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

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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.*

62 2010a; Turner *et al.* 2010; Ellison *et al.* 2011; De Wit & Palumbi 2013), and often results from
63 the operation of selection on standing genetic variation as opposed to novel mutations over
64 relatively short time scales (Barrett & Schluter 2008; Stapley *et al.* 2010). Additionally, the
65 quantitative traits involved in local adaptation may involve many genes of small effect working
66 to produce the desired phenotype (Orr 2005; Rockman 2012), and these ecologically relevant but
67 non-conspicuous phenotypes are predicted to be those most involved in urban adaptation (Sih *et*
68 *al.* 2011). However, traits with relatively simple genetic architecture may also be under selection
69 in urban environments (Thompson *et al.* 2016). Investigating the genetic basis of local
70 adaptation has provided insight into a variety of evolutionary processes including speciation,
71 maintenance of genetic diversity, range expansion, and species responses to changing
72 environments (Savolainen *et al.* 2013; Tiffin & Ross-Ibarra 2014), and holds great promise for
73 understanding adaptive evolution in response to urbanization.

74 Landscape genomics has recently produced a number of approaches for studying local
75 adaptation. This field is defined by the spatially explicit study of genomic variation (Sork *et al.*
76 2013) that seeks to identify environmental variables influencing adaptive genomic variation
77 (Rellstab *et al.* 2015). Landscape genomics, and more specifically genotype-by-environment
78 analyses (GEA), can successfully identify associations between urban environmental variables
79 and allele frequencies that indicate adaptation to local urban conditions. These approaches can
80 also help to untangle the interactions between neutral demographic processes and selection
81 (Rellstab *et al.* 2017). Urban populations are influenced by both genetic drift through founder
82 effects and barriers to gene flow, and selection acting on genetic variation linked to increased
83 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

107 pollution was also reported in tomcod (*Microgadus tomcod)* in the Hudson River through a
108 deletion that similarly increases tolerance to PCBs (Wirgin *et al.* 2011). Urban adaptation has
109 also been confirmed in the well-known peppered moth (*Biston betularia*) system. Recent
110 evidence suggests that the industrial melanism trait in this species is linked to an insertion of a
111 transposable element in the *cortex* gene in the early 1800s that spread throughout the population
112 in response to industrial airborne pollution (Hof *et al.* 2016). The study of additional systems
113 will likely identify a complex array of adaptive evolutionary responses in cities (Whitehead *et al.*
114 2017).

115 Here we examined signatures of selection in isolated urban populations of white-footed
116 mice, *Peromyscus leucopus*, in New York City (NYC) using a landscape genomics approach.
117 *Peromyscus* spp. (Rodentia, Cricetidae) are a group of abundant small mammals found across
118 much of North and Central America. They live in a diverse array of habitats that exposes them
119 to a variety of selective pressures, and thus multiple *Peromyscus* spp. have become model
120 systems for studies examining ecology, evolution, and physiology in natural populations
121 (Munshi-South & Richardson 2017). There is also evidence that *Peromyscus* spp. readily adapt
122 to environmental change (Storz *et al.* 2007, 2009, 2010; Mullen & Hoekstra 2008; Linnen *et al.*
123 2009; Weber *et al.* 2013; Natarajan *et al.* 2013; Munshi-South & Richardson 2017), making
124 them good subjects for the study of local adaptation. White-footed mice are one of the few native
125 mammals that thrive in extremely small, fragmented urban forests in North America (Pergams &
126 Lacy 2008; Rogic *et al.* 2013; Munshi-South & Nagy 2014), and tend to be found at higher
127 densities in urban vs. rural patches due to a thick understory providing abundant food resources
128 and exclusion of major predators and competitors (Rytwinski & Fahrig 2007). Increased density
129 may also be due to limited *P. leucopus* dispersal between urban sites. Munshi-South (2012)

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

137 In the current study, we examined SNPs generated from individual transcriptome
138 sequencing for *P. leucopus* from three urban sites in NYC and three rural sites from the
139 surrounding area. We generated a large SNP dataset and produced estimates of nucleotide
140 diversity (π, Tajima 1983), Tajima's *D* (Tajima 1989), and F_{ST} (Wright 1951) to generate per-site
141 estimates and identify loci that deviate from neutral expectations. We then used a variety of
142 genome scan methods and outlier tests to identify genes subject to selection in an urban setting.
143 Our approach identified population differentiation, shifts in allele frequencies, and associations
144 between alleles and environmental variables. However, neutral demographic processes such as
145 population bottlenecks can produce signatures of genetic variation similar to those produced by
146 selection (Oleksyk *et al.* 2010; Li *et al.* 2012). We accounted for this possibility by
147 incorporating a simulated neutral SNP dataset from an inferred demographic history (Harris *et al.*
148 2016) directly into our null model for identifying outliers (Excoffier *et al.* 2009; Gutenkunst *et*
149 *al.* 2009; Li *et al.* 2012; Vitti *et al.* 2013; Lotterhos & Whitlock 2015).

150 The three specific aims of this study were the following: 1. identify candidate genes
151 exhibiting signatures of selection in NYC populations of white-footed mice using a variety of
152 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

176 SNPs with the Genome Analysis Toolkit (GATK version 2.8) pipeline using a Bayesian
177 genotype likelihood model (DePristo *et al.* 2011). In order to call a SNP, we required it to occur
178 in at least five individuals, have a nucleotide quality (q-score) ≥ 30, exhibit no strand bias (FS ≥
179 35), and to come from only uniquely mapped reads. We also required SNPs to have an overall
180 depth ≥ 10X and ≤ 350X (to account for paralogous sequences), a minor allele frequency (MAF)
181 ≥ 0.025, and removed SNPs where every individual was heterozygous.
182
183 **Summary statistics**
184 SNP information was stored in a VCF (variant call format) file and summary statistics
185 were calculated using vcftools 0.1.12b (Danecek *et al.* 2011). We calculated per-site nucleotide
186 diversity (π), Tajima's D, and F_{ST}. We also calculated the statistics for each contig (per-site
187 statistic summed across all SNPs per contig divided by total sites) and calculated the average
188 estimate for each population, including all pairwise population comparisons for F_{ST}.
189
190 **Scans for positive selection based on population differentiation**
191 We used the F_{ST} based analysis implemented in BayeScan v. 2.1 (Foll & Gaggiotti 2008)
192 to compare all six population-specific allele frequencies with global averages and identify outlier
193 SNPs. BayeScan identifies loci that exhibit divergence between groups that is stronger than
194 would be expected under neutral genetic processes. Based on a set of neutral allele frequencies
195 under a Dirichlet distribution, BayeScan uses a Bayesian model to estimate the probability that a
196 given locus has been subject to selection. To generate more realistic allele frequency
197 distributions, we used BayeScan for independent coalescent simulations of SNP datasets based
198 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

222 the XtX statistic to identify adaptive divergence. SNPs with XtX estimates greater than the 95%
223 threshold determined from PODs were identified as resulting from adaptive divergence.
224
225 **Analysis for selective sweeps**
226 We also identified outlier regions when the observed SFS showed an excess of low
227 frequency and high frequency minor alleles, a signal indicative of a recent selective sweep. The
228 composite likelihood ratio (CLR) statistic is used to identify regions where the observed SFS
229 matches the expected SFS generated from a selective sweep (Kim & Stephan 2002; Nielsen *et al.*
230 2005; Pavlidis *et al.* 2010). We calculated the CLR along sliding windows across the
231 transcriptome using the software program SweeD (Pavlidis *et al.* 2013). SweeD is an extension
232 of Sweepfinder (Nielsen *et al.* 2005) that is optimized for large next generation sequencing
233 (NGS) datasets. We lacked a genome to provide high-quality linkage information so SweeD was
234 run separately for each population and on individual contigs. We used default parameters except
235 for using a sliding window size of 200 bp and use of a folded SFS, as we lacked an outgroup to
236 infer ancestral alleles. The window within each contig with the highest CLR score is considered
237 the likely location of a selective sweep. Similar to the method used for BayeScan, statistical
238 significance was established from a null distribution generated by running SweeD on SNP
239 datasets simulated under the inferred demographic history for *P. leucopus* populations (Harris *et*
240 *al.* 2016). SweeD does not inherently identify outlier regions. The CLR is computed using a
241 selective sweep model on the observed data and then compared to a neutral model calibrated
242 with a simulated background SFS. As before, we used 100 datasets with 100,000 SNPs each,
243 simulated under the inferred neutral demographic history for white-footed mice in NYC. The
244 CLR was calculated using SweeD for all simulated datasets. We identified outlier contigs if their

245 CLR value was greater than any produced in neutral simulations. We also required outliers to
246 fall within the top 0.01% of the CLR distribution for the observed SNPs.
247
248 **Genotype-environment association tests for environmental selection**
249 We used the GEA approach of LFMM: Latent Factor Mixed Models (Frichot *et al.* 2013)
250 to associate our full SNP dataset with potential environmental selection pressures. LFMM
251 examines associations between environmental and genetic variation while accounting for the
252 neutral genetic background and structure between populations (Frichot *et al.* 2013). We tested
253 three environmental variables associated with urbanization: 1) percent impervious surface (i.e.
254 surfaces such as roads, rooftops, and other human infrastructure that do not absorb water
255 calculated from USGS National Land Cover Data) within a 2 km (the approximate lifetime
256 dispersal distance of white-footed mice) buffer around each sampling site's GPS coordinate, 2)
257 human density within a two-kilometer buffer around each sampling site's GPS coordinate
258 (calculated from US Census blocks), and 3) categorization of each site as urban, within NYC
259 limits, or rural, undeveloped state park outside city limits (Coded as 0 or 1 in LFMM).
260 Calculations were made in ArcGIS v10.1 (ESRI, Redlands, CA, USA) and were previously
261 reported in Munshi-South *et al*. (2016). This previous analysis found that variables 1-2 were
262 significantly associated with genome-wide variation in *P. leucopus* populations in the NYC
263 metropolitan area. LFMM requires the user to define the number of latent factors, K, that
264 describe population structure in the dataset. To identify the appropriate number of K latent
265 factors, we performed a genetic PCA followed by a Tracy-Widom test to find the number of
266 eigenvalues with P values ≤ 0.01 (Patterson *et al.* 2006; Frichot & François 2015). Based on this
267 approach, we ran LFMM with default parameters except for K = 6, number of MCMC cycles =

268 100,000, and burn-in = 50,000. Using author recommendations, we calculated the median |z|-
269 score from 10 replicate runs and then readjusted the p values. LFMM uses |z|- scores to report
270 the probability of a SNP's association with an environmental variable. Again, we controlled for
271 FDR by using a q-value threshold of ≤ 0.1.

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

282

283 **Functional annotation of candidate genes**

284 We used the gene annotation pipeline in Blast2GO (Conesa *et al.* 2005; Götz *et al.* 2008)
285 to identify sequences from the NCBI non-redundant protein database that were homologous to
286 our outlier contigs identified above. We then retrieved associated gene ontology (GO) terms.
287 Blast2GO retrieves GO terms associated with BLASTX hits and uses the KEGG database to
288 describe biochemical pathways linking different enzymes (Ogata *et al.* 1999; Kanehisa *et al.*
289 2014). For downstream enrichment analyses, we also used the Ensembl gene annotation system
290 (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**

313 We used BayeScan to identify 39 outlier SNPs exhibiting patterns of divergent selection
314 between urban and rural populations (Fig. 2A, Table S2). There were no SNPs that exhibited
315 signatures of balancing selection. F_{ST} values for outlier SNPs ranged from 0.21 - 0.33.
316 BayeScan identified zero outlier SNPs in the simulated neutral dataset, and accordingly the 39
317 outlier SNPs from the observed data had q-values that were smaller than the most extreme values
318 for the simulated data (q-value ≤ 0.6). We ran a similar test looking for patterns of divergence
319 using BayPass. This analysis identified 56 SNPs that showed evidence of divergent selection
320 (Table S2). We used PODs to estimate a null distribution and outlier SNPs had XtX values ≥
321 8.35 (top 5% of the null distribution). There were 11 SNPs associated with diversifying
322 selection in both the BayeScan and BayPass analyses.
323 To identify signatures of selective sweeps , we used the CLR statistic implemented in
324 SweeD. We found that CLR scores in the top 5% of the simulated distribution were generally 2-
325 3X lower than values in the top 5% of the observed dataset. We ran SweeD on observed SNPs
326 within individual contigs and identified outliers by filtering for a CLR score ≥ 3.53 (the
327 maximum CLR from simulated data). We also chose regions that fell within the top 0.01% of
328 the observed distribution (Fig. 2B); all outliers had CLR scores ≥ 4.97. SweeD identified regions
329 with SFS patterns that fit a selective sweep model in 45 contigs within urban populations (Table
330 S2). There was no overlap between outlier SNPs identified by SweeD and BayeScan / BayPass.
331 There were 131 SNPs associated with at least one of three environmental variables tested
332 using LFMM (Fig. 3A, Table S2). There was zero overlap with outliers identified from
333 BayeScan and only one SNP that overlapped between SweeD and LFMM. Three SNPs
334 identified in BayPass as outliers showing signatures of diversifying selection were also
335 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.

358 We also looked for overrepresentation in the gene annotations associated with the filtered
359 subset of 19 outliers and found related results (Table 3). There were 15 contigs homologous to
360 known genes with functional annotation. Metabolic pathways were the most overrepresented
361 group of gene ontology terms, and there were two biological functions associated with the most
362 overrepresented GO terms from the full list. These included non-alcoholic fatty liver disease and
363 regulation of protein kinase b (AKT) signaling.
364

365 **DISCUSSION**

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

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

381 to longer-term evolutionary changes in non-synonymous substitutions . However, it is important
382 to note that our study is still relatively small, including only six populations and eight individuals
383 from each population. Increasing the number of individuals and sampling sites, especially
384 including multiple cities as replicates, would likely greatly improve the associations found
385 between environmental variables and allele frequencies (Lotterhos & Whitlock 2015). The latter
386 approach may be unlikely, however, with each urban setting presenting a unique set of selective
387 pressures leading to local adaptive responses, as shown with coat coloration in beach mice
388 (*Peromyscus polionotus*) (Hoekstra *et al.* 2006) and climate related adaptation in the flowering
389 plant (*Arabidopsis halleri)* (Rellstab *et al.* 2017). Despite potential issues with sample size, we
390 did find two of the eleven previously identified candidate genes (Harris *et al.* 2013) to be direct
391 matches to outliers in this current analysis (Serine protease inhibitor a3c and Solute carrier
392 organic anion transporter 1A5), and two other genes were from the same gene families or
393 involved in the same biological processes. One gene, an aldo-keto-reductase protein, is part of
394 the same gene family as the aflatoxin reductase gene (Contig 10636-348) identified in this study.
395 The aldo-keto reductase gene family comprises a large group of essential enzymes for
396 metabolizing natural and foreign substances (Hyndman *et al.* 2003). The other is a cytochrome
397 P450 (CYPA1A) gene involved in metabolism of drugs and lipids. *Peromyscus* directly express
398 CYPA1A and Hsp90 (outlier from current SweeD analysis) when exposed to environmental
399 toxins (Settachan 2001).
400
401 **Population genomics summary statistics**
402 Before performing outlier tests, we initially calculated per-site nucleotide diversity and
403 Tajima's D. The Tajima's D statistic was calculated per contig for each population. We found

404 nucleotide diversity to be lower in all urban population compared to rural populations,
405 supporting previous work that found a negative association between genome-wide SNP diversity
406 and urbanization. That study included the six populations studied here and an additional 18
407 populations distributed along an urban-to-rural gradient (Munshi-South *et al.* 2016). While loss
408 of genetic variation will reduce evolutionary potential and decrease the probability of local
409 adaptation, selection may still act if adequate variation is present and genetic drift is not too
410 strong (Donihue & Lambert 2015; Munshi-South *et al.* 2016). Tajima's D is often used to
411 identify signatures of selection, comparing observed to expected heterozygosity. For all our
412 populations, Tajima's D skewed positive, possibly explained by balancing selection. While
413 balancing selection has been found to maintain variation in immune loci in fragmented urban
414 population of bobcats (*Lynx rufus*) (Serieys *et al.* 2015), it is difficult to distinguish whether
415 demography or selection drives Tajima's D values in many cases (MacManes & Eisen 2014).
416 We have estimated the complex demographic history for *P. leucopus* populations in NYC (Harris
417 *et al.* 2016), suggesting Tajima's D may not be the best tool for identifying selection in this
418 system. Outlier tests are more robust to demography and we explicitly accounted for the specific
419 demographic history of *P. leucopus* in the null models used during analysis of our genome scan
420 methods.
421
422 **Signatures of selection in urban populations from genome-wide scans**
423 Over the past decade, genome scans have become feasible methods to detect and
424 disentangle neutral and adaptive evolutionary processes for non-model organisms (De
425 Villemereuil *et al.* 2014; Hoban *et al.* 2016). One method, BayeScan (Foll & Gaggiotti 2008),
426 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

450 (Pavlidis *et al.* 2013), uses a CLR test based on the ratio between the likelihood of a neutral and
451 selective sweep hypothesis. As above, the weakness of hitchhiking methods is the confounding
452 influence certain demographic processes have on the SFS (Hermisson 2009). However, building
453 a robustly inferred demographic history into the null model substantially reduces false positive
454 rates (Pavlidis *et al.* 2013). We included the *P. leucopus* demographic history into our analysis,
455 and found 0.019% of the sequenced loci to contain SFS patterns indicative of selective sweeps.
456 This rate is in line with other studies that reported that 0.5% of regions in domesticated rice
457 (Wang *et al.* 2014), 0.02% of loci in black cottonwood (Zhou *et al.* 2014), and 0.02% of the
458 gorilla genome (McManus *et al.* 2014) show evidence of selective sweeps or hitchhiking.
459 Several studies have shown that identifying outliers with multiple tests and diverse
460 theoretical approaches is the best way to reduce false positives in genome outlier analyses
461 (Nielsen 2005; Grossman *et al.* 2010; Hohenlohe *et al.* 2010b). We required candidate genes to
462 show a signature of diversifying selection or a signature of a selective sweep, and they had to be
463 associated with an environmental variable. We found several outliers identified in both
464 BayeScan and BayPass (Table S2), however, there was no overlap between BayeScan / BayPass
465 and SweeD outliers. This discrepancy is likely due to the different selection scenarios
466 underlying each test, i.e. divergent local selection versus population-wide positive selection in
467 the form of selective sweeps (Hermisson 2009). F_{ST} based methods respond to allelic divergence
468 relatively quickly, while models for selective sweeps typically require nearly-fixed derived
469 alleles (Hohenlohe *et al.* 2010b). Given the recent history of urbanization in NYC, many
470 selective sweeps may be ongoing or otherwise incomplete. Selection may also be acting on
471 standing genetic variation in the form of soft sweeps (Hermisson & Pennings 2005) that are not
472 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.

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

515 Between divergent allele frequencies, a skewed SFS, environmental associations, and
516 overrepresented GO terms, we find several overlapping lines of evidence that support rapid
517 divergent selection in white-footed mice. Our results support the growing body of evidence
518 (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

542 (Oliveira *et al.* 2008; Balloux 2010). For example, specific mitochondrial haplotypes are
543 associated with more efficient thermogenesis and higher fitness in over-wintering shrews
544 (Fontanillas *et al.* 2005). Pergams & Lacy (2007) found complete mitochondrial haplotype
545 replacement in contemporary *P. leucopus* in Chicago compared to haplotypes sequenced from
546 museum skins collected before urbanization. The agent of selection is not clear, but Munshi-
547 South and Nagy (2014) also identified signatures of selection (or alternatively population
548 expansion) in mitochondrial D-loop haplotypes from contemporary *P. leucopus* in NYC. Many
549 mitochondrial functions are affected by the same environmental variables that change in
550 response to urbanization, such as temperature (Balloux 2010), reduced migration (Lankau &
551 Strauss 2011; Munshi-South 2012), or resource availability (Burcelin *et al.* 2002).
552 Urban *P. leucopus* may experience different energy budgets, physiological stressors or
553 diets compared to rural counterparts. We found a substantial number of candidate genes with
554 functions related to the metabolism and transport of lipids and carbohydrates, and the most
555 common overrepresented GO terms involved lipid metabolism and homeostasis (Table S4). In
556 the full outlier analysis, two genes are particularly interesting as targets of diet-mediated
557 selection. The first gene, *FADS1,* is a fatty acid desaturase important for the biosynthesis of
558 omega-3 and -6 fatty acids (long-chain polyunsaturated fatty acids, LCPUFA) from plant
559 sources. Recent evidence suggests that the *FADS* gene family has been an important target of
560 selection in humans during the transition from hunter-gather to agricultural societies (Ye *et al.*
561 2017). Alleles linked to upregulated biosynthesis of LCPUFAs (naturally low in plant based
562 diets) increased in frequency after the Neolithic Revolution (Ye *et al.* 2017). We aligned our
563 homologous *FADS1* contig with human transcripts to identify whether *P. leucopus* had any
564 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

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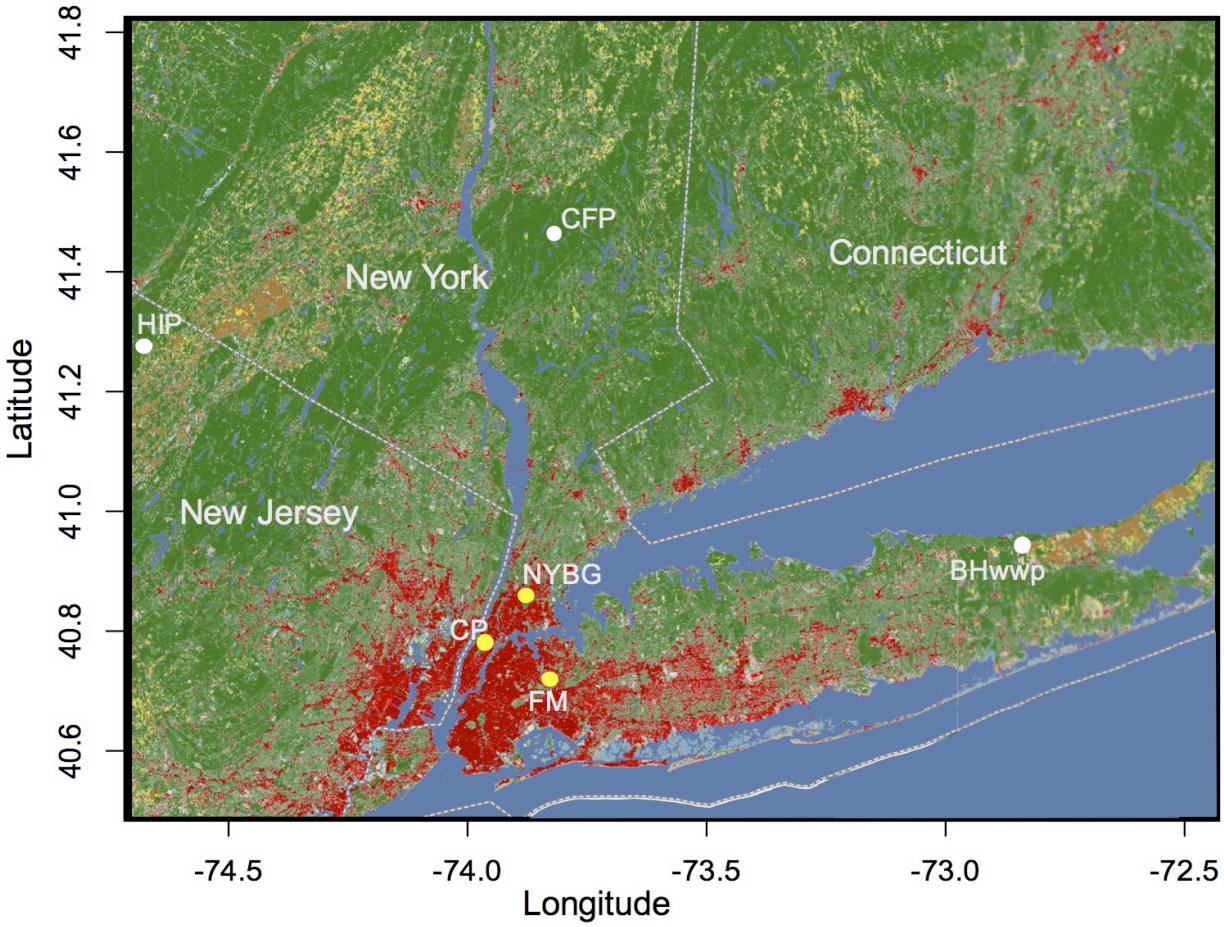
Data Accessibility

-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**



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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.

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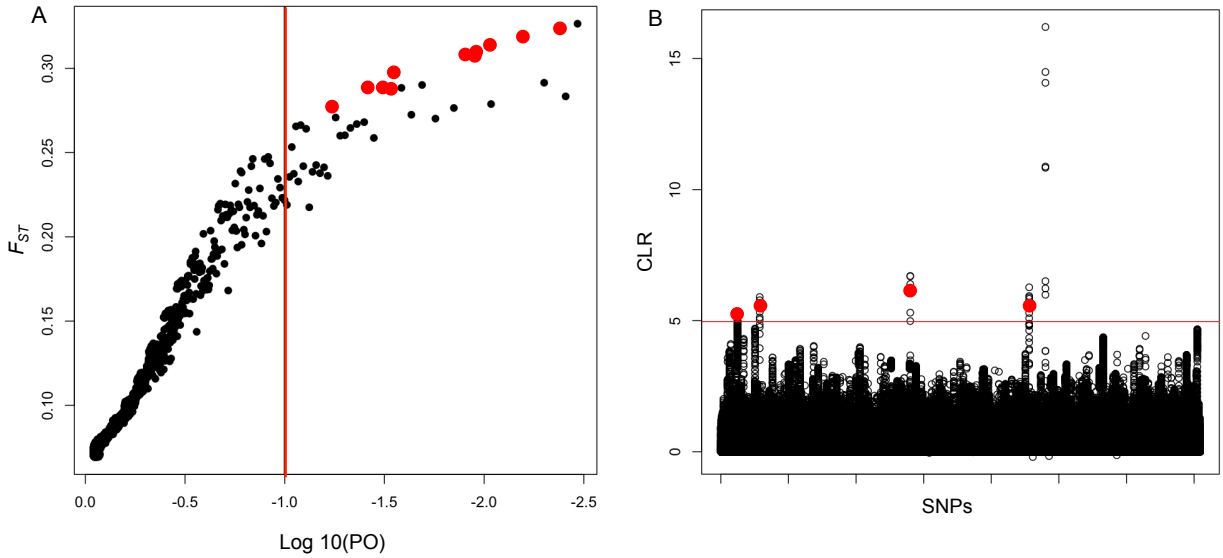

Figure 2. (a) BayeScan 2.1 plot of 154,770 SNPs genome scan analysis between urban and rural populations, including 48 individual white-footed mice from six NYC sampling sites. F_{ST} is on the vertical axis plotted against the $\log_{10}$ of the posterior odds (PO). The vertical red line indicates the 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.

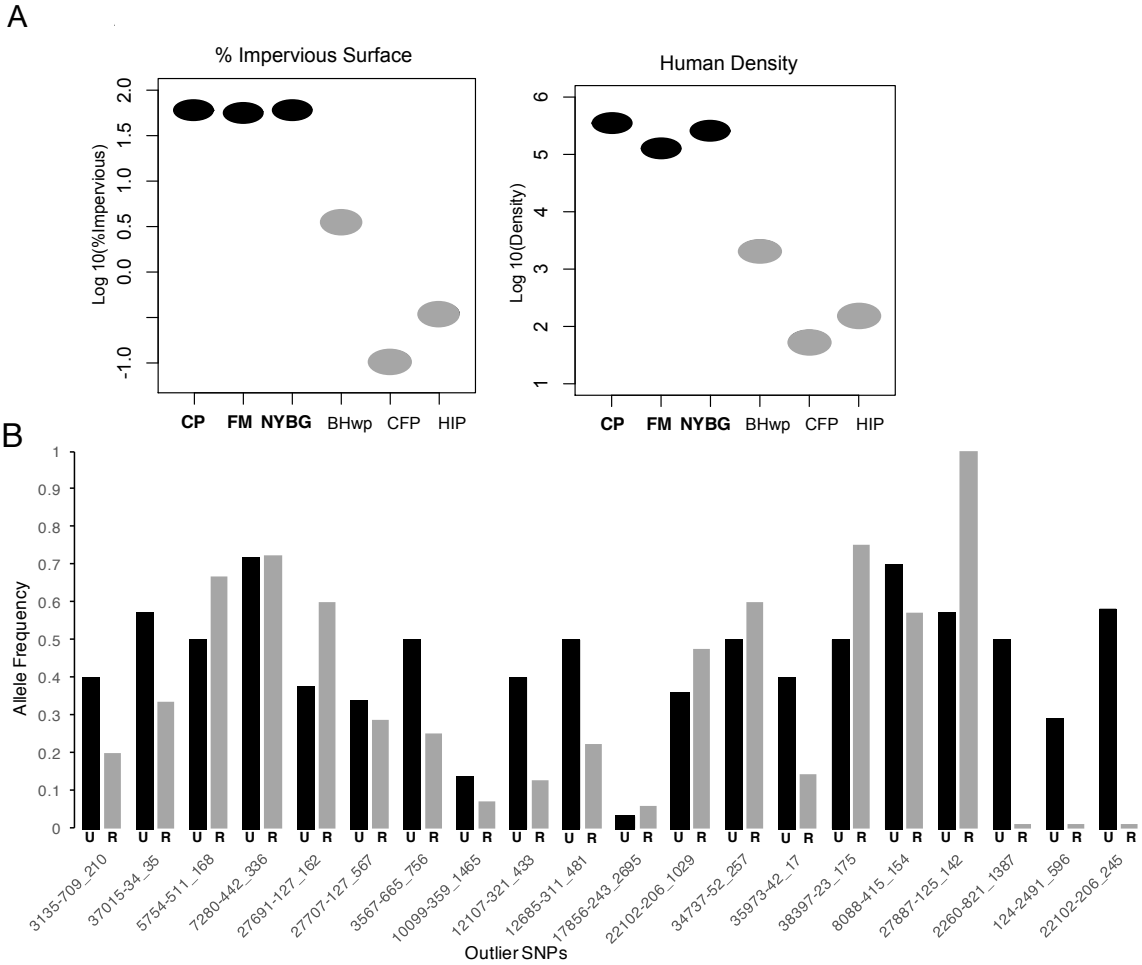

Figure 3. (a) Plot of urbanization metrics for all 6 sampling sites from NYC used in this study. Urban sampling sites are highlighted in bold on the horizontal axis and colored black. Rural sites are colored gray. The log10 value of % Impervious Surface and Human Density are plotted along the vertical 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.

942 **TABLES**

943 **Table 1.** Summary population genomic statistics (mean ± standard error) for three urban and
944 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

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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

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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

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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.