Signatures of positive selection and local adaptation to urbanization in white-footed mice (*Peromyscus leucopus*)

Stephen E. Harris (S.E.H)[1*] and Jason Munshi-South (J.M.-S.)[2]

[1]The Graduate Center, City University of New York (CUNY), New York, NY 10016 USA

[2]Louis Calder Center—Biological Field Station, Fordham University, 31 Whippoorwill Road Armonk, NY 10504 USA

Corresponding author: Stephen E. Harris

E-mail: harris.stephen.e@gmail.com

Current address: Department of Biology, Purchase College, State University of New York (SUNY), Purchase, NY 10577 USA

Running title: Local adaptation in urban white-footed mice

ABSTRACT

Urbanization significantly alters natural ecosystems and has accelerated globally. Urban wildlife populations are often highly fragmented by human infrastructure, and isolated populations may adapt in response to local urban pressures. However, relatively few studies have identified genomic signatures of adaptation in urban animals. We used a landscape genomics approach to examine signatures of selection in urban populations of white-footed mice (*Peromyscus leucopus*) in New York City. We analyzed 154,770 SNPs identified from transcriptome data from 48 *P. leucopus* individuals from three urban and three rural populations, and used outlier tests to identify evidence of urban adaptation. We accounted for demography by simulating a neutral SNP dataset under an inferred demographic history as a null model for outlier analysis. We also tested whether candidate genes were associated with environmental variables related to urbanization. In total, we detected 381 outlier loci and after stringent filtering, identified and annotated 19 candidate loci. Many of the candidate genes were involved in metabolic processes, and have well-established roles in metabolizing lipids and carbohydrates. Our results indicate that white-footed mice in NYC are adapting at the biomolecular level to local selective pressures in urban habitats. Annotation of outlier loci suggest selection is acting on metabolic pathways in urban populations, likely related to novel diets in cities that differ from diets in less disturbed areas.

Keywords: transcriptome, *Peromyscus leucopus*, genotype-environment association, urban evolutionary biology, genome scans, positive selection, landscape genomics, urbanization

INTRODUCTION

Urban habitats are one of the fastest growing and most rapidly changing environments around the world. While urbanization has been traditionally viewed as a driver of declining habitat quality in and around cities, there is growing interest in the idea that urban areas represent novel environments with unique selective pressures (Donihue & Lambert 2015). The recently developed but burgeoning field of urban evolutionary biology aims to determine how urbanization leads to evolutionary change through mutation, genetic drift, gene flow, and natural selection in urban populations.

The ecological changes that occur within cities are likely to have many evolutionary implications. Human infrastructure causes habitat loss and fragmentation and changes resource availability, novel species interactions occur because human movements and commerce introduce a diverse array of nonnative species, and human activity increases exposure to chemical, light, and noise pollution (McKinney 2002; Chace & Walsh 2004; Shochat *et al.* 2006; Sih *et al.* 2011). These changes lead to unique pressures in novel urban habitats that may rapidly drive evolutionary change over short timescales. Increased genetic drift in relatively isolated urban populations, genetic differentiation between populations with restricted gene flow from urban infrastructure, or allele frequency shifts due to local urban adaptation, are all likely outcomes of evolution in cities (Munshi-South 2012; Merilä & Hendry 2014; Donihue & Lambert 2015).

Urban populations are potentially excellent systems for examining how species respond to anthropogenic environmental change, what genes and traits are involved, and how quickly populations locally adapt to changing environments. Local adaptation is a common phenomenon in nature (Stinchcombe & Hoekstra 2008; Bonin 2008; Linnen *et al.* 2009; Hohenlohe *et al.*

2010a; Turner *et al.* 2010; Ellison *et al.* 2011; De Wit & Palumbi 2013), and often results from the operation of selection on standing genetic variation as opposed to novel mutations over relatively short time scales (Barrett & Schluter 2008; Stapley *et al.* 2010). Additionally, the quantitative traits involved in local adaptation may involve many genes of small effect working to produce the desired phenotype (Orr 2005; Rockman 2012), and these ecologically relevant but non-conspicuous phenotypes are predicted to be those most involved in urban adaptation (Sih *et al.* 2011). However, traits with relatively simple genetic architecture may also be under selection in urban environments (Thompson *et al.* 2016). Investigating the genetic basis of local adaptation has provided insight into a variety of evolutionary processes including speciation, maintenance of genetic diversity, range expansion, and species responses to changing environments (Savolainen *et al.* 2013; Tiffin & Ross-Ibarra 2014), and holds great promise for understanding adaptive evolution in response to urbanization.

Landscape genomics has recently produced a number of approaches for studying local adaptation. This field is defined by the spatially explicit study of genomic variation (Sork *et al.* 2013) that seeks to identify environmental variables influencing adaptive genomic variation (Rellstab *et al.* 2015). Landscape genomics, and more specifically genotype-by-environment analyses (GEA), can successfully identify associations between urban environmental variables and allele frequencies that indicate adaptation to local urban conditions. These approaches can also help to untangle the interactions between neutral demographic processes and selection (Rellstab *et al.* 2017). Urban populations are influenced by both genetic drift through founder effects and barriers to gene flow, and selection acting on genetic variation linked to increased fitness in urban settings.

A small but growing number of studies have documented how populations may locally adapt to urban selective pressures through changes in allele frequencies and / or undergo directional shifts in phenotypic traits. Yeh (2004) reported that sexually-selected tail coloration in Juncos (*Junco hyemalis*) was rapidly evolving in urban populations compared to rural ones. European Blackbirds (*Turdus merula*) exhibit evidence of selection on genes underlying anxiety behavior in newly established populations across multiple cities (Partecke *et al.* 2006; Mueller *et al.* 2013). Cheptou *et al.* (2008) reported that a weed (*Crepis sancta)* in urban vegetation plots surrounded by paved surfaces showed heritable changes in seed morphology and dispersal. Reduced snow cover in urban areas leads to colder minimum ground temperatures and Thompson *et al.* (2016) found parallel adaptive evolution in urban white clover (*Trifolium repens*) populations that had increased freezing tolerance. Several studies have also found likely adaptive genetic and morphological changes in urban mammal populations. Suggestive of urban adaptation, a specific mitochondrial genotype rose to fixation in white-footed mice (*Peromyscus leucopus)* populations in Chicago along with morphological changes to skull shape after urbanization (Pergams & Lacy 2008). In urban areas of Italy, Kuhl's pipistrelle (*Pipistrellus kuhlii*) bat populations had significantly larger bodies and longer skulls than natural populations, suggesting urban adaption to a novel diet introduced when artificial illumination attracted an increased number of large hard-bodied moths (Tomassini *et al.* 2014).

Few studies in urban evolutionary biology have been able to measure phenotypic changes, definitively link them to genetic changes, and establish fitness benefits to demonstrate evolutionary adaptation. One exception are urban killifish (*Fundulus heteroclitus*), where selective pressure from polychlorinated biphenyls (PCBs) has led to the evolution of PCB tolerance in urban populations (Whitehead *et al.* 2010; Reid *et al.* 2016). Adaptation to PCB

pollution was also reported in tomcod (*Microgadus tomcod)* in the Hudson River through a deletion that similarly increases tolerance to PCBs (Wirgin *et al.* 2011). Urban adaptation has also been confirmed in the well-known peppered moth (*Biston betularia*) system. Recent evidence suggests that the industrial melanism trait in this species is linked to an insertion of a transposable element in the *cortex* gene in the early 1800s that spread throughout the population in response to industrial airborne pollution (Hof *et al.* 2016). The study of additional systems will likely identify a complex array of adaptive evolutionary responses in cities (Whitehead *et al.* 2017).

Here we examined signatures of selection in isolated urban populations of white-footed mice, *Peromyscus leucopus*, in New York City (NYC) using a landscape genomics approach. *Peromyscus* spp. (Rodentia, Cricetidae) are a group of abundant small mammals found across much of North and Central America. They live in a diverse array of habitats that exposes them to a variety of selective pressures, and thus multiple *Peromyscus* spp. have become model systems for studies examining ecology, evolution, and physiology in natural populations (Munshi-South & Richardson 2017). There is also evidence that *Peromyscus* spp. readily adapt to environmental change (Storz *et al.* 2007, 2009, 2010; Mullen & Hoekstra 2008; Linnen *et al.* 2009; Weber *et al.* 2013; Natarajan *et al.* 2013; Munshi-South & Richardson 2017), making them good subjects for the study of local adaptation. White-footed mice are one of the few native mammals that thrive in extremely small, fragmented urban forests in North America (Pergams & Lacy 2008; Rogic *et al.* 2013; Munshi-South & Nagy 2014), and tend to be found at higher densities in urban vs. rural patches due to a thick understory providing abundant food resources and exclusion of major predators and competitors (Rytwinski & Fahrig 2007). Increased density may also be due to limited *P. leucopus* dispersal between urban sites. Munshi-South (2012)

found barriers to dispersal between isolated NYC parks, with migrants only moving through significantly vegetated corridors throughout the city. There is also substantial genetic structure between NYC parks as measured by microsatellites (Munshi-South & Kharchenko 2010), genome-wide SNPs (Munshi-South *et al.* 2016) and demographic modeling (Harris *et al.* 2016). We have also previously identified signatures of selection in urban populations of NYC white-footed mice (Harris *et al.* 2013), though we used smaller datasets and more limited approaches than presented here.

In the current study, we examined SNPs generated from individual transcriptome sequencing for *P. leucopus* from three urban sites in NYC and three rural sites from the surrounding area. We generated a large SNP dataset and produced estimates of nucleotide diversity (π, Tajima 1983), Tajima's *D* (Tajima 1989), and F_{ST} (Wright 1951) to generate per-site estimates and identify loci that deviate from neutral expectations. We then used a variety of genome scan methods and outlier tests to identify genes subject to selection in an urban setting. Our approach identified population differentiation, shifts in allele frequencies, and associations between alleles and environmental variables. However, neutral demographic processes such as population bottlenecks can produce signatures of genetic variation similar to those produced by selection (Oleksyk *et al.* 2010; Li *et al.* 2012). We accounted for this possibility by incorporating a simulated neutral SNP dataset from an inferred demographic history (Harris *et al.* 2016) directly into our null model for identifying outliers (Excoffier *et al.* 2009; Gutenkunst *et al.* 2009; Li *et al.* 2012; Vitti *et al.* 2013; Lotterhos & Whitlock 2015).

The three specific aims of this study were the following: 1. identify candidate genes exhibiting signatures of selection in NYC populations of white-footed mice using a variety of genome scan methods and outlier tests; 2. distinguish genetic outliers resulting from selection

153 rather than demography by incorporating demographic histories of white-footed mice in NYC
154 into null models of genome scans; and 3. identify genes that are statistically associated with
155 environmental variables representative of urbanization using landscape genomic approaches.
156

157 **MATERIALS AND METHODS**

158 **Sampling, library preparation, and transcriptome assembly**

159 We trapped and collected white-footed mice from 2010 - 2012. For full details on
160 sampling, transcriptome sequencing, assembly and SNP calling, see Harris *et al.* 2013, 2015. In
161 brief, we randomly chose eight adult white-footed mice (equal numbers of males and females)
162 from each of six sampling locations (N = 48 total) representative of urban and rural habitats and
163 with minimal within-site genetic structure (Fig. 1) (Harris *et al.* 2013, 2015). Three sampling
164 sites were within NYC parks: Central Park in Manhattan (CP), New York Botanical Gardens in
165 the Bronx (NYBG), and Flushing Meadows—Willow Lake in Queens (FM). These sites
166 represented urban habitats surrounded by high levels of impervious surface cover and high
167 human population density, as previously quantified in Munshi-South et al. (2016). The
168 remaining three sites occurred ~100 km outside of NYC in rural, undisturbed habitat
169 representative of natural environments for *P. leucopus*. High Point State Park is in the Kittatinny
170 Mountains in New Jersey (HIP), Clarence Fahnestock State Park is located in the Hudson
171 Highlands in New York (CFP), and Brookhaven and Wildwood State Parks occur on the
172 northeastern end of Long Island, New York (BHWWP). Total RNA was extracted separately
173 from livers stored in RNA later for each of the 48 mice, treated with DNase, enriched through
174 ribosomal RNA depletion, fragmented, reverse transcribed, amplified and tagged with a unique
175 barcode, and sequenced in four lanes of one SOLiD 5500XL run (Harris *et al.* 2015). We called

SNPs with the Genome Analysis Toolkit (GATK version 2.8) pipeline using a Bayesian genotype likelihood model (DePristo *et al.* 2011). In order to call a SNP, we required it to occur in at least five individuals, have a nucleotide quality (q-score) ≥ 30, exhibit no strand bias (FS ≥ 35), and to come from only uniquely mapped reads. We also required SNPs to have an overall depth ≥ 10X and ≤ 350X (to account for paralogous sequences), a minor allele frequency (MAF) ≥ 0.025, and removed SNPs where every individual was heterozygous.

Summary statistics

SNP information was stored in a VCF (variant call format) file and summary statistics were calculated using vcftools 0.1.12b (Danecek *et al.* 2011). We calculated per-site nucleotide diversity (π), Tajima's *D*, and F_{ST}. We also calculated the statistics for each contig (per-site statistic summed across all SNPs per contig divided by total sites) and calculated the average estimate for each population, including all pairwise population comparisons for F_{ST}.

Scans for positive selection based on population differentiation

We used the F_{ST} based analysis implemented in BayeScan v. 2.1 (Foll & Gaggiotti 2008) to compare all six population-specific allele frequencies with global averages and identify outlier SNPs. BayeScan identifies loci that exhibit divergence between groups that is stronger than would be expected under neutral genetic processes. Based on a set of neutral allele frequencies under a Dirichlet distribution, BayeScan uses a Bayesian model to estimate the probability that a given locus has been subject to selection. To generate more realistic allele frequency distributions, we used BayeScan for independent coalescent simulations of SNP datasets based on a neutral demographic history inferred by Harris *et al.* (2016) specifically for each *P.*

199 *leucopus* population. Using the coalescent-based fastsimcoal2 software (Excoffier *et al.* 2013),
200 we generated 100 sets of 100,000 SNPs each for every population in this study from a three
201 population isolation-with-migration model using parameter estimates for divergence time,
202 effective population size, migration rate, and population size change previously inferred in Harris
203 *et al.* (2016). In short, the model represented a deep split between an ancestral population into
204 Long Island, NY and the mainland (including Manhattan) 29,440 generations before present
205 (GBP). A third population (representing the sampling sites in this study) later became isolated
206 746 GBP. Urban populations were also modeled to include a population size change event at the
207 time of divergence. BayeScan was run independently on each of the 100 simulated datasets from
208 fastsimcoal2 using default parameters to generate a null distribution of BayeScan statistics.
209 BayeScan was then run on the observed SNP dataset using default parameters. We
210 performed several different analyses including a global analysis, one with two populations
211 representing urban and rural groups, and finally analyses on all sampling site pairwise
212 comparisons. We retained outlier SNPs with a q-value ≤ 0.1 (leading to a FDR of ≤ 0.1) and
213 with a posterior odds probability from BayeScan higher than for any value calculated from the
214 simulated dataset. BayeScan also calculates alpha (α), a locus specific F_{st} coefficient, where a
215 positive value suggests diversifying selection and a negative value suggests balancing or
216 purifying selection. There were no SNPs with negative α values.
217 For comparison to BayeScan results, we used a related method, BayPass (Gautier 2015),
218 that identifies loci subject to selection based on allele frequency patterns that deviate from
219 neutral expectations. We ran BayPass using default parameters under the auxillary covariate
220 (AUX) model, and simulated pseudo-observed datasets (PODs) under the Inference Model in
221 Baypass as suggested by Gautier (2015) to calibrate neutral distributions for XtX. BayPass uses

the XtX statistic to identify adaptive divergence. SNPs with XtX estimates greater than the 95% threshold determined from PODs were identified as resulting from adaptive divergence.

Analysis for selective sweeps

We also identified outlier regions when the observed SFS showed an excess of low frequency and high frequency minor alleles, a signal indicative of a recent selective sweep. The composite likelihood ratio (CLR) statistic is used to identify regions where the observed SFS matches the expected SFS generated from a selective sweep (Kim & Stephan 2002; Nielsen *et al.* 2005; Pavlidis *et al.* 2010). We calculated the CLR along sliding windows across the transcriptome using the software program SweeD (Pavlidis *et al.* 2013). SweeD is an extension of Sweepfinder (Nielsen *et al.* 2005) that is optimized for large next generation sequencing (NGS) datasets. We lacked a genome to provide high-quality linkage information so SweeD was run separately for each population and on individual contigs. We used default parameters except for using a sliding window size of 200 bp and use of a folded SFS, as we lacked an outgroup to infer ancestral alleles. The window within each contig with the highest CLR score is considered the likely location of a selective sweep. Similar to the method used for BayeScan, statistical significance was established from a null distribution generated by running SweeD on SNP datasets simulated under the inferred demographic history for *P. leucopus* populations (Harris *et al.* 2016). SweeD does not inherently identify outlier regions. The CLR is computed using a selective sweep model on the observed data and then compared to a neutral model calibrated with a simulated background SFS. As before, we used 100 datasets with 100,000 SNPs each, simulated under the inferred neutral demographic history for white-footed mice in NYC. The CLR was calculated using SweeD for all simulated datasets. We identified outlier contigs if their

CLR value was greater than any produced in neutral simulations. We also required outliers to fall within the top 0.01% of the CLR distribution for the observed SNPs.

Genotype-environment association tests for environmental selection

We used the GEA approach of LFMM: Latent Factor Mixed Models (Frichot *et al.* 2013) to associate our full SNP dataset with potential environmental selection pressures. LFMM examines associations between environmental and genetic variation while accounting for the neutral genetic background and structure between populations (Frichot *et al.* 2013). We tested three environmental variables associated with urbanization: 1) percent impervious surface (i.e. surfaces such as roads, rooftops, and other human infrastructure that do not absorb water calculated from USGS National Land Cover Data) within a 2 km (the approximate lifetime dispersal distance of white-footed mice) buffer around each sampling site's GPS coordinate, 2) human density within a two-kilometer buffer around each sampling site's GPS coordinate (calculated from US Census blocks), and 3) categorization of each site as urban, within NYC limits, or rural, undeveloped state park outside city limits (Coded as 0 or 1 in LFMM). Calculations were made in ArcGIS v10.1 (ESRI, Redlands, CA, USA) and were previously reported in Munshi-South *et al*. (2016). This previous analysis found that variables 1-2 were significantly associated with genome-wide variation in *P. leucopus* populations in the NYC metropolitan area. LFMM requires the user to define the number of latent factors, K, that describe population structure in the dataset. To identify the appropriate number of K latent factors, we performed a genetic PCA followed by a Tracy-Widom test to find the number of eigenvalues with *P* values ≤ 0.01 (Patterson *et al.* 2006; Frichot & François 2015). Based on this approach, we ran LFMM with default parameters except for K = 6, number of MCMC cycles =

100,000, and burn-in = 50,000. Using author recommendations, we calculated the median |z|-score from 10 replicate runs and then readjusted the p values. LFMM uses |z|- scores to report the probability of a SNP's association with an environmental variable. Again, we controlled for FDR by using a q-value threshold of ≤ 0.1.

BayPass also includes an environmental analysis, so for comparison to LFMM we used the GEA test implemented in the BayPass AUX model that identifies genetic markers associated with population-specific covariates (Gautier 2015). For population covariates, we used the same environmental variables used in LFMM: site classification (i.e. urban or rural) as a binary covariate, human density, and impervious surface. We used the AUX model and again simulated pseudo-observed datasets (PODs) under the Inference Model to calibrate neutral distributions for Bayes Factors (BFs). BayPass uses BFs to associate SNPs with population specific covariates. SNPs with BF estimates greater than the 95% threshold determined from PODs were considered to be associated with population covariates. We further filtered associations by setting a cutoff for BF ≥ 20.

Functional annotation of candidate genes

We used the gene annotation pipeline in Blast2GO (Conesa *et al.* 2005; Götz *et al.* 2008) to identify sequences from the NCBI non-redundant protein database that were homologous to our outlier contigs identified above. We then retrieved associated gene ontology (GO) terms. Blast2GO retrieves GO terms associated with BLASTX hits and uses the KEGG database to describe biochemical pathways linking different enzymes (Ogata *et al.* 1999; Kanehisa *et al.* 2014). For downstream enrichment analyses, we also used the Ensembl gene annotation system (Aken *et al.* 2016) to find homologous *Mus musculus* genes for each *P. leucopus* contig. We

291 further interpreted the outlier gene lists using g:Profiler (Reimand *et al.* 2016) to identify gene
292 ontology terms enriched in our outlier gene list compared to the fully annotated *Mus musculus*
293 genome. We used the g:Profiler webserver and identified enriched terms associated with outlier
294 genes using default parameters and the Benjamini–Hochberg correction for multiple comparisons
295 with an adjusted p-value < 0.05. Finally, we used REViGO to cluster GO terms and summarize
296 them in a subset of terms based on semantic similarity measures (Supek *et al.* 2011).
297
298 **RESULTS**
299 **Genetic diversity statistics**
300 **In total,** we identified 154,770 SNPs for investigating patterns of genetic variation and
301 performing tests of selection. Urban populations had a 50% decrease in nucleotide diversity
302 compared to the rural populations, but mean Tajima's D values for rural parks were consistently
303 higher than for urban parks (Table 1). The average nucleotide diversity for all three rural
304 populations was 0.224 ± 0.034 SE, while the average for urban populations was only 0.112 ±
305 0.019 SE. The average Tajima's *D* within populations did not show substantial differences
306 between populations (Table 1). For all populations, Tajima's *D* was slightly positive. Average
307 pairwise F_{ST} were the lowest between rural populations (0.018 ± 0.364 SE, CFP – HIP Table S1)
308 and highest between urban populations (0.110 ± 0.520 SE, CP – FM Table S1). These F_{ST}
309 values were similar to F_{ST} estimated using genome-wide SNP datasets (Munshi-South *et al.*
310 2016).
311
312 **Outlier detection and environmental associations**

We used BayeScan to identify 39 outlier SNPs exhibiting patterns of divergent selection between urban and rural populations (Fig. 2A, Table S2). There were no SNPs that exhibited signatures of balancing selection. F_{ST} values for outlier SNPs ranged from 0.21 - 0.33. BayeScan identified zero outlier SNPs in the simulated neutral dataset, and accordingly the 39 outlier SNPs from the observed data had q-values that were smaller than the most extreme values for the simulated data (q-value ≤ 0.6). We ran a similar test looking for patterns of divergence using BayPass. This analysis identified 56 SNPs that showed evidence of divergent selection (Table S2). We used PODs to estimate a null distribution and outlier SNPs had XtX values $\geq$ 8.35 (top 5% of the null distribution). There were 11 SNPs associated with diversifying selection in both the BayeScan and BayPass analyses.

To identify signatures of selective sweeps , we used the CLR statistic implemented in SweeD. We found that CLR scores in the top 5% of the simulated distribution were generally 2-3X lower than values in the top 5% of the observed dataset. We ran SweeD on observed SNPs within individual contigs and identified outliers by filtering for a CLR score ≥ 3.53 (the maximum CLR from simulated data). We also chose regions that fell within the top 0.01% of the observed distribution (Fig. 2B); all outliers had CLR scores ≥ 4.97. SweeD identified regions with SFS patterns that fit a selective sweep model in 45 contigs within urban populations (Table S2). There was no overlap between outlier SNPs identified by SweeD and BayeScan / BayPass.

There were 131 SNPs associated with at least one of three environmental variables tested using LFMM (Fig. 3A, Table S2). There was zero overlap with outliers identified from BayeScan and only one SNP that overlapped between SweeD and LFMM. Three SNPs identified in BayPass as outliers showing signatures of diversifying selection were also associated with environmental covariates in LFMM (Table S2). All three SNPs were within

336 genes associated with human density around sampling sites and one was associated with all three
337 environmental covariates. In an analysis similar to LFMM, we used BayPass to also associate
338 environmental variables, called population covariates, with allele frequencies. There were 143
339 SNPs associated with at least one of the three environmental covariates tested using BayPass
340 (Table S2). From these 143, five overlapped with those showing signatures of divergent selection
341 in BayPass and eleven overlapped with outliers in BayeScan.
342 Across all tests, SNPs identified as outliers or associated with environmental variables
343 were found in 381 contigs. We filtered this list down to a subset of 19 contigs (Table 2) that are
344 the most likely candidates for directional selection due to urban selective pressures. We required
345 these filtered candidate contigs to show a signature of diversifying selection between urban and
346 rural populations (BayScan or BayPass) or a signature of a selective sweep (SweeD), and they
347 had to be associated with an environmental variable (human density around parks, impervious
348 surface) as identified in GEA tests (LFMM or BayPass).
349
350 **Functional annotation**
351 The full contig sequences containing outlier SNPs were obtained from the *P. leucopus*
352 transcriptome (Harris *et al.* 2015) and used for functional annotation and analysis. We first tested
353 the full set of 381 contigs identified by all outlier tests for overrepresented GO terms using
354 g:Profiler. There were 260 overrepresented GO terms from the full outlier list (Table S3). We
355 summarized this list using REViGO into 23 representative terms. The top representative term
356 was lipid metabolism, followed by organic substance catabolism (Table S4). The list also
357 includes lipid homeostasis and immune system processes.

We also looked for overrepresentation in the gene annotations associated with the filtered subset of 19 outliers and found related results (Table 3). There were 15 contigs homologous to known genes with functional annotation. Metabolic pathways were the most overrepresented group of gene ontology terms, and there were two biological functions associated with the most overrepresented GO terms from the full list. These included non-alcoholic fatty liver disease and regulation of protein kinase b (AKT) signaling.

DISCUSSION

In this study, we investigated patterns of divergent positive selection between urban and rural populations of *P. leucopus*, and identified significant associations between outlier SNPs and environmental variables relevant to urbanization. The majority of candidate loci were annotated with GO terms that are significantly associated with dietary metabolism, particularly breakdown of lipids and carbohydrates. We discuss what these findings mean for organisms inhabiting novel urban ecosystems, and more generally for understanding the ecological processes and time frame of local adaptation in changing environments.

Our previous study investigated non-synonymous polymorphisms in pooled transcriptome samples and we reported evidence for positive selection in genes dealing with metabolism, immunity, and methylation in NYC white-footed mice (Harris *et al.* 2013). This current study supports the phenotypic traits likely under selection in urban environments, identifying outlier genes that play major roles in metabolism, and to a lesser extent, immunity, but few outlier genes were identified in both the current and previous studies. The dataset analyzed here was much larger, included more sampling sites, and changed the inclusion criteria for outlier genes by using analyses that identify more recent signatures of selection, as opposed

to longer-term evolutionary changes in non-synonymous substitutions . However, it is important to note that our study is still relatively small, including only six populations and eight individuals from each population. Increasing the number of individuals and sampling sites, especially including multiple cities as replicates, would likely greatly improve the associations found between environmental variables and allele frequencies (Lotterhos & Whitlock 2015). The latter approach may be unlikely, however, with each urban setting presenting a unique set of selective pressures leading to local adaptive responses, as shown with coat coloration in beach mice (*Peromyscus polionotus*) (Hoekstra *et al.* 2006) and climate related adaptation in the flowering plant (*Arabidopsis halleri)* (Rellstab *et al.* 2017). Despite potential issues with sample size, we did find two of the eleven previously identified candidate genes (Harris *et al.* 2013) to be direct matches to outliers in this current analysis (Serine protease inhibitor a3c and Solute carrier organic anion transporter 1A5), and two other genes were from the same gene families or involved in the same biological processes. One gene, an aldo-keto-reductase protein, is part of the same gene family as the aflatoxin reductase gene (Contig 10636-348) identified in this study. The aldo-keto reductase gene family comprises a large group of essential enzymes for metabolizing natural and foreign substances (Hyndman *et al.* 2003). The other is a cytochrome P450 (CYPA1A) gene involved in metabolism of drugs and lipids. *Peromyscus* directly express CYPA1A and Hsp90 (outlier from current SweeD analysis) when exposed to environmental toxins (Settachan 2001).

Population genomics summary statistics

Before performing outlier tests, we initially calculated per-site nucleotide diversity and Tajima's D. The Tajima's D statistic was calculated per contig for each population. We found

nucleotide diversity to be lower in all urban population compared to rural populations, supporting previous work that found a negative association between genome-wide SNP diversity and urbanization. That study included the six populations studied here and an additional 18 populations distributed along an urban-to-rural gradient (Munshi-South *et al.* 2016). While loss of genetic variation will reduce evolutionary potential and decrease the probability of local adaptation, selection may still act if adequate variation is present and genetic drift is not too strong (Donihue & Lambert 2015; Munshi-South *et al.* 2016). Tajima's D is often used to identify signatures of selection, comparing observed to expected heterozygosity. For all our populations, Tajima's D skewed positive, possibly explained by balancing selection. While balancing selection has been found to maintain variation in immune loci in fragmented urban population of bobcats (*Lynx rufus*) (Serieys *et al.* 2015), it is difficult to distinguish whether demography or selection drives Tajima's D values in many cases (MacManes & Eisen 2014). We have estimated the complex demographic history for *P. leucopus* populations in NYC (Harris *et al.* 2016), suggesting Tajima's D may not be the best tool for identifying selection in this system. Outlier tests are more robust to demography and we explicitly accounted for the specific demographic history of *P. leucopus* in the null models used during analysis of our genome scan methods.

Signatures of selection in urban populations from genome-wide scans

Over the past decade, genome scans have become feasible methods to detect and disentangle neutral and adaptive evolutionary processes for non-model organisms (De Villemereuil *et al.* 2014; Hoban *et al.* 2016). One method, BayeScan (Foll & Gaggiotti 2008), calculates the posterior probability that a site is under the influence of selection by testing

427 models with and without selection. While BayeScan is relatively robust to confounding
428 demographic processes (Pérez-Figueroa *et al.* 2010; De Villemereuil *et al.* 2014), population
429 bottlenecks, hierarchical structure, recent migration, or variable times to most-recent-common-
430 ancestor (MRCA) between populations can artificially inflate F_{ST} values (Hermisson 2009;
431 Lotterhos & Whitlock 2014) and may still impact BayeScan (Savolainen *et al.* 2013; Lotterhos &
432 Whitlock 2014). We minimized false positives by incorporating population structure and a
433 specific demographic history for *P. leucopus* in NYC directly into the null distribution of F_{ST}
434 (Harris *et al.* 2016). We only included outliers if their posterior probability was greater than
435 probabilities calculated from these simulations. The outliers from BayeScan comprised 0.024%
436 of the total number of loci analyzed from our RNASeq dataset, and 0.036% of the total loci using
437 BayPass. These percentages are in line with candidates uncovered from a similar study (0.05%)
438 that looked at high and low altitude populations of the plant *Senecio chrysanthemifolius*
439 (Chapman *et al.* 2013). Many studies find higher percentages of outlier loci using BayeScan; for
440 example, 4.5% in the American pika across its range in British Columbia (Henry & Russello
441 2013), and 5.7% in Atlantic herring across their range (Limborg *et al.* 2012). Our lower overall
442 percentage of outliers may be due to differences in species or datasets between studies (false
443 positive rate, power, sampling, genome size and composition are all variables that influence
444 numbers of SNPs), or alternatively because of relatively recent isolation or moderate to weak
445 selection in urban populations.
446 SweeD, another genome scan approach, examines patterns within a population's SFS
447 rather than allelic differentiation between populations. The main footprint that selective sweeps
448 leave on the SFS is an excess of low- and high-frequency variants (Nielsen 2005). The
449 SweepFinder method (Nielsen *et al.* 2005), recently upgraded to the NGS compatible SweeD

(Pavlidis *et al.* 2013), uses a CLR test based on the ratio between the likelihood of a neutral and selective sweep hypothesis. As above, the weakness of hitchhiking methods is the confounding influence certain demographic processes have on the SFS (Hermisson 2009). However, building a robustly inferred demographic history into the null model substantially reduces false positive rates (Pavlidis *et al.* 2013). We included the *P. leucopus* demographic history into our analysis, and found 0.019% of the sequenced loci to contain SFS patterns indicative of selective sweeps. This rate is in line with other studies that reported that 0.5% of regions in domesticated rice (Wang *et al.* 2014), 0.02% of loci in black cottonwood (Zhou *et al.* 2014), and 0.02% of the gorilla genome (McManus *et al.* 2014) show evidence of selective sweeps or hitchhiking.

Several studies have shown that identifying outliers with multiple tests and diverse theoretical approaches is the best way to reduce false positives in genome outlier analyses (Nielsen 2005; Grossman *et al.* 2010; Hohenlohe *et al.* 2010b). We required candidate genes to show a signature of diversifying selection or a signature of a selective sweep, and they had to be associated with an environmental variable. We found several outliers identified in both BayeScan and BayPass (Table S2), however, there was no overlap between BayeScan / BayPass and SweeD outliers. This discrepancy is likely due to the different selection scenarios underlying each test, i.e. divergent local selection versus population-wide positive selection in the form of selective sweeps (Hermisson 2009). F_{ST} based methods respond to allelic divergence relatively quickly, while models for selective sweeps typically require nearly-fixed derived alleles (Hohenlohe *et al.* 2010b). Given the recent history of urbanization in NYC, many selective sweeps may be ongoing or otherwise incomplete. Selection may also be acting on standing genetic variation in the form of soft sweeps (Hermisson & Pennings 2005) that are not readily identified by SweeD.

473

474 **Environmental associations strengthen evidence of local adaptation to urbanization**

475 GEA tests are a growing class of methods that identify loci that are associated with
476 environmental factors (Joost *et al.* 2007; Coop *et al.* 2010; Frichot *et al.* 2013), and by
477 accounting for underlying correlation structure of allele frequencies, may often be more powerful
478 than traditional outlier tests (Savolainen *et al.* 2013). GEA tests come from the field of landscape
479 genomics which incorporates tools from landscape genetics and population genomics to examine
480 the effects of demography, migration, and selection, and ultimately identify local adaptation
481 (Sork *et al.* 2013; Rellstab *et al.* 2015). Here we used LFMM (Frichot *et al.* 2013) and the AUX
482 covariate model from BayPass on the full SNP dataset with environmental metrics of
483 urbanization. LFMM performs better than other methods in the presence of hierarchical
484 structure and when polygenic selection is acting on many loci with small effect (De Villemereuil
485 *et al.* 2014). Hierarchical structure in our dataset includes urban and rural differentiation (Harris
486 *et al.* 2015; Harris *et al.* 2016), patterns of geographic structure between mainland mice and
487 Long Island, NY (Harris *et al.* 2016), and population structure between individual urban parks
488 (Munshi-South & Kharchenko 2010). Simulations also suggest that LFMM is superior when
489 sample size is less than 10 individuals per population, there is no pattern of IBD, and the study
490 compares environmentally divergent habitats (Lotterhos & Whitlock 2015). We sampled eight
491 white-footed mice per population, found no evidence of IBD (Munshi-South *et al.* 2016), and
492 sampled environmentally divergent rural and urban locations.

493 Using GEA tests implemented in BayPass and LFMM, we found that 17 (12 %) and 4
494 (2.8 %) outliers, respectively, were significantly associated with one or more urbanization
495 variables. These results are lower than other studies combining genome scans and GEA tests.

Limborg *et al.* (2012) found 62.5% of the outliers identified in BayeScan were correlated with temperature or salinity in Atlantic herring, and 26.3% of genome scan outliers were associated with temperature or latitude in a tree species (De Kort *et al.* 2014). The lower overlap found in our study is likely due to the difficult nature in quantifying urbanization. Percent impervious surface, human population density, or binary classification as urban versus rural may not capture the specific, causative selection pressures acting on white-footed mouse populations (See Table S5 for environmental data). We used these metrics as general proxies for changing ecological processes in urbanized habitats. The percent of impervious surface around a park is likely representative of habitat fragmentation, as urban infrastructure changes the net primary productivity due to increasing percentages of impervious surface or artificial landscapes, parks and yards (Shochat *et al.* 2006). This fragmentation then leads to changing species interactions as migration is impeded or organisms are forced into smaller areas (Shochat *et al.* 2006). The percent human density surrounding an urban park can serve as a proxy for the multitude of ecological changes humans impose on their surrounding environment. Urbanization and increasing human density change the types and availability of resources in the altered habitat (McKinney 2002; Sih *et al.* 2011). Finally, classifying our sites as urban or rural can generally capture the main differences in urban and natural sites. For example, pollution is a major consequence of urbanization (Donihue & Lambert 2015), and urban areas often include increased chemical, noise, or light pollution (Sih *et al.* 2011).

Between divergent allele frequencies, a skewed SFS, environmental associations, and overrepresented GO terms, we find several overlapping lines of evidence that support rapid divergent selection in white-footed mice. Our results support the growing body of evidence (Donihue & Lambert 2015) that finds urbanization directly impacts the ecology and evolution of

519 species. However, to fully support the hypothesis that organisms adapt to urban habitats, it is still
520 necessary to link genetic changes to measurable phenotypic differences and measure direct
521 fitness benefits. Past urban evolutionary studies often focus solely on phenotypic (Yeh 2004;
522 Partecke *et al.* 2006; Cheptou *et al.* 2008; Thompson *et al.* 2016) or genetic (Wandeler *et al.*
523 2003; Noël & Lapointe 2010; Mueller *et al.* 2013; Lourenco *et al.* 2017) differences between
524 populations in and outside of cities. However, researchers are beginning to examine both the
525 genotype and phenotype in parallel instances of urban evolution (Whitehead *et al.* 2010; Wirgin
526 *et al.* 2011; Hof *et al.* 2016), which is key to understanding how urbanization affects the
527 evolution of species. In the future, the gene annotations for our predicted outlier genes can help
528 determine which phenotypic traits to measure in urban *P. leucopus* populations.
529
530 **Functional roles of candidate genes: quality of urban diet?**
531 The model rodents *Mus musculus, Rattus norvegicus,* and *Cricetulus griseus* all have
532 deeply sequenced, assembled and annotated reference genomes. These resources allowed us to
533 annotate 89.5% of outlier loci with high quality functional information. Urban *P. leucopus*
534 exhibited signatures of positive selection in genes with GO terms overrepresented for organismal
535 metabolic processes, specifically digestion and metabolism of lipids and carbohydrates.
536 Mitochondrial genes identified as outliers (Table S2) were largely responsible for the
537 overrepresentation of metabolic process. While we can only speculate until further physiological
538 studies are conducted, our evidence suggests that the evolution of mitochondrial and metabolic
539 processes has been important to the success of *P. leucopus* living in NYC's urban forests.
540 Mitochondrial genes have often been used to describe neutral population variation, but
541 researchers have found ample evidence of selection acting on the mitochondrial genome

(Oliveira *et al.* 2008; Balloux 2010). For example, specific mitochondrial haplotypes are associated with more efficient thermogenesis and higher fitness in over-wintering shrews (Fontanillas *et al.* 2005). Pergams & Lacy (2007) found complete mitochondrial haplotype replacement in contemporary *P. leucopus* in Chicago compared to haplotypes sequenced from museum skins collected before urbanization. The agent of selection is not clear, but Munshi-South and Nagy (2014) also identified signatures of selection (or alternatively population expansion) in mitochondrial D-loop haplotypes from contemporary *P. leucopus* in NYC. Many mitochondrial functions are affected by the same environmental variables that change in response to urbanization, such as temperature (Balloux 2010), reduced migration (Lankau & Strauss 2011; Munshi-South 2012), or resource availability (Burcelin *et al.* 2002).

Urban *P. leucopus* may experience different energy budgets, physiological stressors or diets compared to rural counterparts. We found a substantial number of candidate genes with functions related to the metabolism and transport of lipids and carbohydrates, and the most common overrepresented GO terms involved lipid metabolism and homeostasis (Table S4). In the full outlier analysis, two genes are particularly interesting as targets of diet-mediated selection. The first gene, *FADS1,* is a fatty acid desaturase important for the biosynthesis of omega-3 and -6 fatty acids (long-chain polyunsaturated fatty acids, LCPUFA) from plant sources. Recent evidence suggests that the *FADS* gene family has been an important target of selection in humans during the transition from hunter-gather to agricultural societies (Ye *et al.* 2017). Alleles linked to upregulated biosynthesis of LCPUFAs (naturally low in plant based diets) increased in frequency after the Neolithic Revolution (Ye *et al.* 2017). We aligned our homologous *FADS1* contig with human transcripts to identify whether *P. leucopus* had any relevant alleles, but our sequenced populations did not contain SNPs at any relevant loci. The

565 full list of outliers also contained *APOB-100*, which is the primary apolipoprotein that binds and
566 transports lipids, including both forms of cholesterol (HDL and LDL).

567 When we investigated only candidate genes that were identified by both an outlier test
568 and GEA test, we found similar patterns suggesting *P. leucopus* in urban environments may be
569 adapting to novel food resources. These genes were strongly correlated with environmental
570 measures of urbanization, with clearly divergent allele frequencies between urban and rural sites
571 (Fig. 3B), suggesting that selection is acting on standing genetic variation in urban environments.
572 The most significant overrepresented GO term involved regulation of protein kinase B (*AKT*).
573 *AKT* is a key molecule in the insulin signaling pathway, important for promoting glucose storage
574 and regulating glucose in the bloodstream between fed and fasting states (Boucher *et al.* 2014).
575 Glycine metabolism was also overrepresented; increased amounts of glycine may be important
576 for regulating high-fat, high-sugar diets by decreasing concentrations of free fatty acids and
577 triglycerides (Wang *et al.* 2013). Finally, our candidate list contained genes significantly
578 associated with non-alcoholic fatty liver disease (NAFLD). NAFLD is a major hallmark of
579 obesity and diabetes and can be induced through increased uptake of dietary fatty acids (Fabbrini
580 *et al.* 2010).

581 These candidate genes suggest that white-footed mice in isolated urban parks may be
582 evolving in response to food resource differences between urban and rural habitats. This finding
583 is corroborated by recent evidence that urban white-footed mice in NYC have shorter upper and
584 lower tooth rows than rural mice (Yu *et al.* 2017). Lower quality food in the diet often requires
585 increased chewing and is accompanied with larger occlusal surfaces, and subsequently, longer
586 toothrows (Ungar 2010). One prediction is that urban *P. leucopus* consume a diet with a
587 substantially higher fat content than diets of rural populations. The typical diet of *P. leucopus*

588 across its range consists of arthropods, fruits, nuts, various green vegetation, and fungus (Wolff
589 *et al.* 1985). Given that white-footed mice are opportunistic generalists, many different food
590 resources could differ between urban and rural habitats. Urbanization in NYC has produced
591 relatively small green patches that are surrounded by a dense urban matrix, and *P. leucopus* in
592 NYC may successfully take advantage of invasive plant species, different arthropod
593 communities, or increased human food waste in and around their urban habitats. Local
594 adaptation in urban populations may allow these mice to more efficiently metabolize different
595 types or amounts of lipids and carbohydrates, although field studies are needed to examine the
596 link between these genetic changes and diet in NYC.
597

598 **ACKNOWLEDGMENTS**

599 We thank Mike Hickerson for his helpful comments and advice on many analyses and for access
600 to lab space for analyses and writing. We thank Diego Alvarado-Serrano, Alexander T. Xue,
601 Tyler Joseph, and Champak Reddy for their invaluable comments and advice concerning
602 bioinformatics and demographic analyses. Three anonymous reviewers and Prof. Stuart B.
603 Piertney also provided very helpful comments on the manuscript through Axios Review, as did
604 Dr. Aurélie Bonin and four anonymous reviewers for this journal. This research was supported
605 by the National Institute of General Medical Sciences of the National Institutes of Health under
606 award number R15GM099055 to JM-S and a NSF Graduate Research Fellowship to SEH. The
607 content is solely the responsibility of the authors and does not represent the official views of the
608 National Institutes of Health.
609

610 **REFERENCES**

611 Aken BL, Ayling S, Barrell D *et al.* (2016) The Ensembl gene annotation system. *Database*,

612 **2016**, baw093.

613 Balloux F (2010) The worm in the fruit of the mitochondrial DNA tree. *Heredity*, **104**, 419–420.

614 Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. *Trends in Ecology*

615 *& Evolution*, **23**, 38–44.

616 Bonin A (2008) Population genomics: a new generation of genome scans to bridge the gap with

617 functional genomics. *Molecular Ecology*, **17**, 3583–4.

618 Boucher J, Kleinridders A, Kahn CR (2014) Insulin Receptor Signaling in Normal and Insulin-

619 Resistant States. *Cold Spring Harbor Perspectives in Biology*, **6**, a009191–a009191.

620 Burcelin R, Crivelli V, Dacosta A, Roy-Tirelli A, Thorens B (2002) Heterogeneous metabolic

621 adaptation of C57BL/6J mice to high-fat diet. *American Journal of Physiology.*

622 *Endocrinology and Metabolism*, **282**, E834–E842.

623 Chace JF, Walsh JJ (2004) Urban effects on native avifauna: a review. *Landscape and Urban*

624 *Planning*, **74**, 46–69.

625 Chapman MA, Hiscock SJ, Filatov DA (2013) Genomic Divergence during Speciation Driven by

626 Adaptation to Altitude. *Molecular Biology and Evolution*, **30**, 2553–67.

627 Cheptou P-O, Carrue O, Rouifed S, Cantarel A (2008) Rapid evolution of seed dispersal in an

628 urban environment in the weed *Crepis sancta*. *Proceedings of the National Academy of*

629 *Sciences of the United States of America*, **105**, 3796–9.

630 Conesa A, Götz S, García-Gómez JM *et al.* (2005) Blast2GO: a universal tool for annotation,

631 visualization and analysis in functional genomics research. *Bioinformatics*, **21**, 3674–6.

632 Coop G, Witonsky D, Di Rienzo A, Pritchard JK (2010) Using environmental correlations to

633 identify loci underlying local adaptation. *Genetics*, **185**, 1411–23.

634 Danecek P, Auton A, Abecasis G *et al.* (2011) The variant call format and VCFtools.

Bioinformatics, **27**, 2156–2158.

DePristo MA, Banks E, Poplin R *et al.* (2011) A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nature Genetics*, **43**, 491–8.

Donihue CM, Lambert MR (2015) Adaptive evolution in urban ecosystems. *AMBIO*, **44**, 194–203.

Ellison CE, Hall C, Kowbel D *et al.* (2011) Population genomics and local adaptation in wild isolates of a model microbial eukaryote. *Proceedings of the National Academy of Sciences*, **108**, 2831–2836.

Excoffier L, Dupanloup I, Huerta-Sanchez E, Sousa VC, Foll M (2013) Robust Demographic Inference from Genomic and SNP Data. *PLoS Genetics*, **9**, e1003905.

Excoffier L, Hofer T, Foll M (2009) Detecting loci under selection in a hierarchically structured population. *Heredity*, **103**, 285–98.

Fabbrini E, Sullivan S, Klein S (2010) Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications. *Hepatology*, **51**, 679–689.

Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics*, **180**, 977–93.

Fontanillas P, Dépraz A, Giorgi MS, Perrin N (2005) Nonshivering thermogenesis capacity associated to mitochondrial DNA haplotypes and gender in the greater white-toothed shrew, *Crocidura russula*. *Molecular Ecology*, **14**, 661–670.

Frichot E, François O (2015) LEA : An R package for landscape and ecological association studies. *Methods in Ecology and Evolution*, **6**.

Frichot E, Schoville SD, Bouchard G, François O (2013) Testing for associations between loci and environmental gradients using latent factor mixed models. *Molecular Biology and*

Evolution, **30**, 1687–1699.

Gautier M (2015) Genome-wide scan for adaptive divergence and association with population-specific covariates. *Genetics*, **201**, 1555–1579.

Götz S, García-Gómez JM, Terol J *et al.* (2008) High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Research*, **36**, 3420–35.

Grossman SR, Shylakhter I, Karlsson EK *et al.* (2010) A composite of multiple signals distinguishes causal variants in regions of positive selection. *Science*, **327**, 883–6.

Gutenkunst RN, Hernandez RD, Williamson SH, Bustamante CD (2009) Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. *PLoS Genetics*, **5**, e1000695.

Harris SE, Munshi-South J, Obergfell C, O'Neill R (2013) Signatures of Rapid Evolution in Urban and Rural Transcriptomes of White-Footed Mice (*Peromyscus leucopus*) in the New York Metropolitan Area. *PLoS ONE*, **8**, e74938.

Harris SE, O'Neill RJ, Munshi-South J (2015) Transcriptome resources for the white-footed mouse (*Peromyscus leucopus*): new genomic tools for investigating ecologically divergent urban and rural populations. *Molecular Ecology Resources*, **15**, 382–394.

Harris SE, Xue AT, Alvarado-Serrano D *et al.* (2016) Urbanization shapes the demographic history of a native rodent (the white-footed mouse, *Peromyscus leucopus*) in New York City. *Biology Letters*, **12**, 20150983-.

Henry P, Russello MA (2013) Adaptive divergence along environmental gradients in a climate-change-sensitive mammal. *Ecology and Evolution*, **3**, 3906–3917.

Hermisson J (2009) Who believes in whole-genome scans for selection? *Heredity*, **103**, 283–284.

Hermisson J, Pennings PS (2005) Soft sweeps: Molecular population genetics of adaptation from

681 standing genetic variation. *Genetics*, **169**, 2335–2352.

682 Hoban S, Kelley JL, Lotterhos KE *et al.* (2016) Finding the Genomic Basis of Local Adaptation:
683 Pitfalls, Practical Solutions, and Future Directions. *The American Naturalist*, **188**, 379–397.

684 Hoekstra HE, Hirschmann RJ, Bundey RA, Insel PA, Crossland JP (2006) A Single Amino Acid
685 Mutation Contributes to Adaptive Beach Mouse Color Pattern. *Science*, **313**, 101–104.

686 Hof AEV, Campagne P, Rigden DJ *et al.* (2016) The industrial melanism mutation in British
687 peppered moths is a transposable element. *Nature*, **534**, 102–105.

688 Hohenlohe PA, Bassham S, Etter PD *et al.* (2010a) Population genomics of parallel adaptation in
689 threespine stickleback using sequenced RAD tags. *PLoS Genetics*, **6**, e1000862.

690 Hohenlohe PA, Phillips PC, Cresko WA (2010b) Using Population Genomics to Detect Selection
691 in Natural Populations: Key Concepts and Methodological Considerations. *International*
692 *Journal of Plant Sciences*, **171**, 1059–1071.

693 Hyndman D, Bauman DR, Heredia V V., Penning TM (2003) The aldo-keto reductase
694 superfamily homepage. *Chemico-Biological Interactions*, **143–144**, 621–631.

695 Joost S, Bonin A, Bruford MW *et al.* (2007) A spatial analysis method (SAM) to detect
696 candidate loci for selection: towards a landscape genomics approach to adaptation.
697 *Molecular Ecology*, **16**, 3955–69.

698 Kanehisa M, Goto S, Sato Y *et al.* (2014) Data, information, knowledge and principle: Back to
699 metabolism in KEGG. *Nucleic Acids Research*, **42**, 199–205.

700 Kim Y, Stephan W (2002) Detecting a local signature of genetic hitchhiking along a
701 recombining chromosome. *Genetics*, **160**, 765–777.

702 De Kort H, Vandepitte K, Bruun HH *et al.* (2014) Landscape genomics and a common garden
703 trial reveal adaptive differentiation to temperature across Europe in the tree species *Alnus*

glutinosa. *Molecular Ecology*, 4709–4721.

Lankau RA, Strauss SY (2011) Newly rare or newly common: evolutionary feedbacks through changes in population density and relative species abundance, and their management implications. *Evolutionary Applications*, **4**, 338–353.

Li J, Li H, Jakobsson M *et al.* (2012) Joint analysis of demography and selection in population genetics: where do we stand and where could we go? *Molecular Ecology*, **28**, 28–44.

Limborg MT, Helyar SJ, De Bruyn M *et al.* (2012) Environmental selection on transcriptome-derived SNPs in a high gene flow marine fish, the Atlantic herring (*Clupea harengus*). *Molecular ecology*, **21**, 3686–703.

Linnen CR, Kingsley EP, Jensen JD, Hoekstra HE (2009) On the origin and spread of an adaptive allele in deer mice. *Science*, **325**, 1095–8.

Lotterhos KE, Whitlock MC (2014) Evaluation of demographic history and neutral parameterization on the performance of F ST outlier tests. *Molecular Ecology*, **23**, 2178–2192.

Lotterhos KE, Whitlock MC (2015) The relative power of genome scans to detect local adaptation depends on sampling design and statistical method. *Molecular Ecology*, **24**, 1031–1046.

Lourenco A, Alvarez D, Wang IJ, Velo-Anton G (2017) Trapped within the city: integrating demography, time since isolation and population-specific traits to assess the genetic effects of urbanization. *Molecular Ecology*, **26**, 1498–1514.

MacManes MD, Eisen MB (2014) Characterization of the transcriptome, nucleotide sequence polymorphism, and natural selection in the desert adapted mouse *Peromyscus eremicus*. *PeerJ*, **2**, e642.

McKinney ML (2002) Urbanization, biodiversity, and conservation. *Bioscience*, **52**, 883–890.

McManus KF, Kelley JL, Song S *et al.* (2014) Inference of Gorilla Demographic and Selective History from Whole-Genome Sequence Data. *Molecular Biology and Evolution*, **32**, 600–612.

Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, **7**, 1–14.

Mueller JC, Partecke J, Hatchwell BJ, Gaston KJ, Evans KL (2013) Candidate gene polymorphisms for behavioural adaptations during urbanization in blackbirds. *Molecular Ecology*, **22**, 3629–3637.

Mullen LM, Hoekstra HE (2008) Natural selection along an environmental gradient: a classic cline in mouse pigmentation. *Evolution*, **62**, 1555–70.

Munshi-South J (2012) Urban landscape genetics: canopy cover predicts gene flow between white-footed mouse (*Peromyscus leucopus*) populations in New York City. *Molecular Ecology*, **21**, 1360–1378.

Munshi-South J, Kharchenko K (2010) Rapid, pervasive genetic differentiation of urban white-footed mouse (*Peromyscus leucopus*) populations in New York City. *Molecular Ecology*, **19**, 4242–4254.

Munshi-South J, Nagy C (2014) Urban park characteristics, genetic variation, and historical demography of white-footed mouse (*Peromyscus leucopus*) populations in New York City. *PeerJ*, **2**, e310.

Munshi-South J, Richardson JL (2017) *Peromyscus* transcriptomics: Understanding adaptation and gene expression plasticity within and between species of deer mice. *Seminars in Cell & Developmental Biology*, **61**, 131–139.

750 Munshi-South J, Zolnik CP, Harris SE (2016) Population genomics of the Anthropocene: urbani
751 zation is negatively associated with genome-wide variation in white -footed mouse
752 populations. *Evolutionary Applications*, doi:10.1111/eva.12357.
753 Natarajan C, Inoguchi N, Weber RE *et al.* (2013) Epistasis Among Adaptive Mutations in Deer
754 Mouse Hemoglobin. *Science*, **340**, 1324–1327.
755 Nielsen R (2005) Molecular signatures of natural selection. *Annual Review of Genetics*, **39**, 197–
756 218.
757 Nielsen R, Williamson S, Kim Y *et al.* (2005) Genomic scans for selective sweeps using SNP
758 data. *Genome research*, **15**, 1566–75.
759 Noël S, Lapointe F (2010) Urban conservation genetics : Study of a terrestrial salamander in the
760 city. , **143**, 2823–2831.
761 Ogata H, Goto S, Sato K *et al.* (1999) KEGG: Kyoto encyclopedia of genes and genomes.
762 *Nucleic Acids Research*, **27**, 29–34.
763 Oleksyk TK, Smith MW, O'Brien SJ (2010) Genome-wide scans for footprints of natural
764 selection. *Philosophical transactions of the Royal Society of London. Series B, Biological*
765 *sciences*, **365**, 185–205.
766 Oliveira DCSG, Raychoudhury R, Lavrov D V, Werren JH (2008) Rapidly evolving
767 mitochondrial genome and directional selection in mitochondrial genes in the parasitic wasp
768 *Nasonia* (Hymenoptera: Pteromalidae). *Molecular Biology and Evolution*, **25**, 2167–2180.
769 Orr HA (2005) The genetic theory of adaptation: a brief history. *Nature Reviews Genetics*, **6**,
770 119–27.
771 Partecke J, Schwabl I, Gwinner E (2006) Stress and the city: Urbanization and its effects on the
772 stress physiology in European Blackbirds. *Ecology*, **87**, 1945–1952.

773 Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. *PLoS Genetics*, **2**,
774 e190.
775 Pavlidis P, Jensen JD, Stephan W (2010) Searching for Footprints of Positive Selection in
776 Whole-genome SNP Data from Non-equilibrium Populations. *Genetics*.
777 Pavlidis P, Živkovic D, Stamatakis A, Alachiotis N (2013) SweeD: likelihood-based detection of
778 selective sweeps in thousands of genomes. *Molecular Biology and Evolution*, **30**, 2224–34.
779 Pérez-Figueroa A, García-Pereira MJ, Saura M, Rolán-Alvarez E, Caballero A (2010)
780 Comparing three different methods to detect selective loci using dominant markers. *Journal*
781 *of Evolutionary Biology*, **23**, 2267–2276.
782 Pergams ORW, Lacy RC (2008) Rapid morphological and genetic change in Chicago-area
783 *Peromyscus*. *Molecular Ecology*, **17**, 450–63.
784 Reid NM, Proestou DA, Clark BW *et al.* (2016) The genomic landscape of rapid repeated
785 evolutionary adaptation to toxic pollution in wild fish. *Science*, **354**, 1305–1308.
786 Reimand J, Arak T, Adler P *et al.* (2016) g:Profiler—a web server for functional interpretation of
787 gene lists (2016 update). *Nucleic Acids Research*, **44**, W83–W89.
788 Rellstab C, Fischer MC, Zoller S *et al.* (2017) Local adaptation (mostly) remains local:
789 reassessing environmental associations of climate-related candidate SNPs in *Arabidopsis*
790 *halleri*. *Heredity*, **118**, 193–201.
791 Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R (2015) A practical guide to
792 environmental association analysis in landscape genomics. *Molecular Ecology*, **24**, 4348–
793 4370.
794 Rockman M (2012) The QTN program and the alleles that matter for evolution: all that's gold
795 does not glitter. *Evolution*, **66**, 1–17.

796 Rogic A, Tessier N, Legendre P, Lapointe F-J, Millien V (2013) Genetic structure of the white-
797 footed mouse in the context of the emergence of Lyme disease in southern Québec. *Ecology*
798 *and Evolution*, **3**, 2075–88.
799 Rytwinski T, Fahrig L (2007) Effect of road density on abundance of white-footed mice.
800 *Landscape Ecology*, **22**, 1501–1512.
801 Savolainen O, Lascoux M, Merilä J (2013) Ecological genomics of local adaptation. *Nature*
802 *Reviews Genetics*, **14**, 807–20.
803 Serieys LEK, Lea A, Pollinger JP, Riley SPD, Wayne RK (2015) Disease and freeways drive
804 genetic change in urban bobcat populations. *Evolutionary Applications*, **8**, 75–92.
805 Settachan D (2001) Mechanistic and molecular studies into the effects of 2,3,7,8-
806 tetrachlorodibenzo-p-dioxin and similar compounds in the deer mouse, *Peromyscus*
807 *maniculatus*. Texas Tech University.
808 Shochat E, Warren PS, Faeth SH, McIntyre NE, Hope D (2006) From patterns to emerging
809 processes in mechanistic urban ecology. *Trends in Ecology and Evolution*, **21**, 186–91.
810 Sih A, Ferrari MCO, Harris DJ (2011) Evolution and behavioural responses to human-induced
811 rapid environmental change. *Evolutionary Applications*, **4**, 367–387.
812 Sork VL, Aitken SN, Dyer RJ *et al.* (2013) Putting the landscape into the genomics of trees:
813 Approaches for understanding local adaptation and population responses to changing
814 climate. *Tree Genetics and Genomes*, **9**, 901–911.
815 Stapley J, Reger J, Feulner PGD *et al.* (2010) Adaptation Genomics: the next generation. *Trends*
816 *in Ecology & Evolution*, **25**, 705–712.
817 Stinchcombe JR, Hoekstra HE (2008) Combining population genomics and quantitative genetics:
818 finding the genes underlying ecologically important traits. *Heredity*, **100**, 158–70.

819 Storz JF, Runck AM, Moriyama H, Weber RE, Fago A (2010) Genetic differences in
820 hemoglobin function between highland and lowland deer mice. *The Journal of*
821 *Experimental Biology*, **213**, 2565–74.
822 Storz JF, Runck AM, Sabatino SJ *et al.* (2009) Evolutionary and functional insights into the
823 mechanism underlying high-altitude adaptation of deer mouse hemoglobin. *Proceedings of*
824 *the National Academy of Sciences of the United States of America*, **106**, 14450–5.
825 Storz J, Sabatino S, Hoffmann F (2007) The molecular basis of high-altitude adaptation in deer
826 mice. *PLoS Genetics*, **3**.
827 Supek F, Bosnjak M, Skunca N, Smuc T (2011) Revigo summarizes and visualizes long lists of
828 gene ontology terms. *PLoS ONE*, **6**.
829 Tajima F (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics*,
830 **105**, 437–460.
831 Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA
832 polymorphism. *Genetics*, **123**, 585–95.
833 Thompson KA, Renaudin M, Johnson MTJ (2016) Urbanization drives the evolution of parallel
834 clines in plant populations. *Proceedings of the Royal Society B: Biological Sciences*, **283**,
835 20162180.
836 Tiffin P, Ross-Ibarra J (2014) Advances and limits of using population genetics to understand
837 local adaptation. *Trends in Ecology & Evolution*, **29**, 673–680.
838 Tomassini A, Colangelo P, Agnelli P *et al.* (2014) Cranial size has increased over 133 years in a
839 common bat, *Pipistrellus kuhlii*: a response to changing climate or urbanization? *Journal*
840 *Biogeography*, **41**, 944–953.
841 Turner TL, Bourne EC, Von Wettberg EJ, Hu TT, Nuzhdin S V (2010) Population resequencing

reveals local adaptation of *Arabidopsis lyrata* to serpentine soils. *Nature Genetics*, **42**, 260–3.

Ungar PS (2010) *Mammal Teeth: Origin, Evolution, and Diversity*. Johns Hopkins University Press, Baltimore, MD.

De Villemereuil P, Frichot É, Bazin É, François O, Gaggiotti OE (2014) Genome scan methods against more complex models: When and how much should we trust them? *Molecular Ecology*, **23**, 2006–2019.

Vitti JJ, Grossman SR, Sabeti PC (2013) Detecting natural selection in genomic data. *Annual review of genetics*, **47**, 97–120.

Wandeler P, Funk SM, Largiadèr CR, Gloor S, Breitenmoser U (2003) The city-fox phenomenon: genetic consequences of a recent colonization of urban habitat. *Molecular Ecology*, **12**, 647–56.

Wang W, Wu Z, Dai Z *et al.* (2013) Glycine metabolism in animals and humans: Implications for nutrition and health. *Amino Acids*, **45**, 463–477.

Wang M, Yu Y, Haberer G *et al.* (2014) The genome sequence of African rice (*Oryza glaberrima*) and evidence for independent domestication. *Nature Genetics*, 982–988.

Weber JN, Peterson BK, Hoekstra HE (2013) Discrete genetic modules are responsible for complex burrow evolution in *Peromyscus* mice. *Nature*, **493**, 402–405.

Whitehead A, Triant D, Champlin D, Nacci D (2010) Comparative transcriptomics implicates mechanisms of evolved pollution tolerance in a killifish population. *Molecular Ecology*, **19**, 5186–5203.

Wirgin I, Roy NK, Loftus M *et al.* (2011) Mechanistic basis of resistance to PCBs in Atlantic tomcod from the Hudson River. *Science (New York, N.Y.)*, **331**, 1322–5.

De Wit P, Palumbi SR (2013) Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. *Molecular Ecology*, **22**, 2884–97.

Wolff JO, Dueser RD, Berry K (1985) Food Habits of Sympatric *Peromyscus leucopus* and *Peromyscus maniculatus*. *Journal of Mammalogy*, **66**, 795–798.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, 323–354.

Ye K, Gao F, Wang D, Bar-Yosef O, Keinan A (2017) Dietary adaptation of FADS genes in Europe varied across time and geography. *Nature Ecology & Evolution*, **1**, 167.

Yeh PJ (2004) Rapid evolution of a sexually selected trait following population establishment in a novel habitat. *Evolution*, **58**, 166–174.

Yu A, Munshi-south J, Sargis EJ *et al.* (2017) Morphological Differentiation in White-Footed Mouse (Mammalia : Rodentia : Cricetidae : *Peromyscus leucopus*) Populations from the New York City Metropolitan Area. *Bulletin of the Peabody Museum of Natural History*, **58**, 3–16.

Zhou L, Bawa R, Holliday JA (2014) Exome resequencing reveals signatures of demographic and adaptive processes across the genome and range of black cottonwood (Populus trichocarpa). *Molecular Ecology*, **23**, 2486–2499.

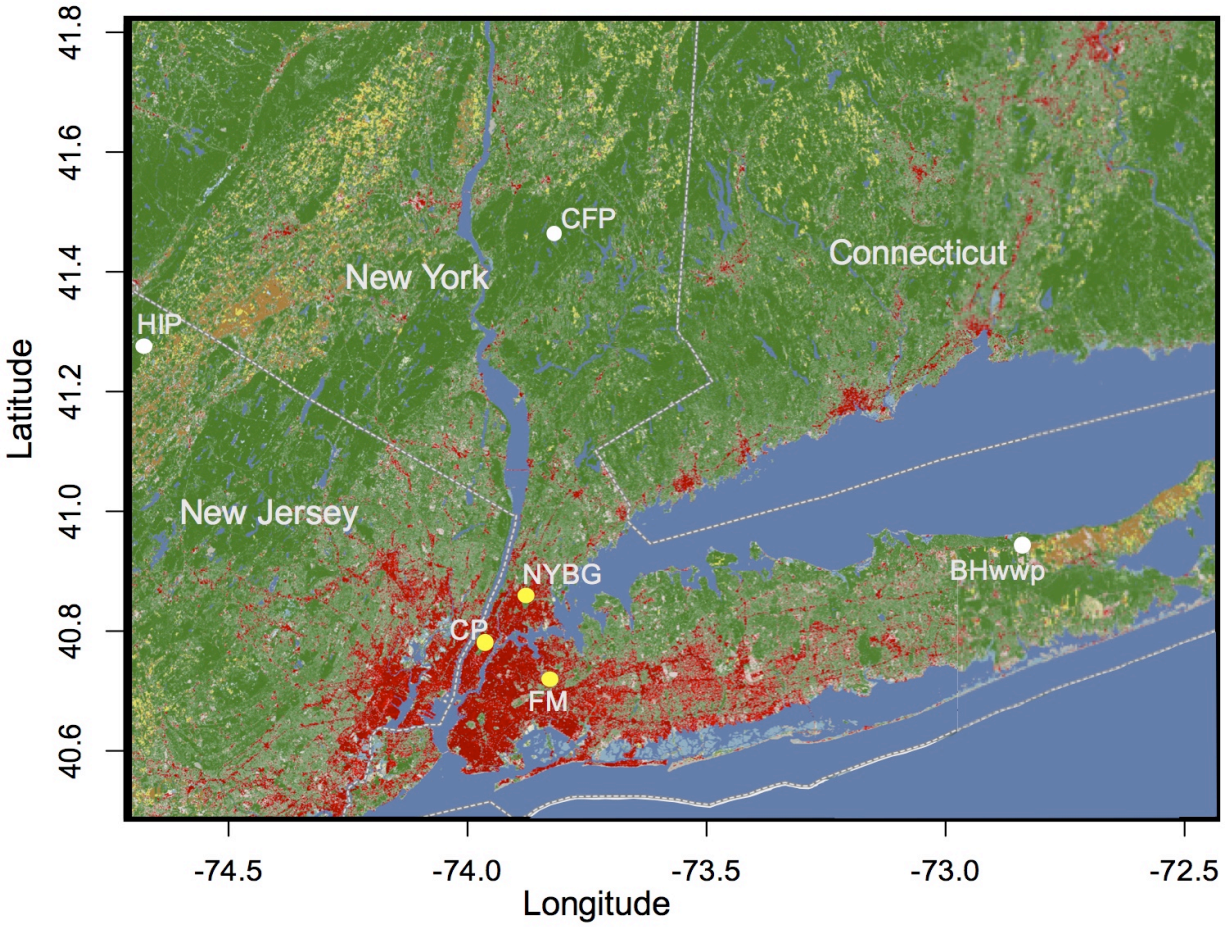
Data Accessibility

-VCF file of SNP genotypes used for demographic inference: Dryad doi:10.5061/dryad.d48f9

-raw sequencing files for transcriptome data: GenBank Sequence Read Archive (SRA Accession no. SRP020005)

-Transcriptome contigs: Dryad doi:10.5061/dryad.6hc0f

889 **FIGURE LEGENDS**



890

891

892 **Figure 1.** Map of sample localities in the NYC metropolitan area. Sites in yellow are urban
893 parks within New York City, CP = Central Park; FM = Flushing Meadows—Willow Lake;
894 NYBG = New York Botanical Gardens. Sites in white are rural parks, BHwwp = Brookhaven
895 and Wildwood State Park; CFP = Clarence Fahnestock State Park; HIP = High Point State Park.
896 The map includes data from the National Land Cover Database. All non-green colors are shaded
897 according to land use. Yellows and browns equal cultivated land and reds represent developed
898 areas (Darker red = increased development). Green colors are shaded according to canopy cover
899 (Darker green = increased canopy cover) and come from the 2011 National Land Cover Canopy
900 database. Full legends for the colors are shown in Figure S1.

901

902

903

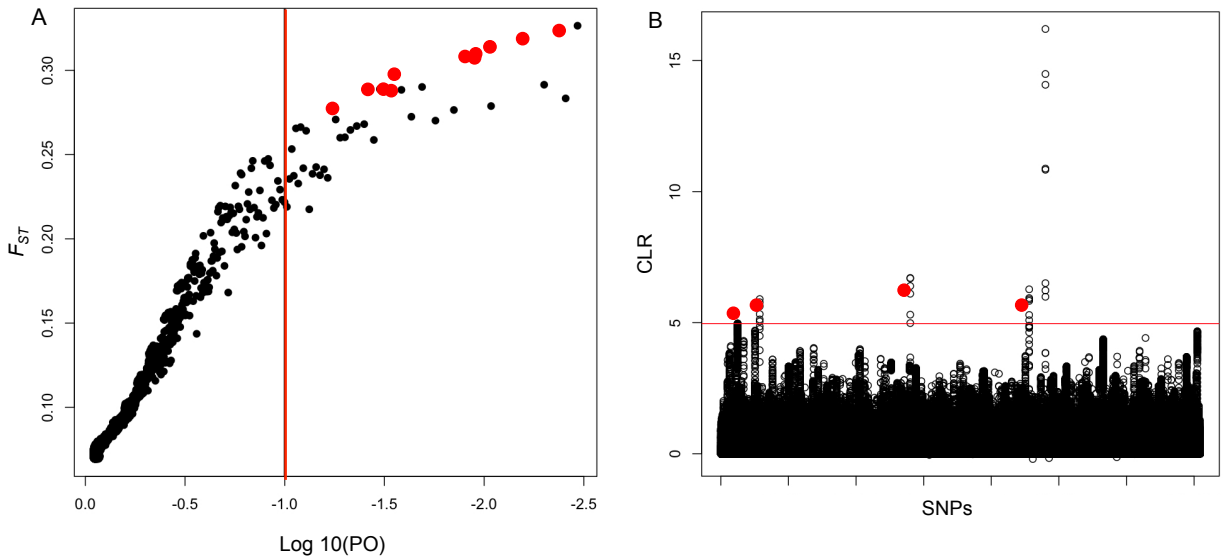


Figure 2. (a) BayeScan 2.1 plot of 154,770 SNPs genome scan analysis between urban and rural populations, including 48 individual white-footed mice from six NYC sampling sites. F_{ST} is on the vertical axis plotted against the $\log_{10}$ of the posterior odds (PO). The vertical red line indicates the cutoff (q-value = 0.1) used for identifying outlier SNPs. The markers on the right side of the vertical line show all outlier SNP candidates and the red circles represent the final accepted outlier SNPs from Table 2. (b) SweeD results with each of the 154,770 SNPs plotted from all 48 individuals. The Composite Likelihood Ratio (CLR) is plotted along the vertical access and each unfilled point represents an individual SNP. The x-axis has SNPs ordered by contig, but not by genomic position. The horizontal red line indicates the cutoff used for identifying outlier SNPs at $P \leq 0.0001$. The red circles represent the final accepted outlier SNPs from Table 2.

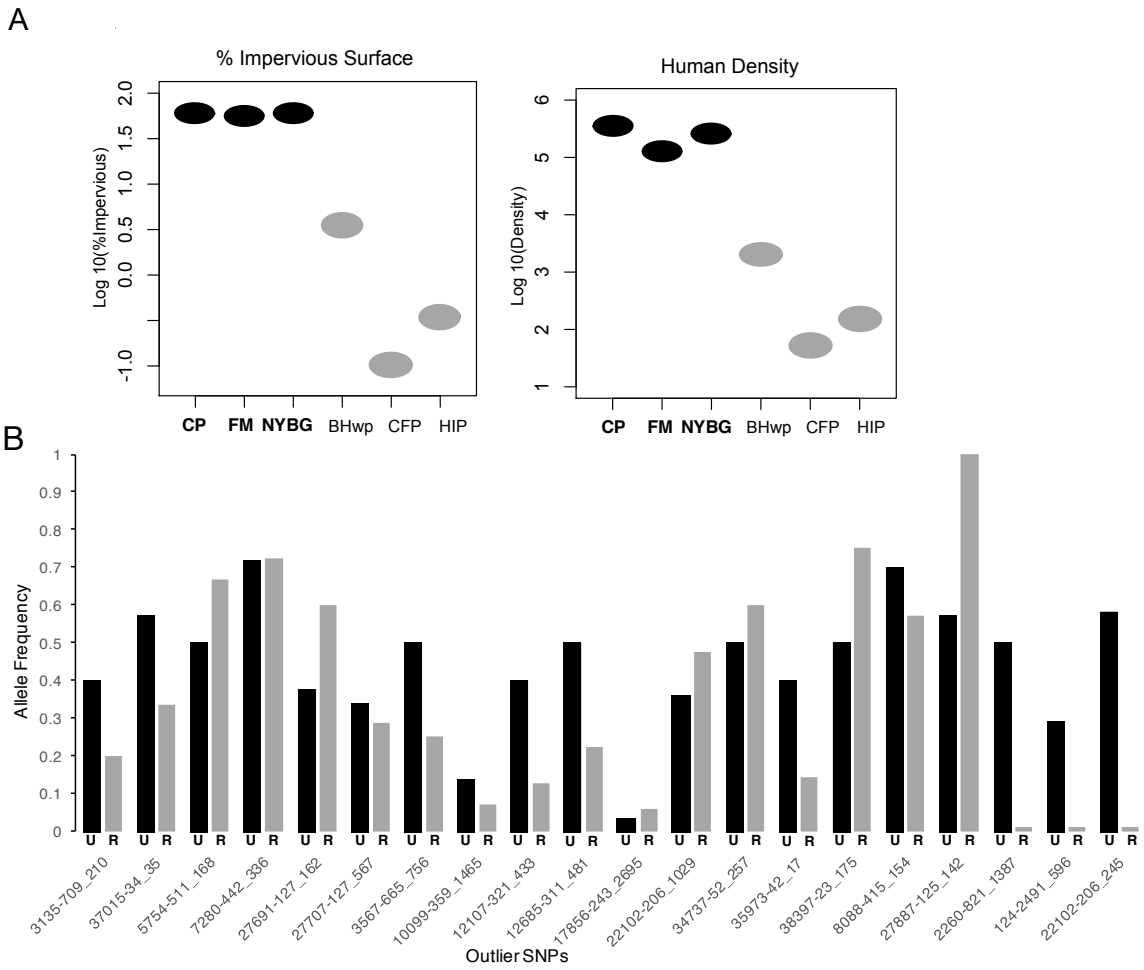


Figure 3. (a) Plot of urbanization metrics for all 6 sampling sites from NYC used in this study. Urban sampling sites are highlighted in bold on the horizontal axis and colored black. Rural sites are colored gray. The log10 value of % Impervious Surface and Human Density are plotted along the vertical axis and the oval represents the value for each sampling site. (b) Allele frequencies for candidate loci identified from both genome scans and GEA tests grouped by urban (U, black) or rural (R, gray) classification. The frequency of the outlier SNP within each type of population is plotted on the vertical axis. Each candidate loci is labeled with the contig and outlier SNP on the horizontal axis; see Table 2 for associated gene names.

TABLES

Table 1. Summary population genomic statistics (mean ± standard error) for three urban and three rural populations of white-footed mice (*Peromyscus leucopus*) examined in this study.

Population	Nucleotide diversity (π)	Tajima's *D*
Urban		
CP	0.131 ±0.001	0.318 ±0.005
FM	0.112 ±0.001	0.301 ±0.006
NYBG	0.092 ±0.001	0.280 ±0.006
Rural		
BHwwp	0.198 ±0.001	0.350 ±0.004
CFP	0.211 ±0.001	0.336 ±0.004
HIP	0.263 ±0.001	0.349 ±0.004

957 **Table 2.** Outlier loci (*N* = 19) identified in at least one test for selection (BayeScan, BayPass, or
958 SweeD) and one GEA test (LFMM or BayPass_GEA). SNP shows the position in contig
959 containing the outlier loci. Tests show which tests identified the SNP as an outlier: BPG =
960 BayPass_GEA; BPD = BayPass_Diversifying; BS = BayeScan; SW = SweeD; LFMM = LFMM.

Contig	SNP	Ensemble Gene ID	Gene	Tests
27887-125	142	ENSMUSG00000029440	proteasome 26S subunit, non-ATPase, 9	BPG, BS
3135-709	210	ENSMUSG00000002320	transmembrane 9 superfamily member 1	BPG, BS
37015-34	35	ENSMUSG00000037287	tubulin folding cofactor E-like	BS, BPD
5754-511	168	ENSMUSG00000041161	OTU domain containing 3	BPG, BS
7280-442	336	ENSMUSG00000021287	X-ray repair complementing defective repair in CHC3	BPD, LFMM
2260-821	1387	ENSMUSG00000024045	A kinase (PRKA) anchor protein 8	BPG, BS
27691-127	162	NA	NA	BPG, BS
27707-127	567	ENSMUSG00000106907	autophagy related 2A	BPG, BS, BPD
3567-665	756	ENSMUSG00000001700	GRAM domain containing 3	BPG, BS, BPD
10099-359	1465	ENSMUSG00000024066	xanthine dehydrogenase	BPG, SW
12107-321	433	NA	NA	BPG,BS
124-2491	596	ENSMUSG00000064358	cytochrome c oxidase III	BPG, SW
12685-311	481	ENSMUSG00000035637	glyoxylate reductase/hydroxypyruvate reductase	BPG, BPD
17856-243	2695	ENSMUSG00000021091	serine peptidase inhibitor, clade A, member 3N	LFMM, SW
22102-206	245, 1029	ENSMUSG00000045868	GTPase, very large interferon inducible 1	BPG, BPD, LFMM
34737-52	125, 257	NA	NA	BPG, BPD
35973-42	17	ENSMUSG00000001173	oculocerebrorenal syndrome of Lowe	BPG, BS
38397-23	175	NA	NA	BPG, BS
8088-415	154	ENSMUSG00000002379	NADH dehydrogenase 1 alpha subcomplex 11	BPG, BPD, LFMM

961

962 **Table 3.** Overrepresented gene ontology (GO) terms from g:Profiler (q-value < 0.05) for the 19
963 outlier loci from tests for both selection and GEA. Associated genes shows which ensemble gene
964 homologs from Table 2 are associated with each overrepresented term.

Description	Annotation ID	P-value	Associated Genes
Negative regulation of protein kinase B signaling	GO:0051898	0.05	ENSMUSG00000024066, ENSMUSG00000041161
Cytochrome c oxidase, mitochondrial	CORUM:538	0.05	ENSMUSG00000064358
Ocrl-Cdc42 complex	CORUM:975	0.00914	ENSMUSG00000001173
Glyoxylate and dicarboxylate metabolism	KEGG:00630	0.0355	ENSMUSG00000035637
Homologous recombination	KEGG:03440	0.0512	ENSMUSG00000021287
Pyruvate metabolism	KEGG:00620	0.0464	ENSMUSG00000035637
Oxidative phosphorylation	KEGG:00190	0.00791	ENSMUSG00000002379, ENSMUSG00000064358
Alzheimer's disease	KEGG:05010	0.0133	ENSMUSG00000002379, ENSMUSG00000064358
Huntington's disease	KEGG:05016	0.0158	ENSMUSG00000002379, ENSMUSG00000064358
Non-alcoholic fatty liver disease (NAFLD)	KEGG:04932	0.0101	ENSMUSG00000002379, ENSMUSG00000064358
Glycine, serine and threonine metabolism	KEGG:00260	0.05	ENSMUSG00000035637
Metabolic pathways	KEGG:01100	0.0029	ENSMUSG00000001173, ENSMUSG00000002379, ENSMUSG00000024066, ENSMUSG00000035637, ENSMUSG00000064358
Parkinson's disease	KEGG:05012	0.00899	ENSMUSG00000002379, ENSMUSG00000064358
Autophagy - other	KEGG:04136	0.0404	ENSMUSG00000106907
Caffeine metabolism	KEGG:00232	0.00742	ENSMUSG00000024066

965

966

967

968

969 **SUPPORTING INFORMATION**

970 **Table S1.** Average pairwise F_{ST} among six *P. leucopus* populations based on transcriptome-
971 derived SNPs.

972 **Table S2.** Excel file containing the full list of outlier contigs (N = 381), the outlier SNP position,
973 and test(s) that identified the outlier SNP. Remaining columns list the homologous Ensemble
974 *Mus musculus* gene ID and name.

975 **Table S3.** Excel file containing results from g:Profiler for overrepresented GO terms from the
976 full list of outlier contigs in Table S2. The table also includes the homologous *Mus musculus*
977 genes that are associated with each GO term.

978 **Table S4.** Excel file containing Revigo results. Enriched GO terms from g:Profiler are sorted
979 into largest parent terms and listed based on the frequency of occurrence.

980 **Table S5.** Environmental variable values for each individual mouse. Impervious = mean %
981 impervious surface in 2 km buffer. Density = human population per 2 km buffer. Urban or Rural
982 = classification as urban or rural site.