Parallel evolution of urban-rural clines

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Abstract

Urbanization is the dominant trend of global land use change. The replicated nature of environmental change associated with urbanization should drive parallel evolution, yet insight into the repeatability of evolutionary processes in urban areas has been limited by a lack of multi-city studies. Here we leverage community science data on coat color in >60,000 eastern gray squirrels (*Sciurus carolinensis*) across 43 North American cities to test for parallel clines in melanism, a genetically based trait associated with thermoregulation and crypsis in gray squirrels. We show the prevalence of melanism in these mammals was positively associated with urbanization. Urban-rural clines in melanism were strongest in the largest cities with extensive forest cover and weakest or absent in cities with warm winter temperature, where thermal selection likely limits the prevalence of melanism. Our results demonstrate that novel traits can evolve in a highly repeatable manner among urban areas, modified by factors intrinsic to individual cities, including size, land cover, and climate.

Introduction

Urban land expansion is occurring at a faster rate than urban population growth[1], transforming land cover, climate, hydrology, and biogeochemical cycling[2]. The dramatic environmental change associated with urbanization renders cities far more similar to one another in many abiotic and biotic characteristics than to adjacent rural lands[3,4]. For example, cities have extensive impervious cover (e.g., buildings, transportation networks), increased air temperature, and increased air, water, light, and noise pollution[2].

35 The convergence of environmental conditions in cities makes urban ecosystems an ideal
36 location to address longstanding questions in evolutionary biology about the degree to which
37 populations exhibit parallel evolution, that is, when multiple populations evolve very similar
38 traits[5]. Cities are thought to drive parallel evolution as urban populations adapt to similar
39 selection pressures[6,7,8,9]. Cities are not homogenous, however, and variation in their
40 characteristics likely mediates processes that facilitate or inhibit parallel evolution, generating
41 variation in the shape of genotypic or phenotypic clines along urbanization gradients. Clines may
42 be strongest in large cities where the environmental gradients driving divergent selection
43 between urban and rural areas are steepest. Conversely, clines may be weak or nonexistent in
44 small cities or cities with extensive greenspace connecting urban and rural populations via gene
45 flow. Environmental conditions at large spatial scales – such as regional climate – may also
46 modulate the evolution of urban-rural clines at a smaller scale. For example, directional selection
47 for traits that reduce thermal stress at climate extremes may overwhelm selective pressures
48 associated with local land use[10]. Advances in our understanding of how landscape-scale
49 differences among cities facilitate or constrain parallel evolution have been limited by a lack of
50 studies conducted across multiple, replicate cities[7,8,9]. Addressing this knowledge gap will help
51 clarify how urbanization, the megatrend of global land use change, will shape biodiversity.
52 Here we test for parallel evolution along urban-rural clines and examine how landscape
53 features mediate the shape of clines. Our focus is on melanism in eastern gray squirrels (*Sciurus*
54 *carolinensis*) in 43 cities in North America (Fig. 1). Gray squirrels are among the most
55 ubiquitous of fauna in cities and rural woodlands in eastern North America[11]. Coat color in gray
56 squirrels is typically gray or black (melanic) and inherited in a simple Mendelian fashion at the
57 melanocortin 1 receptor gene (*Mc1R*)[12]. Evidence suggests divergent selection associated with

crypsis favors the melanic morph in cities and the gray morph in rural woodlands[13], but urban-rural divergence in melanism has been documented in only two cities (Syracuse, NY[13] and Wooster, OH[14]). We used a dataset on coat color from squirrel observations collected via the community science project *SquirrelMapper* to test for parallel clines in melanism along gradients of impervious cover. We included city size, forest cover across each urbanization gradient, and winter temperature in a linear mixed model to test how these landscape-scale characteristics affect the prevalence of melanism in each city (i.e., main effects of each covariate) and drive variation in cline shape among cities (i.e., interaction effects with impervious cover). Gray squirrels can disperse long distances (>10 km)[15], but movements are limited by forest fragmentation[16], so we expected clines to be weakest in small cities and cities with extensive forest cover where gene flow between urban and rural areas should be greatest. We included winter temperature in the model because melanic squirrels are known to have greater thermogenic capacity than the gray morph at cold temperature[17]. Clines may be weak in cities with cold winter temperature if thermal selection overwhelms the selection pressures on melanism associated with urbanization.

Results and Discussion

Our analyses revealed the prevalence of melanism in these mammals was positively related to the degree of urbanization across multiple cities in North America (main effect of impervious cover, Figs. 1-2; Supplementary Table 2). Repeated shifts in phenotype along environmental gradients – such as the general increase in melanism from rural to urban areas documented here – is a hallmark of parallel evolution. Melanism in gray squirrels is a simple Mendelian trait coded

81 by a 24-bp deletion at *Mc1R*[12], so clines in melanism very likely occur at phenotypic and
82 genotypic levels.

83 The presence of parallel clines in heritable traits is often considered evidence of adaptive
84 evolution because gene flow should homogenize populations in the absence of natural
85 selection[18]. Known drivers of parallel adaptive evolution in cities from other systems include the
86 urban heat island[19] and pollution[20]. Evidence suggests multiple selective mechanisms involving
87 crypsis help maintain clines in melanism in gray squirrels. Predation – including hunting of
88 squirrels by humans – is often the main driver of squirrel mortality in rural woodlands[21,22], and
89 the gray morph is more concealed than the melanic morph in regrown, secondary forests that
90 dominate rural woodlands in our study area[13].

91 Conversely, predation pressure on tree squirrels is relaxed in cities, where the primary
92 driver of mortality tends to be vehicular collisions[22]. The melanic morph is more visible to
93 vehicle drivers than the gray morph on asphalt roads, which may lead to the melanic morph
94 having greater survival in areas with high car traffic[13]. An alternative explanation for the urban-
95 rural clines documented here is that melanic squirrels have been introduced to some cities as
96 novelty[23]. However, these introductions do not explain the low prevalence of the melanic morph
97 in adjacent rural woodlands given the dispersal ability of the species. Moreover, melanism was
98 common in northern old growth forests prior to the 1800s[24,25,26], including regions where it is
99 now rare except in cities (e.g., upstate New York[13,25]). We hypothesize that the dramatic shift
100 from old growth to secondary forests following European settlement in North America[27,28,29]
101 changed the degree to which gray and melanic morphs are concealed from visual predators and
102 hunters, favoring the gray morph in regrown forests[13]. Additional selective factors may play a
103 role in driving urban melanism (e.g., parasite pressure, pollution)[30], and ultimately genomic tools

104 and field experiments are needed to clarify the role of natural selection in generating urban-rural
105 clines[31].

106 Although clines in melanism tended to take the same form among cities, the strength of
107 urban-rural clines varied, supporting the idea that repeated evolution is often imperfect[5]. First,
108 clines most commonly evolved in northern cities with cold winter temperature (Fig. 1b), whereas
109 clines in melanism were absent in cities with warm winters where melanism was rare or
110 nonexistent (main effect of winter temperature, Figs. 1-2; Supplementary Table 2). The melanic
111 morph has greater capacity for non-shivering thermogenesis and retains more heat than the gray
112 morph at cold temperature[17,32,33]. Our results suggest thermal selection at a regional scale plays
113 an important role in maintaining melanism in the north at a level where processes operating at a
114 finer spatial scale cause the evolution of urban-rural clines. Winter temperature has also been
115 shown to affect the strength of urban-rural clines in cyanogenesis in white clover (*Trifolium*
116 *repens*)[34].

117 Second, the positive relationship between urbanization and melanism was strongest in
118 large cities with extensive forest cover (interaction effects of city size and forest cover with
119 impervious cover, Fig. 2; Supplementary Table 2), where melanism increased by nearly 20%
120 from rural to urban areas (Fig. 2). Although gray squirrels have substantial capacity for long-
121 distance dispersal, gene flow between urban and rural areas should be most limited in large cities
122 due to isolation-by-distance and mortality caused by extensive road networks during dispersal.
123 Gene flow may be strongest between urban and rural populations in smaller cities, maintaining
124 maladapted morphs across the urbanization gradient[9]. Selection associated with road mortality in
125 urban areas may also be weak in small cities with limited traffic, which could explain why
126 melanism was rare in smaller cities (main effect of city size, Fig. 2).

The finding of strong clines in cities with extensive forest cover (Fig. 2) did not support our prediction that forest cover facilitates gene flow and weakens clines along the urbanization gradient. Gray squirrels require trees, and squirrel abundance and distribution along urbanization gradients is limited by greenspace availability, particularly in large cities[35]. It is possible that cities with more extensive forest cover support a greater abundance of gray squirrels, increasing effective population size and thereby making selection more efficient in overcoming the effects of genetic drift on morph frequencies.

Our work reinforces the idea that ecological homogenization among cities drives parallel evolution[19,20], and it reveals how cities can be a refuge for unique traits generated via rapid evolutionary response[36]. Our study also provides insight into how landscape-scale differences among cities alter evolutionary processes associated with urbanization. We show the degree of parallelism in trait evolution among cities is imperfect, with environmental heterogeneity among cities contributing to variation in the shape of urban-rural clines. Greenspace availability in particular has been a focus in the ecological literature for understanding among-city variation in the response of species distributions to urbanization[35] – our results show that greenspace availability in the form of forest cover likely mediates evolutionary responses to urbanization as well. Finally, our work underscores the value of conducting studies in urban evolution across multiple cities, for which leveraging community science data has great utility.

Methods

Squirrel datasets. Squirrel records were sourced from the community science program *SquirrelMapper*. Starting in 2010, participants used a web platform to submit sightings of *S.*

150 *carolinensis*, including the date of observation and color morph of each squirrel ("gray",
151 "melanic", or "other"). Spatial coordinates for each observation were secured via Google Maps
152 JavaScript API. A total of 5,899 records were submitted to the original *SquirrelMapper* program
153 from the native range of *S. carolinensis* in North America. Beginning in 2019, *SquirrelMapper*
154 was integrated with *iNaturalist*, enabling users to submit georeferenced photos of each squirrel
155 observation. All images of *S. carolinensis* submitted to *iNaturalist* through 21 December 2021
156 and classified as "research grade" were included for analysis (n = 75,130).

157 We used the participatory science portal *Zooniverse* to crowdsource the classification of
158 coat color of squirrels in images submitted to *iNaturalist*. Participants were asked to classify the
159 number of squirrels observed in each image ("zero", "one", "two or more") and the coat color of
160 squirrels present in each image ("gray", "melanic", "other", or "unclear"). Each image was
161 classified by a minimum of 10 participants (range: 10-291; median = 10). We retained images
162 with a minimum agreement threshold of 80% for the number of squirrels observed. Of the
163 72,239 images (96%) that met the agreement threshold, 865 were classified as having no squirrel
164 present (e.g., track or other sign), 70,258 had a single squirrel, and 1,116 had two or more
165 squirrels. Of the images classified as having at least one squirrel, we retained 63,506 images that
166 had a minimum agreement threshold of 80% for coat color of at least one squirrel in the image.
167 We removed 5,182 images that did not have location data available and 4,035 images taken
168 outside of North America where *S. carolinensis* is introduced. The final *iNaturalist* dataset
169 consisted of 54,289 images, of which 54,215 were classified as having a single coat color present
170 (gray: 46,630, melanic: 6,728, other: 857) and 74 had two color morphs present (gray and
171 melanic: 68, gray and other: 5, melanic and other: 1). A random sample of 500 images classified
172 by the authors showed that the classification scheme we used with 80% agreement thresholds

was 100% accurate for the number of squirrels observed in the image and 99.8% accurate for coat color, the only discrepancy being a single image classified as melanic that the authors classified as unclear. The final *iNaturalist* dataset was merged with squirrel records from the original *SquirrelMapper* project, yielding 60,262 squirrel records in total. Multiple coat colors observed at the same location were treated as separate records in this final dataset. Observations in the final dataset were made between 1969 and 2020 (median = 2019), with 95% of observations being reported since 2010.

City selection. We selected cities in the native range of *S. carolinensis* that had at least 100 squirrel observations in the city and surrounding rural area. We defined the spatial extent of each city's footprint using the Urban Area National Shapefile Record Layout (United States Census Bureau) for the United States and the Population Center Boundary File (Statistics Canada Catalogue no. 92-160-G) for Canada. We calculated the distance from the centroid of each city footprint to its maximum extent in each of the four cardinal directions. We then buffered each urban area by 25% of the average distance from the city centroid to its extent. This buffer distance was small enough to include a significant area of rural land surrounding each city but not so large as to produce excessive overlap between polygons of adjacent cities. We determined the number of squirrels in each city polygon (encompassing the city footprint and hinterland) after spatially thinning the squirrel records to a minimum distance of 10 m using the s*pThin* package (v. 0.2.0)[37] in R[38], which helps minimize spatial sampling bias. If two cities with ≥100 squirrels had polygons that overlapped, we retained the city with a greater sample size or spatial spread of squirrel observations. The final dataset included 26,924 squirrels among 43 cities (Fig. 1), and the median number of squirrels in each city was 254 (range: 101 – 4,731; Supplementary

Table 1; maps of squirrel observations in each city available for download at https://github.com/bcosentino/squirrelMapper).

Environmental covariates. We measured percent impervious cover within 1 km around each squirrel observation using the Global Man-made Impervious Surface Dataset (30-m resolution)[39]. A 1-km buffer was chosen to encompass the typical home range size of *S. carolinensis* (<5 ha) and long-distance movements typical of males during winter and summer mating seasons[40]. For each city and its surrounding rural area we measured forest cover and winter temperature. Forest cover was measured using the Global 2010 Tree Cover database (30-m resolution)[41], and we used WorldClim (version 2.1) to measure winter temperature as mean temperature of the coldest quarter (BIO11, 30s resolution)[42]. We also measured the area of each city footprint as a metric of city size. All spatial analyses were conducted in R using the *raster* (v. 3.3-13)[43] and *exactextractr* packages (v. 0.5.1)[44].

Statistical analyses. We fit a generalized linear mixed model with a binomial error distribution to test for effects of impervious cover, city size, winter temperature, and forest cover on the distribution of melanism. Melanism was the response variable and represented by a binary indicator, with 1 corresponding to melanic and 0 to other coat colors for each squirrel observation. Impervious cover within 1 km of each observation was included as a fixed effect to represent each city's urbanization gradient. Because the range of impervious cover varied among cities, we rescaled impervious cover within each city to range from 0 (lowest impervious cover) to 1 (greatest impervious cover)[34]. This ensured the slope term for impervious cover has a consistent interpretation among cities, allowing us to test for a general urban-to-rural cline in

219 melanism. City size (log-transformed), forest cover, and winter temperature were included as
220 fixed effects to test whether these variables predict variation in the prevalence of melanism
221 among cities. Two-way interaction terms were included between each predictor and impervious
222 cover to test how city characteristics affect the shape of urban-to-rural clines in melanism. We
223 standardized each predictor so that each variable had mean = 0 and SD = 1, which aided in
224 model convergence and allowed us to compare the effect size of each slope term[45]. We also
225 included city as a random effect to account for the nested observations within each city and to
226 estimate unexplained variance in the prevalence of melanism among cities (Supplementary Table
227 3). The model was fit with the *lme4* package in R (v. 1.1-26)[46]. Two-sided Wald tests were
228 conducted for each parameter in the model. Binned residual plots were generated using the *arm*
229 package (v. 1.11-2)[47] as recommended for models with a binary response variable[48]. Plots of
230 binned residuals on fitted values and each predictor suggested adequate model fit and no
231 substantial deviation from model assumptions.

232 We used the residual autocovariate approach[49] to account for spatial autocorrelation in
233 the residuals of the model. We extracted residuals from the global model and created a spatial
234 autocovariate for the residuals using the *spdep* package (v. 1.1-7)[50]. We set the radius
235 neighborhood to 31 km (the greatest nearest neighbor distance in the thinned dataset) and used
236 an inverse distance weighting scheme with style "B" for symmetric weights[51]. Our global model
237 was then refit with the spatial autocovariate term as an additional predictor, which reduced
238 spatial autocorrelation in the residuals from a maximum of $r = 0.11$ within 1 km to $r < 0.05$
239 (Moran's I; Supplementary Fig. 1).

240 We refit our model using different spatial thinning distances (10 m, 50 m, 100 m) and
241 buffer distances to measure impervious cover (500 m, 1 km, 10 km). These analyses showed the

model results were not sensitive to spatial thinning distances (Supplementary Table 4) or buffer distances to quantify impervious cover around squirrel observations (Supplementary Table 5).

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Author Contributions

B.J.C. and J.P.G designed the study. B.J.C. led the data analysis and wrote the first draft of the manuscript. J.P.G. contributed ideas for data analysis and edited the manuscript.

Data Availability

The observations of eastern gray squirrels (*Sciurus carolinensis*) generated and analyzed for this project can be found at https://github.com/bcosentino/squirrelMapper.

Competing Interests

The authors declare no competing interests.

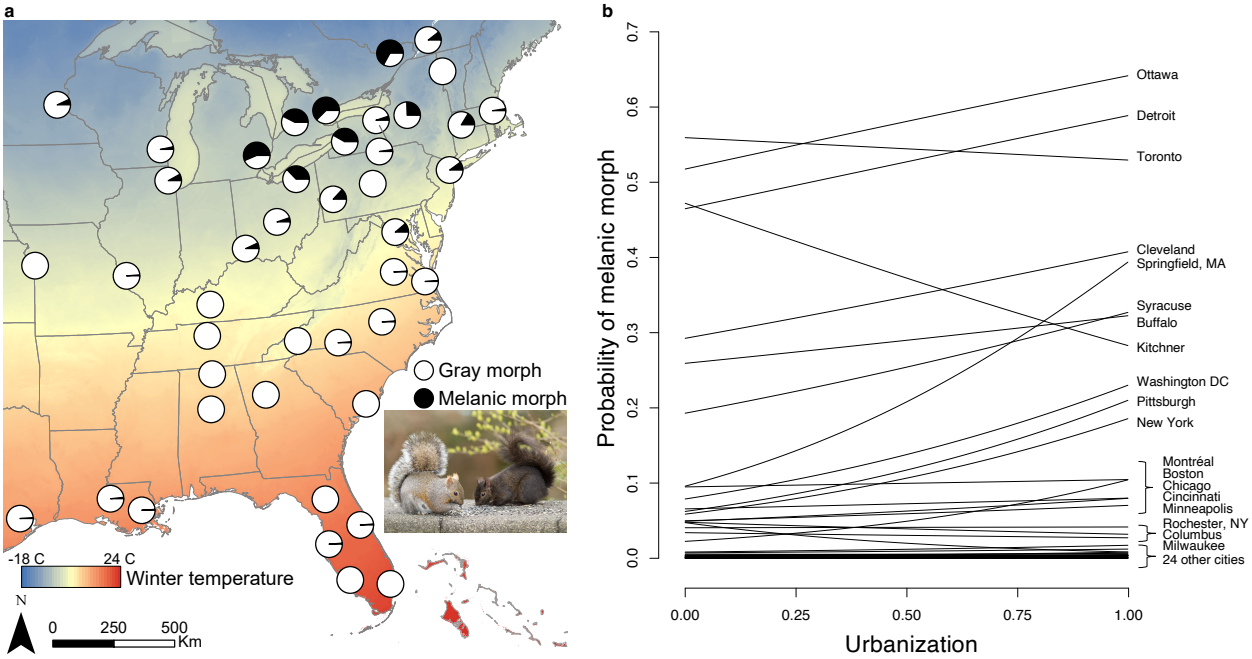

Fig. 1. a, Distribution of coat color morphs of 26,924 eastern gray squirrels (*Sciurus carolinensis*) among 43 cities in North America. Pie charts show the proportion of melanic and gray color morphs in each city. Gray morph included rare color morphs that accounted for 2% of observations (Supplementary Table 1). **b,** Clines in melanism along urbanization gradients in each city. Urbanization was measured as standardized impervious cover. Regression lines represent predicted effect of urbanization on melanism in each city based on a linear mixed model including effects of city size, forest cover, and winter temperature (Fig. 2, Supplementary Table 2). Photo 'grey squirrel, black squirrel' (tinyurl.com/pauha835) by Eyesplash Photography is licensed under CC BY-NC-ND 2.0 (https://creativecommons.org/licenses/by-nc-nd/2.0/).

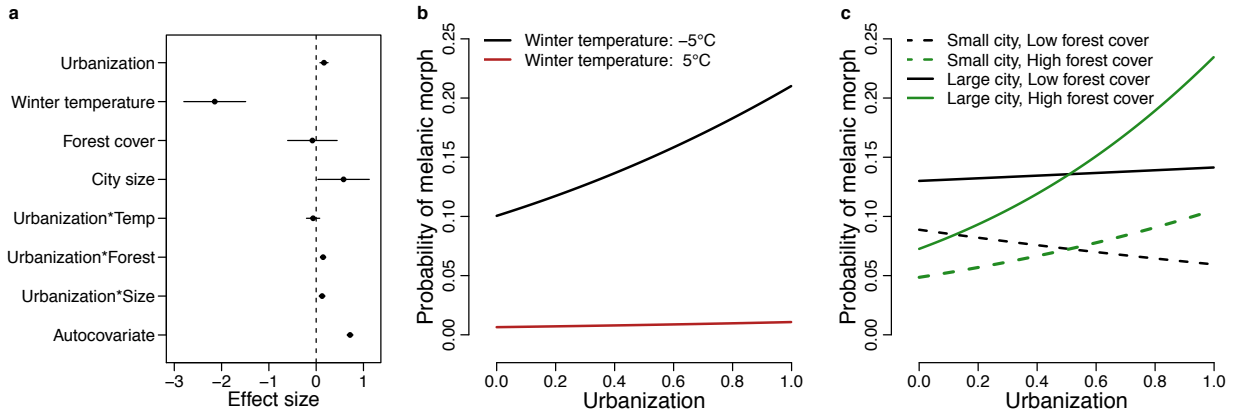

Fig. 2. a, Effect sizes for fixed effects of urbanization, winter temperature, forest cover, city size, interaction terms with urbanization, and a spatial autocovariate from a linear mixed model of melanism in 26,924 eastern gray squirrels *(Sciurus carolinensis*) among 43 cities in North America. Effect sizes represent standardized regression coefficients and include 95% confidence intervals. Urbanization was measured as standardized impervious cover. **b**, Relationship of the probability of melanism to urbanization at low (-5°C) and high (5°C) winter temperature while holding city size and forest cover constant. **c**, Relationship of the probability of melanism to urbanization at varying levels of city size and forest cover. Effects of urbanization on melanism are shown for combinations of small cities (25,000 ha), large cities (350,000 ha), low forest cover (15%), and high forest cover (45%) while holding winter temperature constant at -5°C.