**The effects of elevated population density on North American red squirrel (*Tamiasciurus hudsonicus*) behavior**

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**Abstract**

Urbanization is one of the most significant drivers of changes in wildlife population densities, as it often results in habitat fragmentation, degradation, and loss, decreasing the suitability of habitat for wildlife. Many species in urban areas experience higher population densities compared to undisturbed areas. My study is predicated on an experiment that elevated the population density of wild North American red squirrels (*Tamiasciurus hudsonicus*). The purpose of this study was to investigate the behavioral changes as a result of this increase in population density and whether any resulting behavioral changes are a result of natural selection, as opposed to behavioral plasticity. I leveraged a long-term behavioral database from the Kluane Red Squirrel Project to assess the differences in two behaviors (activity and aggression) recorded from squirrels in a standardized behavioral assay (open-field trial and mirror-image simulation) between the high-density experimental area and lower-density control areas. I then performed behavioral tests on individuals from the high-density area to assess how the number of maternal generations an individual's lineage had experienced in the high-density area affected activity level, aggression, and docility. Previous studies show that female red squirrels that were more aggressive and less active in conditions where competition was elevated had higher reproductive success. Because of this, I predicted a decrease in activity level and increase in aggression with elevated population density. Because these behaviors have been shown to be genetically heritable in red squirrels, I predicted this would be a result of natural selection and there would be an increase in the frequency of these behaviors with increasing maternal generation time in the area of elevated density. My results showed squirrels from the area with elevated population density were significantly less active than those from the control areas. There was no relationship with aggression between density levels, or with any behavior and maternal generation time. These results show that elevated population density could influence behavior but suggests that perhaps these changes are due to behavioral plasticity rather than natural selection.

**Keywords:** Behavior, evolution, population density, red squirrel, urbanization.

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## Introduction

Globally, more people now live in urban areas than in rural ones (Knox et al., 2013). Urbanization results in momentous changes to the environment. These changes impact the suitability of habitat for wildlife, as urbanization often results in habitat fragmentation, degradation, and loss (McKinney, 2002). As a result, urbanization is one of the most significant drivers of changes in wildlife population densities (Marzluff, 2001). The loss of suitable habitat has led to an increase in the number of threatened and endangered species (Czech et al., 2000). Species often go locally extinct from newly urbanized habitat or experience a decrease in population density (Thompson & Jones, 1999; McKinney, 2002). Although urbanization can lead to a decrease in the population densities of many wildlife species, it can also result in an increase in population densities of other species, such as white-tailed deer (*Odocoileus virginianus*) with increased habitat edge from habitat fragmentation (Farrell & Tappe, 2007). Many species in urban areas experience higher densities compared to undisturbed areas (McKinney, 2002; Parker & Nilon, 2008). This is important, as there may be problematic consequences to increased population density. For example, populations experiencing elevated density can result in an increase in disease transmittance and overall physiological stress levels of individuals (Creel et al., 2013; Hassell et al., 2017; Bradley & Altizer, 2007; Scott, 1988). Species experiencing elevated population density as a result of urbanization may also be less fearful of predators, both human and non-human (Beausoleil et al., 2008; Dickman, 2010). For conservation management and decision-making, it is important to understand how urbanization impacts wildlife and how individuals respond to their changing environment.

I investigated how wildlife behavior changes as a response to elevated intraspecific population density. Behavioral ecology is one of the longest-studied aspects of ecology, as humans have recorded detailed descriptions of behavioral observations of animals since the dawn of our species (Lima & Zollner, 1996). Animal behavior is known to have ecological and evolutionary consequences (Reale et al., 2010). Personality is defined by Reale et al. (2007) as individual differences in behavior that are repeatable over time and across situations. Categories of behaviors that contribute to personality include activity, aggression, shyness-boldness, exploration-avoidance, and sociability (Reale et al. 2007). Personalities and behavior of individuals have been shown to have important fitness effects in many ecological systems, as certain behaviors may be more helpful in mate acquisition or territory defense (Dingemanse & Reale, 2005; Smith & Blumstein, 2008). Studying behavioral ecology helps understand current ecological and evolutionary processes and allow us to make wildlife management decisions based on behavioral patterns. Understanding behavior to make predictions is especially important during a time of rapid global environment changes. For example, knowing what behaviors are considered beneficial in terms of fitness and what behaviors are selected for may indicate the how suitable an altered habitat is for a population.

Biologists often use “model” organisms to answer questions and make predictions in their respective fields. In this study I used the North American red squirrel (*Tamiasciurus hudsonicus*) to answer questions about behavioral changes with the elevation of population density. My study has two components. The first component investigates how population density affects red squirrel behavior. Similar studies have taken place in the same population of red squirrels in the Yukon, Canada, that I studied.

Taylor et al. (2014) studied behavioral changes as a result of increased juvenile competition. Using behavioral tests, they found that under conditions with increased juvenile competition, there was natural selection for more aggressive and less active squirrel mothers. Squirrel mothers that were less active and more aggressive were more successful at rearing their pups to being recruited into the population. This is relevant to my study on population density, as increased juvenile competition often occurs in conditions of elevated population density (Taylor et al., 2014). Based on this study, I expected to see changes in behavior with elevated population density. Specifically, I predicted I would find that populations with increased population density would, on average, be more aggressive and less active than individuals from a lower density.

The second component asked whether the observed behavioral changes are a result of natural selection or behavioral plasticity. The experiment that my study was predicated on took place in the same system I studied. Dantzer et al. (2013) supplemented an experimental research area with food for 14 winter seasons, resulting in a doubling of squirrel population density that was still present at the time of my study (Figure 1). Another study by Taylor et al. (2012) showed that red squirrel behavior is genetically heritable and variation in personalities can partially be explained by genetics. I used this system and assumed that individuals with more generations of their family lineage from the experimental area have experienced longer selective pressures of the increased population density to make my second hypothesis. I predicted that if these behaviors were being selected for, then individuals that had a longer family history of experiencing elevated population density would exhibit these behaviors more strongly. I predicted to see more aggressive, less docile, and less active individuals with increasing length of generational lineage. If there was selection for behaviors but no relationship with the length of generational lineage under increased population density conditions, then I predicted these behaviors were not being selected for but are rather a result of behavioral plasticity.

## Methods

### *Study Site*

This study took place in near Kluane National Park in southwestern Yukon, Canada (61° N, 138° W) within Champagne and Aishihik First Nations traditional territory along the Alaska Highway. This study was done in conjunction with a long-term study of North American red squirrels, *Tamiasciurus hudsonicus*, by the Kluane Red Squirrel Project, which has been monitoring a population of free-ranging red squirrels continuously since 1987 (McAdam et al. 2007). Study areas are flagged and staked at 30-meter intervals, allowing locations of squirrel territories and behavioral observations to be recorded to the nearest three meters. This study took place on two control areas (grids, 40 hectares each) that experience natural variation in food abundance and population density, and an experimental area (45 hectares) that was food supplemented from 2004 to 2017 (Dantzer et al. 2013). This experimental area began supplementing food in the winter of 2004, where a kilogram of peanut butter (no salt or sugar added) was hung from a bucket in the center of the area an individual squirrel owns and defends ("midden"). Each bucket was refilled approximately every 6 weeks beginning in October until May or when a female was done lactating, whereupon the peanut butter was removed. Squirrels cannot hoard or cache peanut butter, and it does not appear to affect body mass (Dantzer et al. 2012). This food supplementation experiment doubled the squirrel population density, on average, compared to control grids (Figure 1, Dantzer et al. 2013). In 2018 the high-density experimental area population density was 2.8 individuals/hectare, compared to 1.7 individuals/hectare as the average of the two control areas (Figure 1), a 64.7% increase in density on the high-density area compared to the average of the control areas.

*Population*

North American red squirrels defend their middens year-round (Price & Boutin 1990). Individuals harvest mature white spruce cones (*Picea glauca)* in late summer through early autumn. Males and females are territorial and can acquire middens from bequeathal (from their mother) or competition over vacant territories (Price et al. 1993). As part of the Kluane Red Squirrel Project, the individual squirrels used in this study were ear-tagged and monitored since birth. The territorial nature of red squirrels makes them an ideal animal to track, as they can be easily trapped at the midden they defend. Live-trapping is done with Tomahawk traps (Tomahawk, WI, USA). At each trapping event, individuals are identified by unique alphanumeric ear tags and unique combinations of colored wires (National Band and Tag Company, Newport, KY, USA). They are weighed with spring scales (Pesola) and their reproductive status is assessed via abdominal palpation. Radio-collars are fastened to pregnant individuals, allowing their nests to be located with VHF telemetry. After mothers have given birth, DNA samples are taken from squirrel pups and they are assigned a unique squirrel identification number. I assumed any pup in the nest of a female was her offspring, as adoption is very rare (Gorrell et al., 2010). Knowing the mother of each individual squirrel allowed me to determine how many maternal generations an individual's lineage has been in an area (i.e. resided in an experimental versus study areas). For example, an individual with 0 maternal generations on the high-density area would have migrated to the area after birth. An individual with 4 maternal generations in the area means itself, its mother, grandmother, and great-grandmother were all born in the high-density area. For the investigation of the effects of maternal generation, I selected individuals with a maternal generation time of 0 (n = 14) or greater than or equal to 3 (n = 23; Table 3).

*Behavioral Tests*

I used two tests to measure how population density and maternal generation time affected the behavior of squirrels. After a trapping event, squirrels targeted in this study underwent two behavioral tests to measure their personality. I used open field trials (OFT) to quantify “activity” in a novel environment and mirror-image simulation test (MIS) to quantify “aggression”, as previously interpreted in this species (Boon et al. 2007, 2008; Taylor et al. 2012, 2014). These behavioral tests took place in the same testing session. Individuals can habituate to these tests, but the behavior of individuals over time is known to be repeatable, meaning their quantifiable “personality” is consistent over time (Boon et al. 2007, 2008; Taylor et al. 2014). The OFT and MIS took place in the same testing arena; a 60 x 80 x 50 cm white corrugated plastic box with a clear acrylic lid (Figure 2). The arena floor had four blind holes that served as “escape routes” and help to separate exploratory behaviors from activity, since they are often correlated (Martin & Reale 2007). A 45 x 30 cm mirror was fixed to one end of the arena and exposed at the beginning of the MIS. Trials were recorded with a digital video camera through the clear lid. All tests were performed at the point of capture on the territory of the target individual, conducted by the same observer (ZOG), using a standardized procedure. The OFT was 7.5 minutes, beginning when the squirrel entered the arena. This test also served as a habituation period for the individual prior to the MIS (Svendsen & Armitage 1973). The 5-minute MIS trial began after uncovering the mirror and the squirrel noticed its reflection. After the tests, the individual was released and the arena was cleaned with 70% isopropyl alcohol.

Videotaped trials were scored manually with JWatcher Video 1.0 using an ethogram developed and used in previous red squirrel studies in this population (Boon et al. 2007, 2008; Taylor et al. 2012; Kelley et al. 2015; Table 1). The OFT and MIS videos were scored using a standardized procedure described by Boon et al. (2008). I leveraged the Kluane Red Squirrel Project long-term database of behavioral tests in this study. I used OFT and MIS data from 2005 to 2018. I measured "activity" in 405 individuals and "aggression" in 409 individuals (Table 2). I combined these data with behavioral data I collected from June to August of 2018 (38 individuals; Table 3). All individuals were mature adults (>1 year old) at the time of their trials (Table 3). Behavioral videos from 2018 were scored by two observers (ELW & BFC).

An additional test was done to measure the docility of an animal by quantifying its response to handling (Carere & van Oers 2004). After transferring an individual from a trap to a canvas handling bag, I recorded the number of seconds (out of 30) that the individual spent struggling (defined as actively moving in the bag). One measure of docility was collected per individual. This behavioral test was only analyzed for the second component of my study, as data were not available from the long-term database to make comparisons between high- and low-density areas.

*Statistical Analyses*

All analyses were conducted in R version 3.4.3 (R Core Team 2016). I conducted principal component analyses (PCA) for both OFT and MIS behaviors, to reduce redundancy among behavioral measurements and calculate composite behavioral scores for each trial, as has previously been done in this species (Boon et al. 2007, 2008; Taylor et al. 2012, 2014; Kelley et al. 2015). I used R package ‘ade4’ version 1.7-10 (Dray and Dufour 2007) to conduct the PCAs. PCAs were done using the entire dataset using trials conducted from 2005-2018. Variables with a loading greater than 0.25 or less than -0.25 were considered most important in contributing to PC1 (Budaev, 2010; Franklin et al. 1995). All further analyses used the scores calculated from the PC loadings for each trial.

I used linear mixed-effects models to assess the behavioral differences between the individuals from the experimental area and the control areas. Squirrels on the two control areas were combined into one area for the purposes of this study. I used R package ‘lme4’ version 1.1-21 to run each model (Bates et al. 2007). This model for the first component of this study included fixed effects for sex, age (both linear and non-linear [$age^2$] terms were included), treatment (high-density experimental area versus control area), birth year as a random effect, and an interaction between sex and treatment. To assess if maternal generation time affected squirrel activity, aggression, or docility, I used three separate linear mixed-effects, each of which included fixed effects for sex, age (both linear and non-linear [$age^2$] terms were included), a two-level categorical variable for maternal generation time (0 generations or ≥3), and an interaction between sex and maternal generation time. Although maternal generation time was effectively a continuous variable, ranging from 0 to 5, the sample sizes across these generation times were unequal (0 generations, n = 14; 1 generation, n = 0; 2 generations, n = 0; 3 generations, n = 8; 4 generations, n = 7; 5 generations, n = 8) so I used a two-level categorical variable where generation time was either 0 generations or ≥3. I included an interaction between sex and treatment (study 1) or sex and maternal generation time (study 2) because males and females may experience selection differently (Boutin & Schweiger 1998; Reale et al. 2007) and Taylor et al. (2014) only explored selection on the behavior of females, not males.

## Results

### *Principal Component Analyses*

Results of the OFT principal component analysis (PCA) showed that walking, hanging, chewing, hole investigation, and jumping characterized a more active squirrel ad resting and grooming characterized a less active squirrel, such that high scores of PC1 from the OFT PCA corresponded to squirrels that were more active in the trial (Table 4). Results of MIS PCA showed that number of attacks and time spent in front of the testing arena characterized a more aggressive squirrel and time spent in the back of the arena, latency to approach the mirror, and latency to attack the mirror all characterized a less aggressive squirrel, such that high PC1 scores from the MIS correspond to squirrels that were more aggressive (Table 4).

*Effect of Density on Behavior*

Average density of the experimental area in 2018 was 64.75% greater than the average of the two control areas (Figure 1). Squirrels on the high-density area were less active in the open-field trial than those on the lower density control areas ($t=-3.54$, $df=268$, $p<0.001$; Figure 3). There was no difference between aggression of individuals on the high-density area and the lower density control study areas ($t=-1.85$, $df=215$, $p=0.06$; Figure 4). There was no interaction with sex and density for activity ($t=-0.3$, $df=279$, $p=0.77$) or aggression ($t=1.09$, $df=243$, p-value=0.28), meaning males and females did not differ in their response to increased population density. There was no relationship between age and activity ($t=0.71$, $df=32$, $p=0.48$; $t=-1.07$, $df=45$, $p=0.29$) or aggression ($t=-1.43$, $df=19$, $p=0.17$; $t=1.56$, $df=28$, $p=0.13$), with linear nor non-linear terms, respectively.

*Effect of Maternal Generation Time on Behavior*

There was no statistical difference in activity level between individuals with 0 maternal generations and 3 or more maternal generations from the high-density experimental area ($t=-0.47$, $df=31$, $p=0.64$; Figure 5). Results of the linear model comparing aggression and maternal generation time spent on the high-density experimental area did not show a statistically significant relationship ($t=-0.33$, $df=31$, $p=0.74$; Figure 6). There was no relationship between maternal generation time and docility ($t=-0.73$, $df=31$, $p=0.47$; Figure 7) There was no interaction with sex and maternal generation time for activity ($t=0.21$, $df=31$, $p=0.84$), aggression ($t=-0.26$, $df=31$, $p=0.8$), or docility ($t=-0.11$, $df=31$, $p=0.91$), meaning males and females did not differ in their response to number of maternal generations spent in the high density area. There was no relationship between age and activity (t=0.69, df=31, p=0.5; t=-1.2, df=31, p=0.24), aggression (t=-0.44, df=31, p=0.66; t=0.14, df=31, p=0.88), or docility (t=0.2, df=31, p=0.85; t=-0.01, df=31, p=0.99), with linear nor non-linear terms, respectively.

**Discussion**

This study focused on whether behaviors can change in response to elevated population density, and whether that change is a result of natural selection or behavioral plasticity. The first component of this study investigated differences in aggression and activity levels between a high-density experimental area and lower density control areas. Based on the results of this portion of the study, I can conclude that population density can alter behavior and personality traits like aggression and activity that were recorded in an open-field trial and mirror-image simulation test, respectively. Squirrels experiencing conditions of elevated population density were less active, on average. This finding supports the results of Taylor et al. (2014) where active behaviors were negatively selected for when juvenile competition was high as it suggests that squirrels on the high-density were behaving adaptively. Taylor et al. (2014) showed that less active mothers were more successful and producing offspring that were recruited into the population, which could help explain the pattern shown in my study. Similar to the relationship with juvenile competition, increased resource competition can also result due to elevated population density (Svanbäck & Bolnick, 2006). Decreased activity level could be selected for under these conditions to save energy for food foraging and caching. A study by Beausoleil (2008) found a negative relationship between activity level and fear of predators. Selecting for more fearful, less active individuals in conditions of high population density could be explained as a mechanism to evade predation, as an increase in one species may increase the number of their predators (Beausoleil, 2008).

Elevated population density affected activity level of squirrels, but it did not affect their level of aggression in their mirror-image simulation test. Taylor et al. (2014) showed that there was a fitness benefit of being more aggressive when competition was high, but there was no difference in aggressiveness between squirrels on the high density and control study areas in my study. The selective forces acting on this behavior could be stabilizing. Previous studies show that intermediate levels of aggression are favored (Knell, 2009; Winters & Jawor, 2016). A review by Knell (2009) showed that during initial population increase, individuals may invest in aggressive behaviors to be more competitive when foraging for food and defending their territories. However, there may be a threshold for how aggressive an individual can be before the behaviors become too costly, as individuals are more likely to get injured and have less energy to invest in other activities. This is shown in male Northern cardinals (*Cardinalis cardinalis*), where there was stabilizing selection on plumage brightness and aggression (Winters & Jawor, 2016). Individuals benefited from bright plumage and aggression when it came to mate acquisition, but being too bright increased their likelihood of being preyed upon. Behaving too aggressively was energetically expensive and led to injury or increased predation. Increased aggression under high densities could be more harmful than beneficial to individuals, resulting in stabilizing selection for aggressive behaviors. This may explain why there was no significant difference of the level of aggression between the high-density area and control area in the first portion of my study.

The second component of this study investigated whether behavioral changes were a result of behavioral plasticity or natural selection. Comparisons of activity level and aggression between recent migrants to the elevated density area and individuals with three to five consecutive maternal generations from the area were made in attempt to answer this question. The phenomenon that is driving the behavioral changes would be distinguished by whether there was a difference in activity level and aggression between recent migrants and individuals with a long maternal lineage in the high-density area. If the increase in frequency of these behaviors, compared to the control area, were a result of natural selection, then I expected to see a difference in activity level and aggression between these two categories because it would suggest a genetic response to selection acting on squirrel activity or aggression. I found no difference in aggression or activity level between squirrels who had experienced three or more generations of selection on their behavior and those who had experienced zero, indicating that the behavioral changes seen under elevated population density are not a result of natural selection, but rather behavioral plasticity. However, I cannot say this with complete confidence. First, my sample size for this component of the study was extremely small compared to the density comparison component of this study. With a larger sample size, I may have seen a significant relationship. Additionally, it is possible that I would see a more apparent result of evolution after more generations of selection for these behaviors. It would be valuable to replicate this study in several years, after allowing more maternal generations to accumulate in the higher density area.

In summation, this study further supports that behavior has ecological consequences. The decrease in activity level found in this study could have cascading effects on the environment these individuals are part of, which is why these questions are important to ask. This study shows that investigating behavior is important in conservation management and decision-making, as population density influences behavior, and population densities are changing with urbanization and habitat loss, fragmentation, and degradation.

## Tables

**Table 1.** Behaviors scored for OFT & MIS behavioral tests. (E) indicates a behavior that is an event and (S) indicates a state behavior.

| **OFT Behavior** | **Description** |
|---|---|
| Jump (E) | Jumping. |
| Hang (S) | Hanging from the top of the arena. Behaviors like chew or scan can be performed while hanging. |
| Chew or scratch (S) | Scratching at or chewing the OFT arena. |
| Groom (S) | Paw or mouth grooming. |
| Hole dips (E) | Interactions with one of the 4 blind holes. |
| Still (S) | When the squirrel is still for 2 seconds |
| Walk (S) | When the squirrel is moving around the arena. |
| **MIS Behavior** | **Description** |
| Attack mirror (E) | Each time the squirrel aggressively contacts the mirror |
| Approach mirror (E) | When the squirrel moves in the direction of the mirror |
| Front (S) | When the squirrel enters the front of the arena |
| Back (S) | When the squirrel enters the back of the arena |

**Table 2.** Sample sizes for first component of study to assess behavioral differences of squirrels on a high-density experimental area vs. a low-density control area. OFT means open-field trial and MIS means mirror-image simulation.

| **Study Individuals for Study 1 (Density Comparisons)** | |
|---|---|
| **OFT individuals total** | **n = 404** |
| Females; males | n = 221; 183 |
| Age range; mean | 1-7; 3 |
| Control study areas | n = 257 |
| Females; males | n = 149; 108 |
| High-density study area | n=147 |
| Females; males | n = 72; 75 |
| **MIS individuals total** | **n = 408** |
| Females; males | n = 212; 196 |
| Age range; average | 1-7; 3 |
| Control study areas | n = 268 |
| Females; males | n = 141; 127 |
| High-density study area | n = 140 |
| Females; males | n = 71; 69 |

**Table 3.** Sample sizes for first component of study to assess behavioral differences of squirrels on with zero maternal generations from the high-density experimental area (presumably a recent migrant) vs. squirrels with three to five maternal generations in the area. OFT means open-field trial and MIS means mirror-image simulation

| **Study Individuals for Study 2 (Maternal Generation Comparisons)** | |
|---|---|
| **OFT individuals total** | **n = 37** |
| Females; males | n = 16; 21 |
| Age range; mean | 1-7; 3 |
| 0 generations | n = 14 |
| Females; males | n = 7; 7 |
| ≥ 3 generations | n = 23 |
| Females; males | n = 9; 14 |
| **MIS individuals total** | **n = 36** |
| Females; males | n = 16; 20 |
| Age range; average | 1-7; 3 |
| 0 generations | n = 14 |
| Females; males | n = 7; 7 |
| ≥ 3 generations | n = 22 |
| Females; males | n = 9; 13 |

**Table 4.** PCA loadings for behaviors in two behavioral tests. Positive OFT PC1 loadings indicate behaviors that contribute to a more active squirrel. Positive MIS PC1 loadings indicate behaviors that contribute to a more aggressive squirrel. Italicized behaviors indicate behaviors that significantly contribute to the overall activity level or aggressiveness of a squirrel based upon guidelines by Budaev (2010).

| OFT Behavior | PC1 Loadings | MIS Behavior | PC1 Loadings |
|---|---|---|---|
| *Jumps* | *0.505* | *Front of arena* | *0.507* |
| *Walking* | *0.465* | *Attacks* | *0.279* |
| *Chewing* | *0.326* | *Back of arena* | *-0.421* |
| Hole dips | 0.231 | *Attack latency* | *-0.477* |
| Hanging | 0.178 | *Approach latency* | *-0.51* |
| Grooming | -0.018 | | |
| *Resting* | *-0.58* | | |

**Table 5.** Results from a linear mixed-effects model for the first component of the study, analyzing the effects of high density on activity level and aggression. Intercept (reference) value represents a female from the low-density control area. Models included birth year as a random effect to control for any early-age environmental variation and controlled for age. Bold face font indicates a statistically significant effect.

| | **β** | **Std. Error** | **d.f.** | ***t*** | ***p*-value** |
|---|---|---|---|---|---|
| **Activity** | | | | | |
| Intercept | 0.58 | 0.37 | 32 | 1.57 | 0.13 |
| **Density (High)** | **-0.53** | **0.15** | **268** | **-3.54** | **0.0004** |
| Sex (M) | -0.02 | 0.13 | 268 | -0.14 | 0.89 |
| Density (High) x Sex (M) | -0.06 | 0.21 | 279 | -0.3 | 0.77 |
| Age | 0.15 | 0.21 | 32 | 0.71 | 0.48 |
| $Age^2$ | -0.03 | 0.03 | 45 | -1.07 | 0.29 |
| **Aggression** | | | | | |
| Intercept | 0.91 | 0.39 | 20 | 2.3 | 0.32 |
| Density (High) | -0.3 | 0.16 | 215 | -1.85 | 0.06 |
| Sex (M) | -0.18 | 0.14 | 210 | -1.26 | 0.21 |
| Density (High) x Sex (M) | 0.24 | 0.22 | 243 | 1.09 | 0.28 |
| Age | -0.32 | 0.23 | 19 | -1.43 | 0.17 |
| $Age^2$ | 0.05 | 0.03 | 28 | 1.56 | 0.13 |

**Table 6.** Results from linear mixed-effects model for the second component of the study, analyzing the effects of number of generations experienced elevated population density on activity, aggression, and docility. Intercept (reference) value represents a female with three to five generations in the elevated population density area. Maternal generation was a two-level categorical variable (0 generations or 3).

| | β | Std. Error | d.f. | *t* | *p*-value |
|---|---|---|---|---|---|
| **Activity** | | | | | |
| Intercept | -0.06 | 0.68 | 31 | -0.09 | 0.93 |
| Generations (0) | -0.14 | 0.29 | 31 | -0.47 | 0.64 |
| Sex (M) | 0.15 | 0.3 | 31 | 0.5 | 0.62 |
| Generations (0) x Sex (M) | 0.08 | 0.39 | 31 | 0.21 | 0.84 |
| Age | 0.22 | 0.32 | 31 | 0.69 | 0.5 |
| $Age^2$ | -0.05 | 0.39 | 31 | -1.2 | 0.24 |
| **Aggression** | | | | | |
| Intercept | 1.03 | 1.17 | 31 | 0.89 | 0.38 |
| Generations (0) | -0.17 | 0.5 | 31 | -0.33 | 0.74 |
| Sex (M) | 0.25 | 0.51 | 31 | 0.48 | 0.63 |
| Generations (0) x Sex (M) | -0.18 | 0.68 | 31 | -0.26 | 0.8 |
| Age | -0.25 | 0.56 | 31 | -0.44 | 0.66 |
| $Age^2$ | 0.01 | 0.68 | 31 | -0.26 | 0.8 |
| **Docility** | | | | | |
| Intercept | 12.05 | 9.31 | 31 | 1.29 | 0.21 |
| Generations (0) | -3.25 | 4.48 | 31 | -0.73 | 0.47 |
| Sex (M) | -0.35 | 3.69 | 31 | -0.1 | 0.92 |
| Generations (0) x Sex (M) | -0.68 | 5.97 | 31 | -0.11 | 0.91 |
| Age | 0.97 | 4.9 | 31 | 0.2 | 0.85 |
| $Age^2$ | -0.01 | 0.6 | 31 | -0.01 | 0.99 |

**Figures**

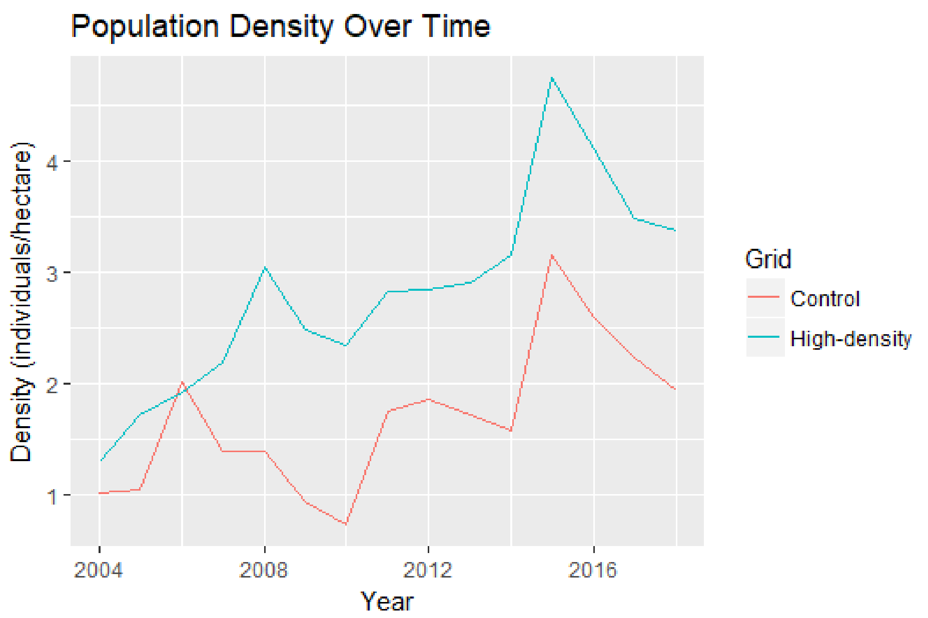


**Figure 1.** Population density increased as a result of food manipulation study (Dantzer et al. 2013). This study was done 2004-2017, where the average population density was 60% higher on the high-density area compared to the control. The control area was the average of two control study areas.

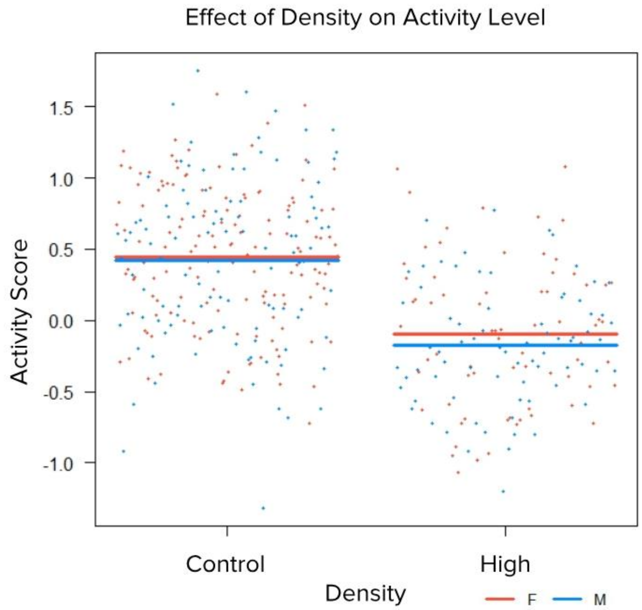


**Figure 2.** Both female and male red squirrels from the high-density experimental area (n = 147 trials from 100 squirrels) were, on average, less active than those from the lower-density control area (n = 257 trials from 159 squirrels). Bars indicate the mean activity score.

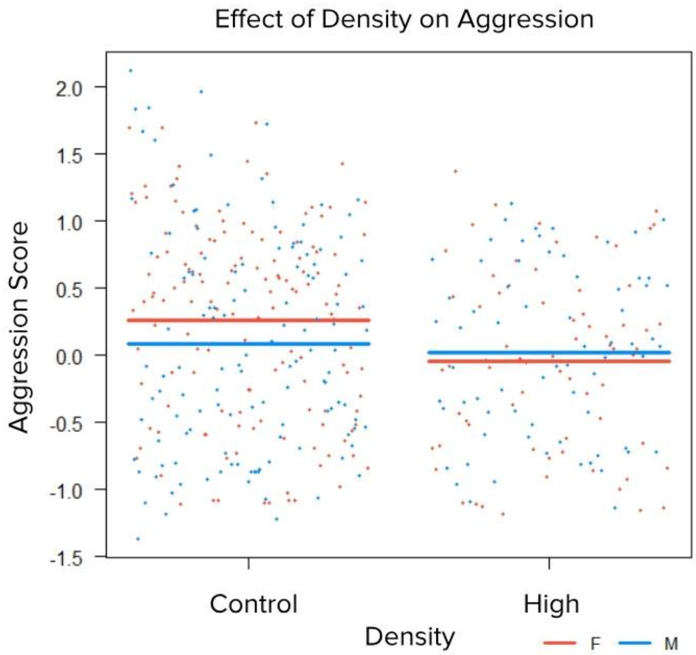


**Figure 3.** Neither males nor females from the high-density experimental area (n = 196 trials from 133 squirrels) were no more or less aggressive than those from the lower-density control areas (n = 268 trials from 180 squirrels). Bars indicate the mean aggression score.

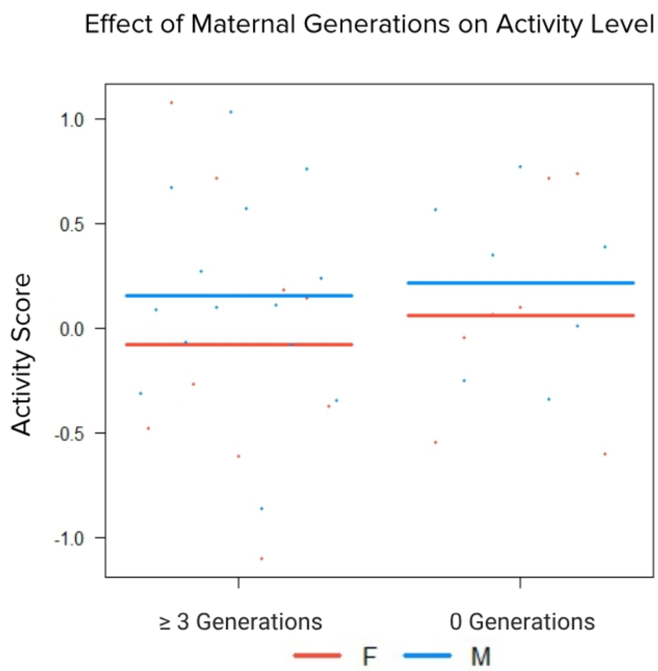


**Figure 4** Neither male nor female red squirrels who had experienced three or more generations of selection (n = 23 trials from 23 squirrels) from the high-density experimental area were no more or less active than those who had experienced zero generations of selection (presumably recent migrants; n = 14 trials from 14 squirrels). Bars indicate mean activity score.

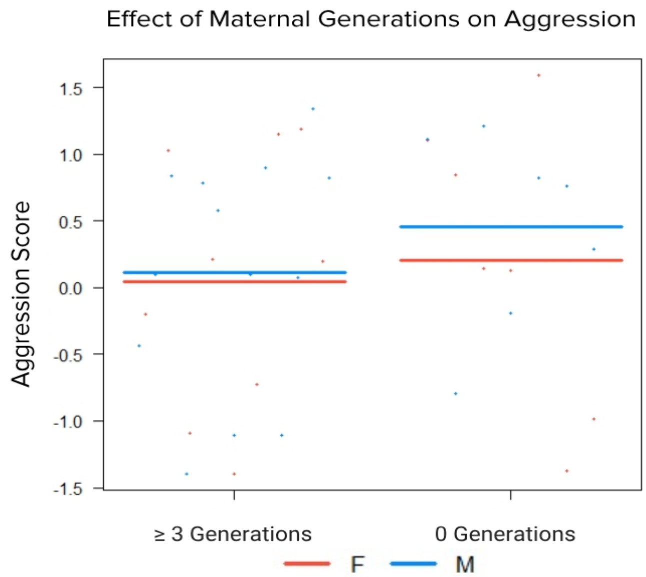


**Figure 5** Neither male nor female red squirrels who had experienced three or more generations of selection (n = 22 trials from 22 squirrels) from the high-density experimental area were no more or less aggressive than those who had experienced zero generations of selection (recent migrants; n = 14 trials from 14 squirrels). Bars indicate mean aggression score.

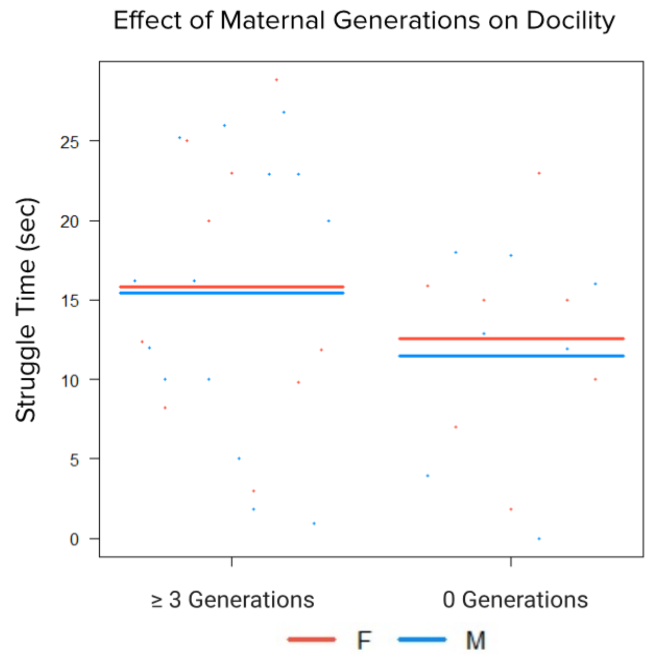


**Figure 6** Neither male nor female red squirrels who had experienced three or more generations of selection (n = 23 trials from 23 squirrels) from the high-density experimental area were no more or less docile than those who had experienced zero generations of selection (presumably recent migrants; n = 14 trials from 14 squirrels). Bars indicate mean struggle time.

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