bw4-13, Bw4-93, Bw4-249
	Over all sites		312	9.2	5.9	5.3	0.746	0.804	0.071	

N, sample size; N_A, mean number of alleles; N_E, mean effective number of alleles; N_P, number of private alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; HWD, loci exhibiting Hardy–Weinberg disequilibrium. Data available at Dryad (doi: 10.5061/dryad.1893).

Table 2 Characteristics of 18 microsatellite loci genotyped in 312 mice from 15 populations

Locus	Source	Allele size range	N_A	H_O	H_E	F_{ST}
Bw2-1	Mullen *et al.* (2006)	135–163	17	**0.783**	0.897	0.126
Bw4-7	Mullen *et al.* (2006)	392–434	24	0.827	0.932	0.111
Bw4-13	Mullen *et al.* (2006)	157–237	22	**0.486**	0.917	0.469
Bw4-54	Mullen *et al.* (2006)	154–246	16	**0.601**	0.735	0.182
Bw4-93	Mullen *et al.* (2006)	269–409	29	**0.719**	0.943	0.236
Bw4-129	Mullen *et al.* (2006)	264–318	33	**0.721**	0.948	0.238
Bw4-137	Mullen *et al.* (2006)	182–254	18	0.803	0.898	0.105
Bw4-178	Mullen *et al.* (2006)	253–393	33	0.864	0.934	0.074
Bw4-200	Mullen *et al.* (2006)	297–341	14	0.753	0.858	0.121
Bw4-249	Mullen *et al.* (2006)	215–339	32	**0.548**	0.936	0.414
PLGT15	Schmidt (1999)	237–267	16	0.827	0.884	0.064
PLGT58	Schmidt (1999)	136–168	18	0.802	0.875	0.082
PLGT62	Schmidt (1999)	148–180	18	0.845	0.879	0.038
PLGT67	Schmidt (1999)	253–283	15	0.833	0.883	0.056
Pml01	Chirhart & Honeycutt (2000)	155–183	19	0.798	0.913	0.125
Pml04	Chirhart & Honeycutt (2000)	191–277	28	0.810	0.907	0.105
Pml06	Chirhart & Honeycutt (2000)	135–169	19	**0.706**	0.896	0.211
Pml10	Chirhart & Honeycutt (2000)	168–252	40	**0.801**	0.939	0.145
Mean			22.8	**0.7515**	0.897	0.161

Deviations from Hardy–Weinberg equilibrium when genotypes from all sites were pooled are denoted in bold for $P < 0.05$ after Bonferroni correction. All locus pairs were in linkage equilibrium.
N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

of dispersal between populations, we also examined isolation by distance in NYC using a Mantel test to compare linearized pairwise F_{ST} and the linearized Euclidean distance between populations to 10 000 randomizations of the values in zt (Bonnet & Van de Peer 2002). We performed Mantel tests for all 15 sites, all six sites in Bronx and Manhattan, and all eight sites in Queens.

We hypothesized that white-footed mice in NYC are genetically structured owing to genetic drift in allopatry both on different landmasses (i.e. Black Rock Forest, Bronx, Manhattan Island, and Queens, Long Island; Fig. 1) and between forest sites fragmented by urbanization on each landmass. To examine hierarchical genetic differentiation, we used analysis of molecular variation (AMOVA) in GenAlex to calculate the genetic variation explained among regions, among populations, and within populations. *P*-values for fixation indices were calculated using 10 000 permutations of the data.

To examine whether individuals were residents of genetically differentiated populations inhabiting the site from which they were trapped, we used GENECLASS2 (Piry *et al.* 2004) with the leave-one-out option to assign or exclude individuals to their hypothesized population (i.e. trapping site) using both a Bayesian (Rannala & Mountain 1997) and likelihood-based approach (Paetkau *et al.* 2004). The latter approach was also used to identify first-generation migrants, and all tests were based on assignment criteria computed from 10 000 simulated populations of the same size as the sample populations. Preliminary analyses showed that the Black Rock Forest mice often could not be assigned and that many NYC individuals were assigned a substantial percentage of ancestry to Black Rock Forest. Because of the biological implausibility of recent migration between NYC and a site ~70 km to the north, we reran these analyses without the Black Rock population.

We used multiple clustering methods to estimate the number of evolutionarily distinct *P. leucopus* populations in NYC because simulations suggest that some analyses perform better than others under certain conditions (*i.e.* Latch *et al.* 2006). First, we used Structure 2.3 to place individual genotypes in evolutionary clusters that minimized departures from Hardy–Weinberg and linkage equilibrium (Pritchard *et al.* 2000). We did not incorporate prior information on population membership and used options for correlated allele frequencies and genetic admixture across populations (Falush *et al.* 2003). Ten replicate runs for each value of $K = 1$ through $K = 20$ were used to calculate the probability of the data, $\Pr(X \mid K)$. For all 200 runs, we used a burn-in of 100 000 Markov chain Monte Carlo (MCMC) steps followed by an additional 500 000 iterations. The mean and standard deviation of $\Pr(X \mid K)$ from the 10 replicate runs were used to find the most likely value of K (Fig. 2). We also used the ΔK statistic, based on the rate of change in log $\Pr(X \mid K)$ for successive assumed K values, to assess the Structure results (Evanno *et al.* 2005). Replicate Structure runs may switch labels for individual clusters or arrive at different clustering patterns altogether, so we used the LargeKGreedy algorithm in CLUMPP 1.1 (Jakobsson & Rosenberg 2007) to align the 10 replicates for the K value with the highest $\Pr(X \mid K)$. The alignment was then visualized as a bar chart using Distruct 1.1 (Rosenberg 2004).

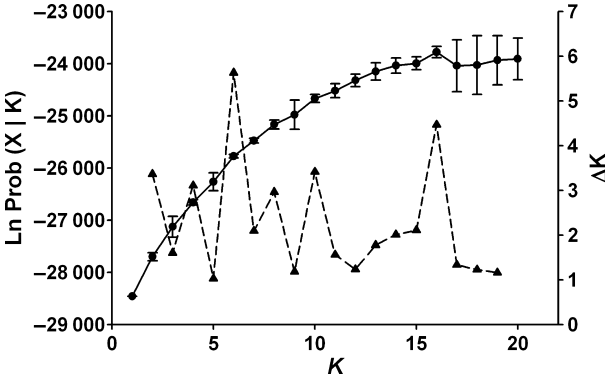

Fig. 2 Average and standard deviation of the log likelihood of the data ($\Pr(X \mid K)$) from 10 replicate Structure runs assuming $K = 1$–20 (solid line, left axis), and ΔK calculated from these runs using the method of Evanno *et al.* (2005; dashed line, right axis).

For comparison with Structure's analytical approach, we used Structurama to calculate the number of evolutionary clusters, assuming that both individual membership and K are random variables that follow a Dirichlet process prior (Huelsenbeck & Andolfatto 2007). All Structurama runs were conducted with a burn-in of 100 000 MCMC steps followed by additional 1 000 000 iterations. Structurama has not been thoroughly assessed in simulation studies, so we ran five replicates assuming that the prior expected K was a random variable, and five replicates with a prior $K = 15$ (i.e. the number of sampling sites), to examine the importance of this prior parameter to inferences about K. We assessed support for K values using (i) the distribution of Bayesian posterior probabilities $\Pr(K = i \mid X)$ that are proportional to the time Structurama samples a particular clustering of individual genotypes and (ii) a mean partition that minimizes the sum of the squared distances to the partitions sampled by MCMC (Huelsenbeck & Andolfatto 2007).

We also evaluated the presence of distinct evolutionary clusters among predefined groups of individuals (N = our 15 sampling sites) using a Bayesian approach (Corander *et al.* 2003) in BAPS 5.2 that incorporates the spatial proximity of the groups as prior information ('spatial clustering of groups' module, Corander *et al.* 2008). The best value of K was estimated using the log marginal likelihood values of the best partitions and the distribution of posterior probabilities for different K values. The best partition was visualized using a Voronoi tessellation in BAPS with spatial clusters of groups represented by unique colours.

Results

Allelic diversity and observed heterozygosity were generally high for all 18 microsatellite loci when considering the full 312 genotypes (Table 2). Approximately half of the loci exhibited a significant heterozygote deficiency, $F_{ST} > 0.1$, and deviation from Hardy–Weinberg equilibrium as a result of a Wahlund effect (Table 2). Within each study site, only one to three loci were typically not in Hardy–Weinberg equilibrium despite small sample sizes for some sites (Table 1). All locus pairs were in linkage equilibrium over the entire data set and within each population. Genetic diversity was relatively high at each site as measured by the mean number of observed and effective alleles; most sites contained substantial numbers of private alleles (Table 1). The single nonurban, undisturbed site, Black Rock Forest (Site 1), had the highest expected heterozygosity, highest number of observed, effective, and private alleles, (Table 1), but also the largest number of loci deviating from Hardy–Weinberg equilibrium.

Allele size permutation tests in SPAGeDi were nonsignificant for the entire data set and all pairs of populations, indicating that F_{ST} is superior to R_{ST} for measuring differentiation among our study sites. Pairwise F_{ST} values were highly significant in all cases. Pairwise $F_{ST} > 0.1$ was observed in 19% of population pairs (20 out of 105 comparisons), and $F_{ST} > 0.05$ was observed in 89.5% of pairs (94 out of 105 comparisons, Table 3). Even, populations separated by a few kilometres on the same landmass typically exhibited $0.05 < F_{ST} < 0.09$ (Table 3). The total data set from 15 trapping sites did not show a pattern of isolation by distance ($r = -0.25$, $P = 0.11$), nor did the eight sites in Queens ($r = 0.05$, $P = 0.41$). The six Bronx and Manhattan sites did show moderately significant isolation by distance ($r = 0.539$, $P = 0.02$).

The AMOVA revealed that much more genetic variance could be explained by differentiation between trapping sites than between landmasses (i.e. regions, Table 4). Most of the genetic variance was contained within the populations themselves, but substantial values were found for fixation indices calculated between study sites compared to the total (F_{ST}) or to landmasses (F_{SR}, Table 4). Population assignment in GENECLASS2 was 93.2% successful (i.e. individual genotypes were assigned to the urban site from which they were trapped) using both the Bayesian (Rannala & Mountain 1997) and likelihood-based approaches (Paetkau *et al.* 2004). The latter analysis identified 23 possible migrants (7.8%), but in all cases, the trapping site was still the population with the highest calculated likelihood.

Genetic clustering analyses indicated the presence of multiple, unique evolutionary clusters of white-footed mice in NYC. The highest probability of the data, $\Pr(X \mid K)$, was calculated using Structure for $K = 16$ (Table 5), and the standard deviation of $\Pr(X \mid K)$ began to increase substantially at $K > 16$ (Fig. 2). The ΔK statistic of Evanno *et al.* (2005) was highest at $K = 6$ (Fig. S1, Supporting Information) but exhibited a multimodal distribution and secondary peak at $K = 16$ (Fig. 2). Alignment of the 10 replicate Structure runs and visualization of cluster membership for $K = 16$ showed that most genotypes were assigned to the site from which they were sampled (Fig. 3). Notable

Table 3 F_{ST} values calculated between all pairs of study sites

Site no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	—														
2	0.064	—													
3	0.047	0.093	—												
4	0.050	0.090	0.078	—											
5	0.030	0.073	0.058	0.059	—										
6	0.062	0.093	0.099	0.111	0.070	—									
7	0.038	0.071	0.062	0.079	0.051	0.072	—								
8	0.036	0.088	0.085	0.092	0.055	0.079	0.057	—							
9	0.041	0.072	0.073	0.082	0.058	0.076	0.053	0.040	—						
10	0.058	0.098	0.089	0.108	0.078	0.095	0.077	0.070	0.045	—					
11	0.040	0.079	0.079	0.077	0.062	0.078	0.060	0.050	0.032	0.048	—				
12	0.082	0.100	0.123	0.134	0.102	0.116	0.105	0.094	0.083	0.127	0.104	—			
13	0.065	0.101	0.110	0.113	0.086	0.094	0.084	0.080	0.067	0.097	0.072	0.119	—		
14	0.062	0.107	0.107	0.118	0.077	0.086	0.083	0.073	0.080	0.096	0.067	0.104	0.101	—	
15	0.058	0.078	0.085	0.091	0.070	0.079	0.059	0.063	0.051	0.070	0.042	0.113	0.088	0.076	—

All values were significant at $P < 0.0001$ based on 10 000 random permutations of the data ($P < 0.05$ in all cases after Bonferroni correction). Site numbers correspond to Fig. 1.

exceptions were Site 1, located north of NYC, and Sites 8, 9, and 11, which are located near each other in a relatively linear pattern in Queens and contained individuals with admixed genotypes. Site 5, Van Cortlandt Park, contained two separate clusters not present in other sites, accounting for an estimated K that was one cluster greater than the number of sampling sites (denoted by two sampling points for Site 5 in Fig. 1).

Table 4 Differentiation among regions [i.e. populations in New York City (NYC) boroughs on different landmasses] and populations determined using analysis of molecular variance (AMOVA)

Source	Variance explained	P-value	Fixation index
Among regions	0.4%	<0.0001	F_{RT} = 0.004
Among populations	7.8%	<0.0001	F_{SR} = 0.079
Within populations	91.8%	<0.0001	F_{ST} = 0.082

P-values were based on 10 000 permutations of the data. Regions include Black Rock Forest (Population 1), Bronx (Populations 2–5), Manhattan (Populations 6–7), and Queens Co., Long Island (Populations 8–15).

Table 5 Results of three computational approaches to estimating the number of evolutionary clusters, K, among 312 white-footed mice trapped from 15 sites

Analytical approach	Criterion	Estimated K	Figures
Structure 2.3	$Pr(X \mid K)$	16	2 and 3
	ΔK	6	2 and 3
BAPS 5.0	Spatial clustering of groups	12	4
Structurama 1.0	Prior K = random variable	7.75	5
	Prior K = 15	8.48	5

See text for full description of analyses and prior parameters.

The BAPS analysis incorporating spatial orientation of sampling sites as prior information estimated K = 12 evolutionary clusters of populations (Table 5). The optimal partition had a posterior probability of 99.8% compared to partitions of different K values. All sampling sites were classified as separate clusters except for one cluster containing sites 1, 8, 9, and 11 (Fig. 4). The latter three sites are in proximity and also contained many admixed genotypes in the Structure analysis (Fig. 3).

The most likely value of K and average posterior probabilities from five runs of Structurama was similar (K = 8 clusters; Table 5, Fig. 5) when the expected prior K was set to the number of sampling sites [$E(K \mid X)$ = 8.48] or treated as a random variable with a relatively flat gamma distribution [$E(K \mid X)$ = 7.75]. Both analyses identified three unique clusters in the Bronx (Sites 2–4), two unique clusters among eight sites in Queens (Sites 8–15), and three remaining clusters, among others, at two sites containing genotypes that could not be readily assigned (Site 1, 5). Genotypes from the two Manhattan sites (Sites 6–7) were distributed among the Bronx and Queens clusters.

Discussion

White-footed mice are urban adapters that occur at high population densities in disturbed habitats (Wilder & Meikle 2005; Rytwinski & Fahrig 2007). Our results from multiple analytical approaches clearly show that urban fragmentation produced rapid, substantial genetic structure of *P. leucopus* populations in NYC. Traditional F-statistics, assignment tests, and Bayesian clustering analyses indicated that all or most of the 14 habitat fragments we sampled within NYC contained genetically differentiated populations. Queens and Bronx counties were not substantially urbanized until the early 20th century, suggesting that this population structure developed in less than a century or two- to three-hundred *P. leucopus* generations (assuming that white-footed mice in our study areas breed at least two to three times every year and breeding females consist

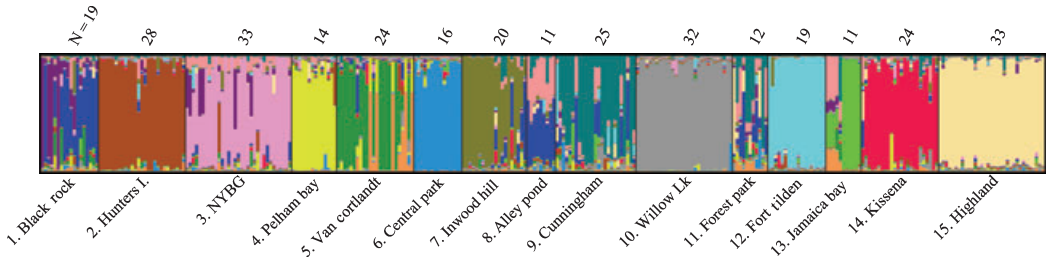

Fig. 3 Sixteen evolutionary clusters inferred from Structure analysis of 312 mice from 15 sites. Each colour represents an inferred cluster, and each individual is represented by a vertical line coloured according to its probability of assignment to each cluster. Sample sizes appear above each site, and numbers before site names correspond to locations in Fig. 1.

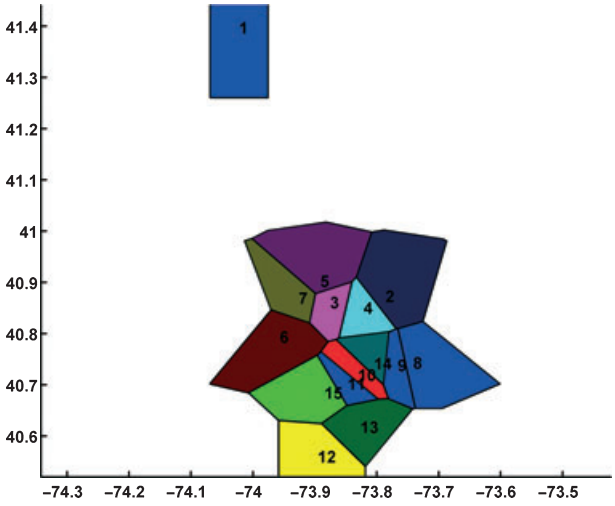

Fig. 4 Twelve evolutionary clusters of populations inferred using the spatial model in BAPS 5.3. Axes represent spatial *X*, *Y* coordinates. All sites classified separately except for one cluster containing Black Rock (Site no. 1), Alley Pond (8), Cunningham (9), and Forest Parks (11).

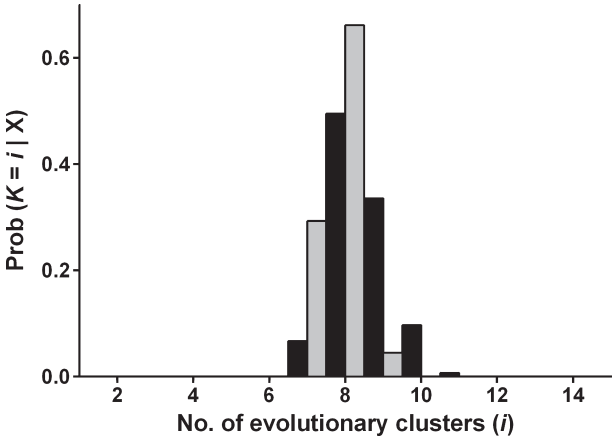

Fig. 5 Average posterior probabilities of the number of evolutionary clusters inferred from five Structurama runs of 312 mice from 15 sites. Black bars correspond to runs with an expected prior $K = 15$ (i.e. the number of sampling sites), and grey bars correspond to runs with the prior K set as a random variable with a relatively flat gamma prior distribution.

of adults from the previous year and female offspring from that year's early litters; J. Munshi-South, unpublished data). However, these fragmented populations have not rapidly lost allelic diversity or heterozygosity at microsatellite loci. Both measures of genetic differentiation varied little across our urban populations and were similar (albeit slightly lower) to values calculated for mice from a large, contiguous forest area outside NYC (Site 1). Additionally, heterozygosity of urban NYC populations was much higher than values calculated at many of the same loci in nonfragmented *P. maniculatus* populations in western North America (Yang & Kenagy 2009).

Our report of relatively high genetic diversity for urban white-footed mice is at odds with many previous studies on the genetic impacts of fragmentation on small mammal populations. Gaines *et al.* (1997) examined five case studies that used allozymes or mitochondrial DNA (mtDNA) sequence data to examine fragmentation in small mammals and reported that extreme fragmentation results in both genetic differentiation and loss of genetic variation. Both outcomes are key predictions of conservation genetics theory (Frankham *et al.* 2002) and are common arguments for maintaining connectivity between populations (i.e. corridors, Mech & Hallett 2001; Segelbacher *et al.* 2010). A recent survey (Keyghobadi 2007) of 32 studies found enhanced genetic structure (69%) and loss of genetic variation (58%) in the majority of cases. Out of five small mammal species examined for population structure, three had become genetically differentiated, and out of six small mammals examined for genetic diversity, three had lost significant genetic diversity in at least one marker type (Keyghobadi 2007). Multiple studies since that review have also reported population differentiation and loss of diversity for small mammals in fragmented habitats (Garner *et al.* 2005; Peakall & Lindenmayer 2006; White & Searle 2007; Biedrzycka & Radwan 2008; Macqueen *et al.* 2008; Booth *et al.* 2009; Kozakiewicz *et al.* 2009; Lampila *et al.* 2009; Vignieri 2010).

In Keyghobadi's (2007) review, *P. maniculatus* and *P. leucopus* were the only two small mammal taxa to show no effect of fragmentation on genetic diversity or differentiation. *P. maniculatus* exhibited genetic distances near zero among individuals trapped from contiguous, fragmented, and corridor-connected landscapes (Mech & Hallett 2001), whereas *P. leucopus* did not exhibit significant differences in F_{ST} calculated between population pairs in fragmented vs. continuous habitat (Mossman & Waser 2001). *P. leucopus* have the ability to disperse through agricultural matrices (Krohne & Hoch 1999; Anderson & Meikle 2010), and dispersal events of several km have been recorded for both *P. leucopus* and *P. maniculatus* (Maier 2002; Jung *et al.* 2005). Given the relatively high dispersal capabilities and flexible habitat use of white-footed mice, it is somewhat surprising that NYC populations have become genetically differentiated so quickly. The intervening matrix between our trapping sites contains corridors of cemeteries, parkway medians, and other manicured vegetation that may have similar permeability to agricultural areas. However, roads, buildings, human barriers, and competition with human commensals (i.e. *Rattus norvegicus*) may counteract any potential corridor effect.

We did not compare differentiation among populations in urban fragments to differentiation among populations in contiguous habitat near NYC, but results from multiple *Peromyscus* studies across North America facilitate interpretation of our findings in the context of natural genetic variation within the genus. *P. leucopus* in forest habitats fragmented by agriculture rather than urbanization exhibit relatively high rates of gene flow (Mossman & Waser 2001; Anderson & Meikle 2010). Studies of *P. maniculatus* offer several important comparisons because this species broadly overlaps *P. leucopus* in morphology, territoriality, population density, habitat use, and dispersal (King 1968). An evolutionary clustering analysis of *P. maniculatus* populations spanning a zone of mtDNA divergence between California and the Pacific NW, USA, identified only five highly admixed clusters using many of the same microsatellite loci as this study. Despite structure among mtDNA haplotypes, the microsatellite results showed evidence of substantial gene flow between populations with little structure over much greater distances than between NYC populations (Yang & Kenagy 2009). Pairwise F_{ST} values calculated between isolated island populations of the southeastern beach mouse, *P. polionotus niveiventris*, and a mainland subspecies of *P. polionotus* in Florida ($0.11 < F_{ST} < 0.22$) were higher but overlapped with the range of pairwise F_{ST} values we calculated between urban populations separated by only a few km (Degner *et al.* 2007). Differentiation between beach and mainland populations was attributed to the most recent Pleistocene sea level fluctuations, whereas we observed similar levels of genetic structuring owing to urbanization over the last century. *P. leucopus* populations on Virginia barrier islands and the Delmarva peninsula exhibited an overall mean $F_{ST} = 0.18$ at allozyme loci (Loxterman *et al.* 1998). *P. maniculatus* in the Great Lakes region (Taylor & Hoffmann 2010) and *P. attwateri* in the south-central USA (Lack *et al.* 2010) also show weaker genetic structure at mtDNA haplotypes than predicted based on putative biogeographic boundaries that arose during the Pleistocene. Taken together, these studies indicate that the levels of genetic structure we observed between NYC white-footed mouse populations are unusually high for the geographic scale. We conclude that urbanization acts as a particularly potent driver of genetic differentiation in *Peromyscus*.

The combination of substantial genetic differentiation but high genetic diversity may be a general characteristic of urban adapters with poor dispersal capabilities, but studies of other taxa are needed for confirmation. White-footed mice achieve their highest population densities in small habitat fragments (Anderson & Meikle 2010); if the populations are large enough, then loss of genetic variation owing to drift may be slowed or prevented altogether. Insular, endemic small mammals can maintain genetic variation at levels similar to mainland populations (Degner *et al.* 2007; Vega *et al.* 2007), possibly attributable to low density or absence of native predators and competitors that would otherwise regulate small mammal density. Another possible explanation is that detectable genetic structure may develop more rapidly than loss of genetic diversity in fragmented populations (reviewed in Keyghobadi 2007). Both theory (Varvio *et al.* 1986) and empirical examples (Keyghobadi *et al.* 2005) support the assertion that within-population heterozygosity reaches equilibrium more slowly than between-population structure. However, high heterozygosity, allelic diversity, and numbers of private alleles within populations suggest that rare alleles are not being lost at a fast rate from *P. leucopus* populations in NYC.

At selectively neutral loci in fragmented habitats, the relative contributions of genetic drift, mutation, and migration determine the degree of population structure. Using a permutation test of microsatellite allele sizes (Hardy *et al.* 2003), we found that R_{ST} does not assess differentiation better than F_{ST} in our data set. This result implies that mutation is a less important force than genetic drift in urban white-footed mouse populations, because R_{ST} should be superior when stepwise mutations contribute significantly to population differentiation. Simulations of island models indicate that F_{ST} performs better than R_{ST} when the mutation rate is much lower than the migration rate (Balloux & Goudet 2002), but white-footed mice and other small taxa probably cannot migrate through the urban matrix at a high rate. We found no evidence of isolation by distance for the city as a whole or for populations in Queens, suggesting that migration between NYC populations is low to nonexistent outside some areas of the Bronx. Under an alternative model of isolated populations, F_{ST} performs better than R_{ST} when the mutation rate is much lower than the reciprocal of the number of generations since isolation (Slatkin 1995). Given that NYC became rapidly urbanized over only the last century, *P. leucopus* populations have been fragmented for at most a few hundred generations. The reciprocal of this time span is unlikely to greatly exceed the mutation rate of most genetic markers. Surprisingly, we found that natural fragmentation between landmasses explains almost no variation at microsatellite loci, whereas populations on each landmass exhibit significant structure. Urbanization seems to be a sufficiently strong force of contemporary differentiation, and microsatellites sufficiently variable, to mask the effects of genetic drift owing to prehistoric allopatry. Microsatellites may generally underestimate the degree of structure compared to other markers owing to limits on the number of unique allelic states (Balloux *et al.* 2000).

Several other analyses beyond F_{ST} were also successful at detecting significant population structure in urban *P. leucopus*. Assignment tests, Bayesian clustering analysis of individual genotypes in Structure, and Bayesian clustering of predefined spatial populations of genotypes in BAPS indicated that most of our sampled areas contained genetically distinct groups. The latter two approaches produced almost indistinguishable results. The only substantial difference was that BAPS grouped together three populations in Queens (Fig. 4) that were in proximity, represented by small sample sizes ($N \leq 12$ at two sites), and contained many highly admixed genotypes in the Structure analysis (Fig. 3). An alternative analysis of the Structure output using the second-order rate of change in the probability of the data, ΔK (Evanno *et al.* 2005), and the clustering approach in Structurama that allows the number of clusters, K, to be a random variable (Huelsenbeck & Andolfatto 2007) both produced estimates of K that were only half to two-thirds of the number of sampling sites. The ΔK statistic is widely used because it offers an alternative to the ad hoc $\Pr(X|K)$ statistic of Pritchard *et al.* (2000) and can be interpreted graphically as a unimodal peak in ΔK at the best estimate of K. Our ΔK analysis produced a multi-modal distribution with the highest peak at six clusters and a lower peak at the same value of $K = 16$ as $\Pr(X|K)$. Evanno *et al.* (2005) reported that ΔK outperforms $\Pr(X|K)$ when overall differentiation is high (i.e. total $F_{ST} = 0.4$) and migration rates vary between populations. However, two other simulations that analysed scenarios of pairwise F_{ST} similar to those we report here found that ΔK does not perform well in many scenarios, whereas Structure and BAPS identify the correct K even when $F_{ST} = 0.02$ (Latch *et al.* 2006; Waples & Gaggiotti 2006).

The Structurama analysis also resulted in a relatively low estimate of K. This approach has not been as rigorously assessed by simulations as Structure and BAPS, although the authors of the program report that it is more accurate when migration rates are low and population sizes and/or mutation rates are high (Huelsenbeck & Andolfatto 2007). In general, we agree with the advice of Pritchard *et al.* (2000) to carefully and sensibly interpret estimates of K from evolutionary clustering analyses. The evolutionary clustering estimated by Structurama and the ΔK analysis of our Structure results present more unlikely biological scenarios than the high estimates of K, such as nearly complete admixture between genotypes from populations located on different landmasses (Fig. S1, Supporting Information). Given high values of K estimated using different methods, similarities between our analytical conditions and those used in simulations that successfully estimated K, and the significant F_{ST} values calculated between all pairs of our populations, we conclude that most of our sampled populations of urban white-footed mice are unique genetic groups.

The results presented here show that urban adapters can become rapidly and pervasively differentiated at neutral loci owing to human-driven landscape change, but also maintain genetic diversity over hundreds of generations. An important unresolved question is whether urban adapters maintain enough standing genetic variation to adapt to urbanization, or whether their high population densities in human-dominated landscapes can be explained by behavioural or phenotypic plasticity alone (Chevin *et al.* 2010). Morphological changes and nearly complete replacement of mitochondrial haplotypes have been reported for *Peromyscus* in the Chicago metropolitan area over the last century (Pergams & Lacy 2008). Evidence for natural selection on the mitochondrial genome has accumulated in recent years (Balloux 2010), and very dense, crowded urban populations with substantial genetic diversity could facilitate selective sweeps at mtDNA and other loci. Insular rodents have repeatedly exhibited rapid morphological change that is positively correlated with human population density (Pergams & Lawler 2009). More generally, phenotypic changes owing to anthropogenic influence occur at an enhanced rate compared to undisturbed landscapes, but the underlying genetic basis of these changes is often unknown or unimportant compared to phenotypic plasticity (Hendry *et al.* 2007). *Peromyscus* spp. are found in many human-dominated landscapes and thus are a potentially excellent model of anthropogenic selection on native wildlife.

Acknowledgements

This research was funded by the National Science Foundation (DEB 0817259) and two grants from the Professional Staff Congress-CUNY. We thank the National Park Service, NY State Dept. of Environmental Conservation, Natural Resources Group of the NYC Dept. of Parks & Recreation, Central Park Conservancy, Black Rock Forest Consortium, Ellen Pehek, and Regina Alvarez for access to NYC study sites. We thank David Burg, Catherine Burns, Paolo Cocco, Jonathan Grindley, Stephen Harris, Kerstin Kalchmayr, Olga Lavinda, Luca Pozzi, and Miguel Rivera for their invaluable help collecting samples in the field and three anonymous reviewers for comments that improved the manuscript.

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Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Six distinct evolutionary clusters inferred from Structure analysis of 312 mice from 15 sites that were identified as the most likely number of clusters using the ΔK statistic developed by Evanno *et al.* (2005).

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