**Title:** Urban fox squirrels exhibit tolerance to humans but respond to stimuli from natural predators

**Running title:** Fox squirrel behavior in urban areas

**Authors:** Anna Kittendorf[1], Ben Dantzer[1,2]

[1]Department of Psychology, University of Michigan, Ann Arbor, MI, 48109, USA

[2]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, 48109, USA

**Correspondence:** Ben Dantzer (dantzer@umich.edu)

**Acknowledgements**

This research was a part of a senior honor's thesis conducted by AK in the Department of Psychology at the University of Michigan. It was funded by the University of Michigan. Thanks to Dantzer Lab members (especially Amy-Charlotte Devitz) for input on this project. We acknowledge that the University of Michigan and some of the other properties that we worked on during this project reside on the traditional Territories of the Three Fire Peoples – the Ojibwe, Odawa, and Bodewadmi.

**Abstract**

Animals in urban areas that experience frequent exposure to humans often behave differently than those in less urban areas, such as less vigilance or anti-predator behavior. These behavioral shifts may be an adaptive response to urbanization but it may be costly if animals in urban areas also exhibit reduced anti-predator behavior in the presence of natural predators. We tested the hypothesis that increased tolerance to humans in urban populations of fox squirrels (*Sciurus niger*) reduces their responses to predators (hawks and domestic dogs). In trials with only a human observer as the stimulus, urban squirrels exhibited reduced vigilance and anti-predator behavior compared to those in less urban areas but there was little evidence that urbanization altered the correlations among the different behaviors. Next, we exposed squirrels in multiple urban and less urban sites to acoustic playbacks of a control stimulus (non-predatory bird calls), a natural predator (hawk), and dogs and recorded their vigilance and three different anti-predator behaviors when a human approached them while either broadcasting one of these three playbacks or no playbacks at all. Urban squirrels exhibited increased vigilance and anti-predator behavior when exposed to a human paired with hawk playbacks compared to the control playbacks. This indicates that urban squirrels did perceive and assess risk to the natural predator appropriately despite exhibiting increased tolerance to humans. These results provide little support for the hypothesis that increased tolerance to humans causes animals to lose their fear of natural predators.

**Keywords**: Anti-predator behavior, cross-habituation, first alert distance, flight initiation distance, stimulus generalization, urbanization

## Introduction

Behavior plays an important role in enabling animals to persist through environmental change (Baldwin, 1896; Bartholomew, 1964; West Eberhard, 1989; Price et al., 2003; Snell-Rood, 2013). Accordingly, it seems to play a major role in facilitating the ability of animals to cope with new challenges that they face in urban environments (Ditchkoff et al., 2006; Tuomainen and Candolin, 2011; Lowry et al., 2013; Ryan and Partan, 2014). One of the most common behavioral adjustments of animals in urban environments is reduced expression of anti-predator behavior in the presence of humans. This is often reflected in measures of flight initiation distance (FID), which is the distance at which an animal flees from an approaching human and is thought to be an approximation of their sensitivity to risk of an approaching predator (Cooke, 1980; Ydenberg & Dill, 1986; Lima and Dill, 1990). Individuals with shorter FIDs are considered to be bolder than individuals with longer FIDs since they demonstrate reduced fear of the "predator".

Substantial evidence supports the hypothesis that animals in more urban environments exhibit less anti-predator behavior, as reflected by a lower FID. For example, a meta-analysis of 180 bird species, 16 lizard species, and 16 mammal species, Samia et al. (2015) showed that populations of these species that experienced elevated levels of human disturbance exhibited lower FID. This could be because anti-predator behaviors carry energetic or time costs as vigilance and escape behaviors take time away for resource acquisition (Ydenberg and Dill, 1986; Cooper and Frederick, 2007; Møller, 2012) and animals in urban environments may optimize resource acquisition by exhibiting lower levels of anti-predator behavior.

Although reductions in the expression of anti-predator in urban environments is generally thought to be adaptive (Møller, 2008; Carrette et al., 2016), there may be potential costs for urban animals if they reduce their overall expression of anti-predator behavior to not only

humans but also natural predators. This could be due to the phenomenon of cross-habituation or stimulus-generalization. For example, birds that are habituated to a threatening stimulus that are then presented with a second simulated predator exhibit an attenuated response to this second stimulus compared to a group of naïve birds (Hinde, 1954; see also Curio, 1993). This type of stimulus generalization can occur where an animal habituated to one stimulus exhibits an attenuated response to a second stimulus from the same *or* different sensory modality (Guttman and Kalish, 1956; Thompson and Spencer, 1966; Rankin et al., 2009). Related concepts occur in the context of "behavioral spillover" where individuals that exhibit high levels of a behavior in one context also exhibit it in another context even though it may not be adaptive, such as animals exhibiting higher levels of boldness in a courtship context also exhibiting higher boldness in the presence of a predator (Arnqvist and Henriksson, 1997; Sih et al., 2004).

If urban animals also exhibit reductions in anti-predator behavior to natural predators, it could conceivably have important impacts on wildlife populations by increasing their vulnerability to predators (Geffroy et al., 2015). To date, there is little consensus about whether animals in urban areas or those exposed to increased human presence exhibit a reduced response to threats from natural predators (Fitzgerald and Stronza, 2016). For example, some studies show that individuals in areas with higher human activity exhibit less of a behavioral response when natural predators were observed visiting the area (Olson and Acevedo-Gutiérrez, 2017) or due to acoustic playbacks of a natural predator (McCleery, 2009). The latter suggests animals experiencing frequent exposure to human activity exhibit reduced responses to other stimuli from natural predators. Other studies show the response of animals in more urban areas to a stimulus from a natural predator is not attenuated compared to those in more rural locations (Labra and

Leonard, 1999; Coleman et al., 2008; Seress et al., 2011; Bokony et al., 2012; Cavalli et al., 2016; Weaver et al., 2018; Vincze et al., 2019).

In this study, we characterized the vigilance and anti-predator of fox squirrels (*Sciurus niger*) in urban and less urban areas to achieve the following three objectives. First, we conducted standard FID trials (with only stimuli from a human observer) to examine if squirrels in urban areas showed reduced vigilance and anti-predator behavior towards a human observer compared to those in less urban areas. If squirrels in urban areas did exhibit reduced vigilance and anti-predator behavior, this would support the hypothesis that squirrels in our urban study populations were more tolerant to human presence, which would be consistent with numerous other studies (Samia et al., 2015). Squirrels were located in their natural habitat and we recorded the following four aspects of their vigilance and anti-predator behavior. First, we recorded the distance to which the observer could get to before they exhibited vigilance behavior towards the observer ("first alert distance" or FAD, similar to Fernández-Juricic and Schroeder, 2003; Blumstein et al., 2005). Second, we recorded how close the observer could get to them before they ran away (FID). Third, was the probability that the squirrel escape by running up a tree. Fourth, the latency following the trial it took them to resume their typical behavior (foraging or traveling off tree).

Our second objective was to assess the degree of phenotypic correlations among the different behaviors we recorded from these trials. We did this because previous studies suggest that phenotypic correlations among different vigilance or anti-predator behaviors (i.e., the behavioral syndrome) may differ between urban and rural populations (Evans et al., 2010; Scales et al., 2011; Carrette and Tella, 2017; Lapiedra et al., 2017). This could be because selection against anti-predator behavior in urban environments produces a correlated response in other

risk-taking behaviors. If there was a strong phenotypic correlation between two behaviors within the same squirrel recorded from less urban sites compared to those in urban sites, this would support the hypothesis that squirrels in urban areas exhibit greater behavioral flexibility.

Our final objective was to examine if urban animals exhibit reduced behavioral responses to stimuli from natural predators when they are in the presence of humans. To do so, we quantified the four behaviors described above when fox squirrels in urban or less urban areas were presented with a human observer with a control acoustic playback (common non-threatening bird), a human observer paired with the playback of a natural predator (hawk), or a human observer paired with a playback of an invasive predator (dog). We predicted that squirrels in the urban areas but not those in the less urban areas would exhibit no change in vigilance and anti-predator behavior when they were exposed to the human+dog or human+hawk stimuli compared to the human+control playback. This would support the hypothesis that animals in urban environments exhibit less vigilance and anti-predator not only to humans but also when faced with natural predators.

## Materials and Methods

*Study species and sites*

Fox squirrels are ubiquitous in urban and suburban environments in the midwestern United States (McCleery, 2008, 2009). Although arboreal tree squirrel species like fox squirrels are common in urban areas worldwide, they continue to experience predation from natural predators, although it may be rare (McCleery et al., 2008), and from domestic cats and dogs (Koprowski, 1994; Wauters et al., 1997; Tumlison, 2012; Loss et al., 2013; Jokimäki et al., 2017).

We studied natural populations of adult fox squirrels from six sites in and around Ann Arbor, Michigan (Table 1). Sites were chosen based upon estimates of human population density (Center for International Earth Science Information Network, 2018) with urban sites having higher human density than less urban sites (see below and Table 1). Urban sites included Prospect Park as well as two locations on the University of Michigan's (UM) main campus (North and Central Campus) that are ~3-4 km away from one another. Prospect Park is near downtown Ypsilanti, Michigan and about 13 km away from UM main campus. Less urban sites included Nichols Arboretum, County Farm Park, and Saginaw Forest. Nichols Arboretum is located ~1 km away UM main campus, County Farm Park is about ~4.5 km away, and Saginaw Forest is ~7 km away. At all research sites, dogs are allowed but hunting is not. Approval to conduct this research at each site was obtained from UM (Central Campus, Nichols Arboretum, North Campus, Saginaw Forest), Washtenaw County Parks & Recreation (County Farm Park), and the City of Ypsilanti (Prospect Park). All procedures were non-invasive and involved behavioral observation or short-term exposure to playbacks of acoustic stimuli. All experiments followed the guidelines set by the Animal Behavior Society/Association for the Study of Animal Behaviour (Anonymous, 2012) and the US National Research Council and were approved by the UM Institutional Animal Care and Use Committee (protocol # PRO00009076). We note that squirrels used in the study may be STRANGE (sensu Webster and Rutz, 2020) in the sense that individual squirrels likely have different rearing histories (though they are unknown and none should have been reared in captivity) and that compliance to take part in the study was likely biased towards squirrels that did not immediately run away when approached by the human observer.

157 Given that increased exposure to humans may cause animals in urban areas to exhibit less
158 anti-predator behavior towards them (McCleery, 2009; Rodriguez-Prieto et al., 2009; Vincze et
159 al., 2016; Uchida et al., 2019), we focused on human presence as the major factor difference
160 between our study sites (which should also reflect general urbanization). Sites were classified as
161 "urban" based upon having a human population density >1000 persons per $km^2$ whereas the less
162 urban sites had anywhere from 25-250 persons per $km^2$ (Saginaw) to 250-1000 persons per $km^2$
163 (County Farm Park, Nichols Arboretum). To support these classifications, we human and dog
164 presence while we were visiting sites conducting our behavioral observations. We counted the
165 total number of dogs (on or off leash) but only counted the number of humans up to 50. If human
166 presence exceeded 50 people, then a rough estimate of 50, 75, or 100 was recorded. Human
167 presence was recorded as 100 for all numbers estimated to be >100. Although human and dog
168 presence varied among the different sites (Table 1), the number of humans observed per hour of
169 observation averaged over all the urban sites (mean ± SE = 7.23 ± 3.33 humans/hr) was higher
170 than those observed averaged over all the less urban sites (0.51 ± 0.40: Mann-Whitney-Wilcoxon
171 Test, W = 1, $p = 0.1$). We observed fewer dogs per hour at the urban sites (0.065 ± 0.06 dogs/hr)
172 compared to the less urban sites (0.33 ± 0.10, Mann-Whitney-Wilcoxon Test, W = 9, $p = 0.1$).
173 These differences in humans or dogs observer per hour were not significant but in general
174 support our assumption that our urban sites likely experience greater exposure to humans.
175 Adult squirrels and sex were visually identified by size (small juvenile squirrels were
176 excluded) and anatomy (males were identified by presence of testes), respectively. Similar to
177 most studies that measure anti-predator using FID, trials were conducted on unmarked squirrels
178 at each site. Squirrels were located by walking around each site and trials were started when
179 squirrels were observed. Focal individuals were selected randomly, however, only squirrels that

180 were feeding or foraging on the ground were included in this experiment. Because we did not
181 mark squirrels individually, it is possible that the same squirrel was observed on different days,
182 although we visited different areas of each study site to try and reduce this possibility. It is
183 unlikely that the same squirrel was observed multiple times on the same day, because after each
184 trial was completed, the observer traveled approximately 20 meters away from the previous
185 location and started a trial with a different squirrel. Additionally, sites were only revisited after at
186 least three days since the previous visit to reduce the possibility of a squirrel becoming
187 habituated to the trials should it be sampled again. Although we cannot address habituation in
188 this study, if squirrels at these sites were habituating to our protocols, we would expect that their
189 behavioral responses would decline with trial number or date when the trial was conducted. The
190 fact that none of our behavioral variables were associated with date of when the trial was
191 conducted (Tables 2-4) supports our assumption that squirrels were not habituating to our
192 protocols.

193 *Quantifying behavioral responses of squirrels*

194 In total, we observed fox squirrels for 52.36 hours over 30 different days. A single
195 observer (AK) conducted all trials. Sites were visited between 800 and 1900 h EST and data
196 were collected from October 2019 through January 2020. Trials with no playbacks were
197 conducted from 25 October 2019 to 16 November 2019 (from 812 to 1810 h) whereas trials
198 using playbacks (playback trials) were conducted from 18 November 2019 to 27 January 2020
199 (from 802 to 1609 h). Both the order in which sites were visited and the order in which playback
200 versions across playback treatments (see below) were randomized. No two sites were visited on
201 the same day. All trials with no playbacks were conducted prior to the playback trials in this
202 study. This was due to personnel limitations and prevents us from directly comparing trials with

203 and without playbacks given that squirrel behavior likely changes seasonally from October to
204 January due to food caching behavior in autumn but not winter. Trials were not conducted when
205 it was raining or snowing. Results from two one-way ANOVAs showed that the time of day for
206 playback trials did not vary among the three different treatment groups ($F_{2,91} = 0.44$, $p = 0.65$)
207 and that the time of day when baseline trials were conducted did not vary among squirrels at the
208 urban or less urban sites (t-test: $t_{75.4} = 0.21$, $p = 0.83$). Air temperature varied during all the trials
209 varied from -6.1° to 10.5°C (mean = 2.7° C).

210 We measured the behavioral responses of squirrels to humans or humans plus different
211 playbacks using protocols developed for tree squirrels (Dill and Houtman, 1989; Gustafson and
212 VanDruff, 1990; McCleery, 2009). At the beginning of each trial, a marker was placed at the
213 starting position of the observer and trial data were recorded (GPS location, time of day,
214 temperature, general weather conditions, and squirrel sex). The squirrel was approached by a
215 single observer (AK) at a slow and steady pace in a direct line to the squirrel (see Fig. S1 in
216 Appendix I). Additional markers were placed when a squirrel displayed the first alert and when
217 they fled. The FAD was defined as the distance between the observer and the squirrel when it
218 first stopped moving (froze) and looked at the observer with one or both eyes. FID was recorded
219 as the distance between the squirrel's initial position and the observer location when it actively
220 fled (stopped feeding and foraging and moved rapidly away from observer). A marker was also
221 placed at an estimate of the squirrel's initial position to the place where they fled to if refuge was
222 not taken in a tree, and the distance between this marker and the squirrel's initial position was
223 recorded as "flight distance". We recorded this because some studies have noted that FID is
224 variable depending on intruder starting distances and distance to a refuge (Dill & Houtman,
225 1989; Blumstein, 2003). Consequently, the distance between the observer and the focal squirrel

at the start of the trial (starting positions, hereafter referred to as "initial distance") and the distance between the focal squirrel's initial position and the nearest tree were also measured ("distance to nearest tree").

If the focal animal took refuge in a tree other than the one nearest, distance between the squirrel's initial position and its refuge tree of choice ("distance to the chosen tree") was also recorded. If an individual took refuge in a tree, a laser rangefinder was used to measure how high they climbed, and this distance was recorded (same as vertical escape distance in Uchida et al., 2017). Lastly, latency to resume behavior was recorded ("latency"). This was measured with a stopwatch to determine how long it took for the animal to cease alert/vigilance behavior and resume typical activity (foraging or traveling off tree). When the human observer was recording latency, they maintained as large a distance as possible from the tree to reduce the influence on the squirrel's behavior. Out of all the trials, nearly all squirrels ceased alert behavior and resumed typical behavior within a couple minutes (n = 171 trials, mean ± SE = 130.5 ± 10.3 s), but there was one individual squirrel that remained alert for longer than ten minutes and we recorded its latency as ten minutes. Distances were measured with a tape measure and presented in meters.

*Playback trials*

Playback trials were conducted using the same protocol shown above, with the addition of an acoustic stimulus being broadcasted while the observer approached the squirrels. The control stimulus consisted of recordings of black-capped chickadee calls (*Poecile atricapillus*). Black-capped chickadees are not known to be predators of fox squirrels (Korschgen, 1981; Koprowski, 1994) and a previous study in another tree squirrel species showed that individuals exhibited a significantly reduced response to black-capped chickadee playbacks compared to

calls of other anthropogenic noises (car alarm, buzzer) and playbacks of red-tailed hawks (Bohls and Koehnle, 2017). We therefore expected that black-capped chickadee recordings would represent a neutral vocalization for fox squirrels and they can act as control to ensure that any differences in squirrel behavior across playback treatments are attributable to the vocalization information of the playback rather than an added exposure to noise. To simulate the threat of a terrestrial predator, recordings of domestic dogs barking were broadcasted. Domestic dogs are terrestrial predators of fox squirrels (Koprowski, 1994; Wauters et al., 1997) and other species of tree squirrels that live in the same habitats as fox squirrels also adjust their risk-taking behavior according to the abundance of domestic dogs (Bowers and Breland, 1996; Cooper et al., 2008). For the avian predator, recordings of red-tailed hawk (*Buteo jamaicensis*) calls were broadcasted to the focal individual. Red-tailed hawks were chosen since they are year-round predators of fox squirrels in Michigan (Koprowski, 1994; personal observations) and other studies illustrate that tree squirrels respond to hawk playbacks with increased anti-predator behavior (McCleery, 2009; Lilly et al., 2019). No post-processing of sound files was performed.

Playbacks of the recordings were broadcast to individuals at the start of the trial and when the observer began the approach and suspended when the squirrel took flight. Vocalizations were broadcast through a JAMBOX speaker (Jawbone, San Francisco, CA) connected to an Apple iPhone 6s (Mountain View, CA) with a constant volume set for the speaker and phone. The amplitude of the playbacks measured from 1 m away from the speaker was variable among the chickadee (67-80 dB), dog (69-77 dB), and hawk (78-86 dB) playbacks (measured using a BAFX Sound Level Meter, BAFX3370). We note that the initial starting distance of the playbacks was inherently variable as we could not standardize the distance between the observer and squirrel when the trials were started. Consequently, the actual realized

sound level of the playbacks experienced by a squirrel varied. Given how the trials were conducted in real time (not video recorded), the single observer (AK) was not blind to the playback treatments or locations of where the experiments took place. All vocalization recordings were found online (Control A: Place, 2015; Control B: Floyd, 2017a; Control C: Floyd, 2017b; Dog A: Simion, 2016; Dog B: Simion, 2018; Dog C: Simion, 2017; Hawk A: Chartier, 2008; Hawk B: Addison, 2017; Hawk C: Wilson, 2010). Each playback treatment (control, dog, or hawk) had three separate recordings/exemplars (A, B, or C). We tested whether there was any exemplar effects in separate ANOVAs that included playback exemplar (A, B, C), playback treatment (chickadee, dog, hawk), and an interaction between the two. We did this for each of our four behavioral response variables and did not find any significant interactions between playback exemplar and treatment ($p$ = 0.12-0.99), suggesting that the version of the playback treatment did not influence the behavioral response.

*Statistical analyses*

We analyzed the data from trials with and without playbacks separately because the two experiments were not conducted synchronously and seasonal changes from Fall to Winter in Michigan may alter squirrel behavior. In trials without playbacks, we used three separate linear mixed-effects models (LMMs) to examine the effects of urbanization on FAD, FID, and latency to resume activity following the trial. Although the linear distance a squirrel climbed up a tree (from base of tree to location of squirrel) has been used in other studies of tree squirrels (e.g., Uchida et al., 2017), the distance a squirrel climbed up a tree in our study exhibited a Poisson distribution where many squirrels did not climb up a tree at all and a few climbed up very high. Consequently, a generalized linear mixed-effect model (GLMM) with binomial errors was used to examine the effects of urbanization on the probability that squirrels climbed a tree to escape

during the trial. Models included site category of data collection (urban, less urban), distance to the nearest tree, sex, Julian date of the trial, and initial distance of the observer as fixed effects. Distance to the nearest tree was included not only because previous studies show it can impact anti-predator behavior (measured using FID: Dill & Houtman, 1989; Blumstein, 2003) but also because it helps control for any differences in vegetation among the different study sites, which could impact their behavior. The same model structure was used in separate LMMs or the GLMM for data from the playback trials to examine the effects of the acoustic playback manipulations on the four squirrel behaviors described above but the models included an interaction between playback treatment (control, dog, hawk) and site category (urban, less urban). We assessed the statistical significance of the latter interaction between playback treatment and site using post-hoc Tukey's Honest Significant Differences that were corrected for multiple comparisons.

To assess the degree of association between FAD or FID and the two other behavioral variables (probability of climbing a tree, latency to resume typical behavior), we ran two separate LMMs (one for FAD and the other for FID). Each model included fixed effects for site category, probability of climbing a tree, latency to resume typical behavior, and interactions between site category and each of the two behavioral variables. We also report the Pearson' or point-biserial (escaping up tree was binary variable) correlations between these variables. We did not run a similar model looking at the association between FAD and FID given that they are clearly not independent of one another (for all squirrels [n = 171] across all treatments in this study: Pearson $r = 0.88$, 95% CI = 0.84-0.91).

Continuous predictor variables were standardized to a mean of 0 and SD of 1. We confirmed model diagnostics visually and all models met the appropriate assumptions (normality

of residuals, constant variance, no high leverage observations). Latency to resume behavior was log+1 transformed (base e) to improve homoscedasticity and normality. There were also no predictor variables that were found to be colinear as all variance inflation factors (VIFs) were less than 3.68 (Zuur et al., 2010), though the higher VIF were due to interaction terms and VIF of variables not in interactions were <1.5. Because we had repeated samples from the same site, we also included a random intercept for site in all of our models. All analyses were conducted in R version 4.02 (R Core Team, 2020) with lme4 (version 1.1.23, Bates et al., 2015) and p-values estimated using lmerTest (version 3.1.2, Kuznetsova et al., 2017). Tukey's post-hoc tests were used to evaluate if the responses to the playback treatments differed between squirrels in urban and less urban areas using emmeans (1.5.2-1: Lenth, 2020) and *p* values from these analyses were adjusted for multiple comparisons. Mean and SE are presented below.

## Results

*Behavioral responses to human-stimuli only*

Overall, urban squirrels (n = 38, 20 females and 18 males) exhibited greater tolerance to humans as they allowed a human observer to get closer to them before they exhibited vigilance (FAD) or fled (FID) and tended to be less likely to climb a tree during the trial and more quickly return to typical behavior following the trial compared to those in less urban sites (n = 39, 19 females and 20 males; Table 2, Fig. 1). FID in the squirrels at the urban site (6.36 ± 0.52 m) was 97.2% shorter compared to those at the less urban sites (12.54 ± 0.62 m, $p = 0.043$, Table 2, Fig. 1B). Although the average FAD for squirrels observed at the urban site (10.09 ± 0.74 m) was 43.8% shorter than for those at the less urban sites (14.51 ± 0.69 m), this difference were not significant ($p = 0.10$, Table 2, Fig. 1A). Squirrels at the urban sites were less likely to climb a

tree while the observer approached (34.2% of trials) compared to those at the less urban sites (48.6%), although this difference was not significant ($p = 0.093$, Table 2, Fig. 1C). Latency to resume behavior following the trial was shorter for urban squirrels (43.9 ± 14.4 s) compared to those at the less urban sites (157.51 ± 25.35 s), but this difference was not significant ($p = 0.11$, Table 2, Fig. 1D). Trials where the observer started the trial at a longer initial distance to the squirrel had significantly higher FAD and FID but not probability of climbing a tree or latency to resume behavior following the trial (Table 2). There were no significant effects of sex, Julian date, or distance to the nearest tree on FAD, FID, probability of climbing a tree, or latency (Table 2).

*Patterns of associations among behaviors*

Squirrels at urban sites (n = 38) that exhibited a higher FAD also exhibited a significantly higher latency to resume typical behavior following the trial ($r = 0.59$, 95% CI = 0.33-0.77, $t_{22.9} = 3.07$, $p = 0.005$) but this association between these two variables was significantly reduced in squirrels at the less urban sites (n = 39, $r = 0.09$, 95% CI = -0.23-0.39), as indicated by the nearly significant interaction between site category and latency ($t_{33.1} = -2.01$, $p = 0.053$, Table 3, Fig. 2A). Squirrels at both urban sites that exhibited a higher FID also tended to exhibit a higher latency to resume typical behavior following the trial ($r = 0.53$, 95% CI = 0.26-0.73, $t_{68.2} = 1.86$, $p = 0.066$), but the association between these two behaviors was slightly lessened in squirrels at less urban sites ($r = 0.18$, 95% CI = -0.14-0.47), although this was not significant ($t_{70.5} = -1.17$, $p = 0.24$, Table 3, Fig. 2B). In squirrels at both urban ($r = 0.46$, 95% CI = 0.17-0.68) and less urban sites ($r = 0.39$, 95% CI = 0.09-0.63), squirrels that were more likely to escape the human observer by climbing a tree also had a longer latency to resume typical behavior following the

trial ($t_{72.9}$ = 2.08, $p$ = 0.041) and this did not depend upon whether the squirrels were at urban or less urban sites ($t_{72.3}$ = 0.05, $p$ = 0.96).

*Behavioral responses to stimuli from natural predators*

The effects of the playbacks on FAD depended upon whether the squirrels were located at the urban or less urban sites (site x playback treatment: $F_{2, 81.7}$ = 6.07, $p$ = 0.003), with urban squirrels exhibiting the highest FAD when exposed to hawk playbacks and squirrels at less urban sites exhibiting higher overall FAD and no response to the playback treatments (Fig. 3A). Average FAD for urban squirrels exposed to the hawk vocalizations (n = 12 trials, 17.17 ± 1.33 m) was 37% longer than squirrels who were exposed to the control playback (n = 11, 12.56 ± 0.94 m, Tukey's $p$ = 0.0016) and 20% longer than those exposed to the dog playbacks (n = 20, 14.30 ± 0.83 m, Tukey's $p$ = 0.0025, Fig. 3A). By contrast, the FAD of squirrels at the less urban sites were just higher overall (Fig. 3A) and the FAD of those who were exposed to the hawk playbacks (n = 16, 14.62 ± 1.14 m) did not differ from those exposed to the control playback (n = 15, 14.46 ± 0.88 m, Tukey's $p$ = 0.95) or dog playbacks (n = 20, 14.85 ± 1.05 m, Tukey's $p$ = 0.64, Fig. 3A). There were no significant differences in FAD for squirrels exposed to dog playbacks and those exposed to the control playback for squirrels at urban sites (Tukey's $p$ = 0.83) or those at less urban sites (Tukey's $p$ = 0.84).

Similar to FAD, the effects of the playbacks on FID also depended upon whether the squirrels were located at the urban or less urban sites (site x playback treatment: $F_{2, 81.2}$ = 6.28, $p$ = 0.003), with urban squirrels exhibiting the highest FID when exposed to hawk playbacks and squirrels at less urban sites exhibiting the highest FID in response to the dog playbacks (Fig. 3B). Average FID for urban squirrels exposed to the hawk vocalizations (n = 12 trials, 14.95 ± 0.98 m) was 44.3% longer than squirrels who were exposed to the control playbacks (n = 11, 10.37 ±

386 3.13 m, Tukey's $p = 0.0006$) and 29.9% longer than those exposed to the dog playbacks (n = 20,
387 11.86 ± 2.65 m, Tukey's $p = 0.0005$, Fig. 3A). In squirrels at urban sites, there was no difference
388 in FID between those exposed to the control playback and those exposed to the dog vocalizations
389 (Tukey's $p = 0.90$). In squirrels at less urban sites, FID for those exposed to the dog playbacks
390 (13.60 ± 0.98 m,) was 12.2% higher than those exposed to the control playback (12.12 ± 0.88 m,
391 Tukey's $p = 0.049$), but was similar to those exposed to the FID of those exposed to hawk
392 playbacks (13.11 ± 0.96 m, Tukey's $p = 0.66$, Fig. 3B). Unlike urban squirrels, the FID of those
393 at the less urban sites who were exposed to hawk playbacks was similar compared to those
394 exposed to the control playback (Tukey's $p = 0.34$).

395 The playback treatments had no significant effects on the probability that squirrels in
396 urban or less urban sites exhibited an escape response by climbing a tree, as indicated by the lack
397 of interactions between playback treatment and site (Table 4, Fig. 3C). Squirrels at both the
398 urban and less urban sites exhibited the same response when it comes to their latency to resume
399 their typical behavior following the trial (hawk > dog > control, Table 4, Fig. 3D), as indicated
400 by the lack of interaction between site and playback treatment (site x playback treatment: $F_{2, 80.5}$
401 $= 0.20$, $p = 0.82$). Squirrels at urban sites that were exposed to the hawk playbacks took 436%
402 longer to resume their pre-trial behavior (213.33 ± 28.9 s) compared to those who were exposed
403 to the control playback (39.82 ± 7.70 s, Tukey's $p < 0.0001$) and 147% longer than those
404 exposed to dog playbacks (86.25 ± 13.30 s, Tukey's $p = 0.011$). Squirrels at the urban sites also
405 took 114% longer to resume typical behavior if they were exposed to dog playbacks compared to
406 those exposed to the control playback (Tukey's $p < 0.001$). Similarly, squirrels at the less urban
407 sites that were exposed to the hawk playbacks took 218% longer to resume their pre-trial
408 behavior (291.25 ± 35.45 s) compared to those who were exposed to the control playback (91.47

409 ± 25.45 s, Tukey's $p < 0.0001$) and 55.7% longer than those exposed to dog playbacks (187.05 ±
410 21.38 s, Tukey's $p = 0.045$, Fig. 3D). Squirrels at the less urban sites also took 95.6% longer to
411 resume typical behavior if they were exposed to dog playbacks compared to those exposed to the
412 control playback (Tukey's $p < 0.001$).

413 There was no effect of sex or Julian date of trial, on any of the behaviors (Table 4). There
414 was no effect of the initial distance that a squirrel was from a tree when the trial started on FAD,
415 FID, or probability to climb a tree, but, squirrels were less likely to climb a tree if they were
416 closer to one when the trial started (Table 4). Trials that started with the human observer a
417 greater distance away from the squirrel had higher FAD, FID, and latency to resume typical
418 behavior, but not the probability to climb a tree (Table 4).

419

420 **Discussion**

421 Overall, squirrels at urban sites in the no playback trials exhibited a significantly shorter
422 FID compared to those at the less urban sites but there minimal differences in the associations
423 between the behaviors in squirrels at urban versus less urban sites. In the trials with playbacks,
424 squirrels at the urban sites exhibited higher FAD (hawk > control > dog) and FID (hawk >
425 control = dog) when exposed to hawk playbacks compared to control or dog playbacks. By
426 contrast, squirrels at the less urban sites had higher overall FAD and FID than those at urban
427 sites regardless of playback treatment, there was no effect of hawk or dog playbacks on FAD
428 (hawk = control = dog) and they exhibited the highest FID when exposed to dog playbacks (dog
429 > control = hawk). Squirrels at both urban and less urban sites were not more likely to climb a
430 tree following playbacks from possible predators (hawk = dog = control) but both urban and less
431 urban squirrels exhibited a longer latency to resume typical behavior following the hawk or dog

432 playbacks compared to the control (hawk > dog > control). In terms of the STRANGEness of our
433 results (Webster and Rutz, 2020), our results may be generalizable to other squirrel populations
434 or different species but we note that our results are biased towards squirrels that voluntarily
435 participated in the trials (i.e., did not run away when approached).

436 Similar to most other studies in terrestrial animals (Samia et al., 2015) and in studies in
437 tree squirrels (McCleery, 2009; Engelhardt and Weladji, 2011; Sarno et al., 2015; Uchida et al.,
438 2020), our results from trials with no playbacks suggest that squirrels in urban sites were more
439 tolerant of humans. Specifically, squirrels in urban areas exhibited a shorter FAD and FID, lower
440 probability to climb a tree to escape the human observer, and a shorter latency to resume typical
441 behavior following the trial, although only FID was significantly different. The congruency of
442 our results with previous studies strongly supports this assumption that squirrels at our urban
443 sites were more tolerant to humans. This is surprising given that these are presumably sympatric
444 populations with large amount of gene flow among them as the linear distance between some
445 urban and less urban sites is ~1 km. Unless selection favoring reductions in anti-predator
446 behavior is extremely strong in urban areas or features of urban landscapes strongly impede gene
447 flow (Johnson and Munshi-South, 2017), it seems likely that these behavioral differences are
448 driven by plasticity given that the likely exchange of individuals between suburban and urban
449 sites prevents local genetic adaptation to these different sites (see discussion in Sol et al., 2013).
450 It is also possible that these behavioral differences are due to personality-dependent colonization
451 of urban habitats (Carrete and Tella, 2010; Sprau and Dingemanse, 2017), but we cannot
452 distinguish among these possibilities at this time.

453 Urban environments may favor individuals with reduced anti-predator behavior (as this
454 may increase resource acquisition) and this could cause some correlated shift in other behaviors.

455 There was little evidence that the correlations among the different behaviors we recorded
456 differed between squirrels in urban and less urban sites. We found that the association between
457 latency to resume typical behavior following a trial and FAD differed between urban and less
458 urban populations. Squirrels in urban but not less urban sites that allowed humans to get closer to
459 them before exhibiting first vigilance (FAD) also took a significantly shorter amount of time to
460 resume typical behavior following the end of the trial. However, there was no significant
461 deviance in the association between FID and latency or probability to escape by climbing a tree
462 and latency in squirrels from urban and less urban sites. As such, our results somewhat differ
463 from previous studies (Evans et al., 2010; Scales et al., 2011; Bokony et al., 2012; Myers and
464 Hyman, 2016; Carrete and Tella, 2017) that indicate that urban environments may enhance or
465 lessen the associations between different behaviors within the same individual. At this point we
466 cannot infer whether the shift in phenotypic correlations we documented here (for FAD and
467 latency) is adaptive, evidence of a constraint or correlated response to selection, or merely due to
468 environmental co-variance.

469 Although squirrels at our urban sites were more tolerant of humans, they still exhibited a
470 robust behavioral response to acoustic stimuli from natural predators. Specifically, they exhibited
471 increased vigilance (FAD) and anti-predator behavior (FID), latency to resume typical behavior
472 after the trial) when exposed to the playbacks of a natural predator (hawk) compared to the
473 control playback or the dog playbacks. These results indicate that urban squirrels do still pay
474 attention to predation risk and can discriminate and respond accordingly by becoming vigilant
475 and fleeing when the human is at a greater distance if the human is also paired with hawk
476 playbacks. By contrast, squirrels at less urban sites did not exhibit differences in FAD when
477 exposed to the different playbacks, perhaps due to some ceiling effect given that FAD of

478 squirrels at less urban sites was much higher than FAD of squirrels at urban sites. Our results
479 therefore reject the hypothesis that urban squirrels are less responsive to natural predators due to
480 increased tolerance to humans. Previous studies (see also Labra and Leonard, 1999; Seress et al.,
481 2011; Cooper et al., 2008; Bokony et al., 2012; Cavalli et al., 2016; Weaver et al., 2018; Vincze
482 et al. 2019) together with our results support that animals in urban habitats or those frequently
483 exposed to humans, even if more tolerant to human presence, still exhibit increases in anti-
484 predator behavior in response to a non-human predator.

485 We expected that squirrels would respond to the dog playbacks in similar way to how
486 they responded to the hawk playbacks as both are stimuli from potential predators. Previous
487 studies in tree squirrels also show that they exhibit increased anti-predator behavior (vigilance or
488 FID) to the physical presence of a dog with a human handler (Gustafson and VanDruff, 1990;
489 Cooper et al., 2008) or behave in such a way in areas with high levels of dogs and cats that
490 suggests that they perceive a higher predation risk in such areas (i.e., giving up density was
491 higher in study areas where cats and dogs are present: Bowers and Breland, 1996). Instead, we
492 found that squirrels at both sites did not differ in their FAD when exposed to dog playbacks
493 compared to the control playbacks. Additionally, only squirrels in less urban sites had a
494 significantly higher FID when exposed to dog playbacks compared to the control playback.
495 These results differ from previous studies in tree squirrels and owls showing that FAD and/or
496 FID were increased in squirrels or owls in urban areas when they were presented with a human
497 plus dog compared to just a human (Gustafson and VanDruff, 1990; Cooper et al., 2008; Cavalli
498 et al., 2016). This suggests that squirrels in urban sites were more tolerant to dog vocalizations
499 when paired with a human observer, whereas squirrels in less urban areas viewed the sounds of
500 dogs paired with humans as threatening. We predict that squirrels in urban environments exhibit

501 selective tolerance where the response to humans or stimuli from their commensals (dogs) is
502 attenuated but the increased response to natural predators is maintained despite this tolerance to
503 humans and their dogs.

504 Together, our results provide insight into how urbanization may shape the behavioral
505 characteristics animals. As most studies on this topic are in birds (Samia et al., 2015), which can
506 escape from humans using flight, it is important to consider if the same patterns are found in
507 terrestrial animals. Our results show that non-volant animals in urban environments exhibit less
508 vigilance and anti-predator behavior. We also show that squirrels in urban environments were
509 more tolerant to humans but still exhibited a strong behavioral response to acoustic stimuli from
510 a natural predator (hawks). Although we do not wish to imply that tolerance or habituation to
511 humans is cost-free, most studies fail to find evidence that populations where individuals are
512 more tolerant of humans (or in some cases habituated to their presence) also exhibit reduced anti-
513 predator behavior to stimuli from natural predators. Given that urbanization is unlikely to slow,
514 increased effort is needed to determine if increased tolerance and/or habituation to humans
515 carries costs (Geffroy et al,. 2015). Some studies suggest the costs of human tolerance may be
516 more nuanced, such as tolerance to humans reducing the latency to return to the nest following a
517 disturbance in nesting shorebirds, but potentially causing increased chick mortality due to the
518 presence of dogs that often are paired with human stimuli (Baudains and Lloyd, 2007). Other
519 studies that increased tolerance of humans could even be beneficial for populations that cannot
520 avoid anthropogenic stimuli due to seasonal food pulses coinciding with a large influx of tourists
521 (Wheat and Wilmers, 2016). Clearly more work is needed on this subject, especially on a greater
522 number of species including species that are not “urban exploiters” like tree squirrels, but the

existing evidence rejects hypothesis that there is a cost to human tolerance in terms of lowering the anti-predator behavior of animals to other natural predators.

**Data Availability Statement**

All data will be archived at Dryad before final acceptance and all regulations set by the journal will be followed.

**References**

Addison, A., (2017, January 27). *Red-tailed Hawk (Buteo jamaicensis)* [XC401311]. https://www.xeno-canto.org/401311

Anonymous. 2012. Guidelines for the treatment of animals in behavioural research and teaching. Anim Behav 83:301-309.

Arnqvist G, Henriksson S. 1997. Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. Evol Ecol 11:255-273.

Baldwin JM. 1896. A new factor in evolution. The American Naturalist 30:441-451.

Bartholomew GA. 1964. The roles of physiology and behaviour in the maintenance of homeostasis in the desert environment. Symp Soc Exp Biol 18:7-29.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Statistical Software, 67:1-48. doi:10.18637/jss.v067.i01.

Baudains TP, Lloyd P. 2007. Habituation and habitat changes can moderate the impacts of human disturbance on shorebird breeding disturbance. Animal Conservation 10: 400-407.

Blumstein DT. 2003. Flight-initiation distance in birds is dependent on intruder starting distance J Wildlife Management 67: 852-857.

Blumstein DT, Fernández-Juricic E, Zollner PA, Garity SC. 2005. Inter-specific variation in avian responses to human disturbance. J Applied Ecology 42:943-953.

Bohls P, Koehnle TJ. 2017. Responses of eastern gray squirrels (*Sciurus carolinensis*) to predator calls and their modulation by coat color. The American Midland Naturalist 178:226-236.

Bókony V, Kulcsár A, Tóth Z, Liker A. 2012. Personality traits and behavioral syndromes in differently urbanized populations of house sparrows (*Passer domesticus*). PLoS One 7:e36639.

Bowers MA, Breland B. 1996. Foraging of gray squirrels on an urban-rural gradient: use of the GUD to assess anthropogenic impact. Ecological Applications 6:1135-1142.

Carrete M, Tella JL. 2010. Individual consistency in flight initiation distances in burrowing owls: a new hypothesis on disturbance-induced habitat selection. Biol Lett 6:167-170.

Carrete M, Tella JL. 2017. Behavioral correlations associated with fear of humans differ between rural and urban burrowing owls. Frontiers in Ecology and Evolution 5:54.

Cavalli M, Baladrón AJ, Isacch JP, Biondi LM, Bó MS. 2016. Differential risk perception of rural and urban Burrowing Owls exposed to humans and dogs. Behavioural Processes 124:60-65.

Center for International Earth Science Information Network - CIESIN - Columbia University. (2018). *Gridded Population of the World, Version 4 (GPWv4): Population Density, Revision 11*. NASA Socioeconomic Data and Applications Center (SEDAC). https://doi.org/https://doi.org/10.7927/H49C6VHW

Chartier A (2008, June 24). *Red-tailed Hawk (Buteo jamaicensis borealis)*. [XC31161]. https://www.xeno-canto.org/31161

Coleman A, Richardson D, Schechter R, Blumstein DT. 2008. Does habituation to humans influence predator discrimination in Gunther's dik-diks (*Madoqua guentheri*)? Biol Lett 4:250-252.

Cooke AS. 1980. Observations on how close certain passerine species will tolerate an approaching human in rural and suburban areas. Biol Cons 18:85-88.

Cooper CA, Neff AJ, Poon DP, Smith GR. 2008. Behavioral responses of eastern gray squirrels in suburban habitats differing in human activity levels. Northeastern Naturalist 15:619-625.

Cooper WE, Frederick WG. 2007. Optimal flight initiation distance. J Theoretical Biology 244:59–67. https://doi.org/10.1016/j.jtbi.2006.07.011

Crooks KR. 2002 Relative sensitivities of mammalian carnivores to habitat fragmentation. Cons Biol 16:488-502.

Dill LM, Houtman R. 1989. The influence of distance to refuge on flight initiation distance in the gray squirrel (*Sciurus carolinensis*). Can J Zool 67:233–235. https://doi.org/10.1139/z89-033

Ditchkoff SS, Saalfeld ST, Gibson CJ. 2006. Animal behavior in urban ecosystems: modifications due to human-induced stress. Urban Ecosyst 9:5-12.

Dochtermann NA. 2011. Testing Cheverud's conjecture for behavioral correlations and behavioral syndromes. Evolution 65:1814-1820.

Engelhardt SC, Weladji RB. 2011. Effects of levels of human exposure on flight initiation distance and distance to refuge in foraging eastern gray squirrels (*Sciurus carolinensis*). Can J Zool 89:823-830.

Evans J, Boudreau K, Hyman J. 2010. Behavioural syndromes in urban and rural populations of song sparrows. Ethology 116:588-595.

Fernández-Juricic E, Schroeder N. 2003. Do variations in scanning behavior affect tolerance to human disturbance? Applied Animal Behaviour Science 84:219-234.

Fitzgerald LA, Stronza AL. 2016. In defense of the ecotourism shield: a response to Geffroy et al. Trends Ecol Evol 31:94.

Floyd, T. (2017a, January 2). *Black-capped Chickadee (Poecile atricapillus)*. [XC348981]. https://www.xeno-canto.org/348981

Floyd T. (2017b, January 25). *Black-capped Chickadee (Poecile atricapillus)*. [XC352827]. https://www.xeno-canto.org/352827

Geffroy B, Samia DSM, Bessa E, Blumstein DT. 2015. How nature-based tourism might increase prey vulnerability to predators. Trends Ecol Evol 30:755-765.

Gustafson EJ, VanDruff LW. 1990. Behavior of black and gray morphs of *Sciurus carolinensis* in an urban environment. The American Midland Naturalist 123:186-192.

Guttman N, Kalish HI. 1956. Discriminability and stimulus generalization. J Exp Psych 51:79-88.

Hinde RA. 1954. Factors governing the changes in strength of a partially inborn response, as shown by the mobbing behaviour of the chaffinch (*Fringilla coelebs*). II. The waning of the response. Proc Roy Soc B 142:331-358.

Johnson MTJ, Munshi-South J. 2017. Evolution of life in urban environments. Science 358:607. https://doi.org/10.1126/science.aam8327

Jokimäki J, Selonen V, Lehikoinen A, Kaisanlahti-Jokimäki M-L. 2017. The role of urban habitats in the abundance of red squirrels (*Sciurus vulgaris*, L.) in Flnland. Urban Forestry & Urban Greening 27:100-108.

Koprowski JL. 1994. *Sciurus niger*. Mammalian Species 479:1–9. https://doi.org/10.2307/3504263

Korschgen LJ. 1981. Foods of fox and gray squirrels in Missouri. J Wildlife Management 45:260-266.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. lmerTest package: tests in linear mixed effects models. Journal of Statistical Software 82:1-26. doi: 10.18637/jss.v082.i13

Labra A, Leonard R. 1999. Intraspecific variation in antipredator responses of three species of lizards (*Liolaemus*): possible effects of human presence. J Herpetol. 33:441-448.

Lapiedra O, Chejanovski Z, Kolbe JJ. 2017. Urbanization and biological invasion shape animal personalities. Global Change Biology 23:592-603.

Lenth R. 2020. emmeans: estimated marginal means, aka least-squares means. R package version 1.5.2-1. https://CRAN.R-project.org/package=emmeans

Lilly MV, Lucore EC, Tarvin KA. 2019. Eavesdropping grey squirrels infer safety from bird chatter. PLoS One 14:e0221279.

Lima SL, Dill L. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68:610-640.

Loss SR, Will T, Marra PP. 2013. The impact of free-ranging domestic cats on wildlife of the United States. Nature Communications 4:1396.

Lowry H, Lill A, Wong BBM. 2013. Behavioural responses of wildlife to urban environments. Biological Reviews 88:537–549. https://doi.org/10.1111/brv.12012

McCleery RA. 2009. Changes in fox squirrel anti-predator behaviors across the urban-rural gradient. Landscape Ecology 24:483–493. https://doi.org/10.1007/s10980-009-9323-2

McCleery RA, Lopez RR, Silvy NJ, Gallant DL. 2008. Fox squirrel survival in urban and rural environments. Journal of Wildlife Management 72:133–137. https://doi.org/10.2193/2007-138

Møller AP. 2008. Flight distance of urban birds, predation, and selection for urban life. Behav Ecol Sociobiol 63:63-75.

Møller AP. 2012. Urban areas as refuges from predators and flight distance of prey. Behav Ecol 23:1030-1035.

Myers RE, Hyman J. 2016. Differences in measures of boldness even when underlying behavioral syndromes are present in two populations of the song sparrow (*Melospiza melodia*). J Ethol 34:197-206.

Olson JK, Acevedo-Gutiérrez A. 2017. Influence of human exposure on the anti-predator response of harbor seals (*Phoca vitulina*). Aquatic Mammals 43:673-681.

Place A. (2015, September). *Black-capped Chickadee (Poecile atricapillus)*. [XC289220]. https://www.xeno-canto.org/289220

Price TD, Qvarnström A, Irwin DE. 2003. The role of phenotypic plasticity in driving genetic evolution. Proc Roy Soc B 270:1433-1440.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton D, Colombo J, Coppola G, Geyer MA, Glanzman DL, Marsland S, McSweeney F, Wilson DA, Wu C-F, Thompson RF. 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol Learn Mem 92:135-138.

Rodriguez-Prieto I, Ferndández-Juricic E, Martin J, Regis Y. 2009. Antipredator behavior in blackbirds: habituation complements risk allocation. Behav Ecol 20:371-377.

Ryan AM, Partan SR. 2014. Urban wildlife behavior. In: McCleery RA, Moorman CE, Peterson MN, editors. Urban Wildlife Conservation. New York, USA: Springer p. 149-173.

Samia DSM, Nakagawa S, Nomura F, Rangel TF, Blumstein DT. 2015. Increased tolerance to humans among disturbed wildlife. Nat Comm 6:8877.

Sarno RJ, Parsons M, Ferris A. 2015. Differing vigilance among gray squirrels (*Sciuridae carolinensis*) along an urban-rural gradient on Long Island. Urban Ecosyst 18:517-523.

Scales J, Hyman J, Hughes M. 2011. Behavioral syndromes break down in urban song sparrow populations. Ethology 117:887-895.

Seress G, Bókony V, Heszberger J, Liker A. 2011. Response to predation risk in urban and rural house sparrows. Ethology 117:896-907.

Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: An ecological and evolutionary overview. Trends Ecol Evol 19:372–378. https://doi.org/10.1016/j.tree.2004.04.009

Simion D. (2016, August 1). *German Shepard Sound* [Audio]. http://soundbible.com/2146-German-Shepard.html

Simion D. (2017, May 12). *Doberman Pinscher Sound* [Audio]. http://soundbible.com/2194-Doberman-Pinscher.html

Simion D. (2018, May 4). *Labrador Barking Dog Sound* [Audio]. http://soundbible.com/2215-Labrador-Barking-Dog.html

Snell-Rood EC. 2013. An overview of the evolutionary causes and consequences of behavioural plasticity. Anim Behav 85:1004-1011.

Sol D, Lapiedra O, González-Lagos C. 2013. Behavioural adjustments for a life in the city. Anim Behav 85:1101-1112.

Sprau P, Dingemanse NJ. 2017. An approach to distinguish between plasticity and non-random distributions of behavioral types along urban gradients in a wild passerine bird. Front Ecol Evol 5:92.

Thompson RF, Spencer WA. 1966. Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychological Review 73:16-43.

Tumlison R. 2012. Unusual foraging tactics by a red-tailed hawk in an urban environment. Wilson J Ornithol 124:818-820.

Tuomainen U, Candolin U. 2011. Behavioural responses to human-induced environmental change. Biol Rev 86:640-657.

Uchida K, Suzuki KK, Shimamoto T, Yanagawa H, Koizumi I. 2017. Escaping height in a tree represents a potential indicator of fearfulness in arboreal squirrels. Mammal Study 42:39-43.

Uchida K, Suzuki KK, Shimamoto T, Yanagawa H, Koizumi I. 2019. Decreased vigilance or habituation to humans? Mechanisms on increased boldness in urban animals. Behav Ecol, 30:1583–1590. https://doi.org/10.1093/beheco/arz117

Uchida K, Shimamoto T, Yanagawa H, Kolzumi I. 2020. Comparison of multiple behavioral trials between urban and rural squirrels. Urban Ecosyst 23:745-754.

Vincze E, Papp S, Preiszner B, Seress G, Bókony V, Liker A. 2016. Habituation to human disturbance is faster in urban than rural house sparrows. Behav Ecol 27:1304-1313.

Vincze E, Pipoly I, Seress G, Preiszner B, Papp S, Németh B, Liker A, Bókony V. 2019. Great tits take greater risk toward humans and sparrowhawks in urban habitats than in forests. Ethology 125:686-701.

Wauters LA, Somers L, Dhondt AA. 1997. Settlement behaviour and population dynamics of reintroduced red squirrels *Sciurus vulgaris* in a park in Antwerp, Belgium. Biological Conservation 82:101-107.

Weaver M, Ligon RA, Mousel M, McGraw KJ. 2018. Avian anthrophobia? Behavioral and physiological responses of house finches (*Haemorhous mexicanus*) to human and predator threats across an urban gradient. Landscape and Urban Planning 179:46-54.

Webster, M.M. & Rutz, C. (2020) How STRANGE are your study animals? Nature 582, 337-340.

West-Eberhard MJ. 1989. Phenotypic plasticity and the origins of diversity. Ann Rev Ecol Syst 20:249-278.

Wheat RE, Wilmers CC. 2016. Habituation reverses fear-based ecological effects in brown bears (*Ursus arctos*). Ecosphere 7:e01408.

Wilson T. (2010, May 29). *Red-tailed Hawk (Buteo jamaicensis)* [XC53797]. https://www.xeno-canto.org/53797

Ydenberg RC, Dill LM. 1986. The economics of fleeing from predators. Advances in the Study of Behavior, 16:229–249.

Zuur AF, Ieno EN, Elphick CS. 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3-14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

**Table 1.** Behavioral data from fox squirrels were collected at six sites around Ann Arbor, Michigan, USA located in areas that were classified as urban or less urban. Urban sites were in areas with human population densities of greater than 1000 persons/km$^2$ whereas less urban sites were in areas with population densities of 250 – 1000 persons/km$^2$ (County Farm Park, Nichols Arboretum) or fewer than 250 persons per square km (Saginaw Forest). Population density estimates obtained from the Center for International Earth Information Network of Columbia University (2018). Total days of observation and hours over these days are provided. When we visited the sites, human and dog presence was quantified (see methods) with mean and 1 standard deviation (SD) shown below.

| Site Name | GPS Location of Site | Site Category | Population Density (persons/ sq km) | Human Presence | | Dog Presence | | Total Trials | Total Observation Days | Total Observation Hours |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mean Count | SD | Mean Count | SD | | | |
| Central Campus | N 42°16.622' W083°44.293' | Urban | > 1000 | 97.7 | 7.3 | 0.39 | 0.61 | 33 | 6 | 9.68 |
| North Campus | N 42°17.753' W083°42.620' | Urban | > 1000 | 68.0 | 11.4 | 0 | 0 | 25 | 4 | 6.18 |
| Prospect Park | N 42°14.825' W083°36.093' | Urban | > 1000 | 3.6 | 10.2 | 0.69 | 1.55 | 23 | 4 | 6.08 |
| County Farm Park | N 42°15.430' W083°42.518' | Less urban | 250 - 1000 | 1.6 | 1.3 | 0.41 | 0.50 | 32 | 5 | 8.73 |
| Nichols Arboretum | N 42°16.940' W083°43.410' | Less urban | 250 - 1000 | 11.9 | 2.3 | 2.32 | 1.19 | 28 | 5 | 9.07 |
| Saginaw Forest | N 42°16.235' W083°48.465' | Less urban | 25 - 250 | 0.37 | 0.50 | 0.20 | 0.41 | 30 | 6 | 12.62 |

**Table 2.** Differences between fox squirrels at urban and less urban sites that were not exposed to any playbacks for first alert distance (FAD), flight initiation distance (FID), probability of escaping the observer by climbing a tree during the trial, and latency to resume behavior following the trial. A random effect for site identity was included in the model or FAD ($\sigma^2$ = 6.9), FID ($\sigma^2$ = 6.24), probability of climbing a tree ($\sigma^2$ = 1.23), and latency ($\sigma^2$ = 1.22). Latency was ln+1 transformed. Results are from 77 trials from six sites.

| Response Variable | Variable | *b* | SE | *t* or *z* | *P* |
|---|---|---|---|---|---|
| First alert distance (FAD) | Intercept (Less urban, Female) | 14.11 | 1.63 | 8.67 | 0.0007 |
| | Site (Urban) | -4.79 | 2.26 | -2.11 | 0.10 |
| | Distance to tree | -0.37 | 0.38 | -0.98 | 0.33 |
| | Sex (Male) | 1.17 | 0.66 | 1.77 | 0.08 |
| | Julian Date | 0.15 | 0.40 | 0.39 | 0.70 |
| | Initial Distance | 2.63 | 0.35 | 7.51 | <0.0001 |
| Flight initiation distance (FID) | Intercept (Less urban, Female) | 12.16 | 1.54 | 7.89 | 0.0009 |
| | Site (Urban) | -6.22 | 2.14 | -2.90 | 0.043 |
| | Distance to tree | -0.56 | 0.35 | -1.59 | 0.12 |
| | Sex (Male) | 0.85 | 0.61 | 1.38 | 0.17 |
| | Julian Date | 0.01 | 0.37 | 0.03 | 0.97 |
| | Initial Distance | 1.38 | 0.32 | 4.24 | <0.0001 |
| Probability of climbing a tree | Intercept (Less urban, Female) | 0.71 | 0.79 | 0.89 | 0.37 |
| | Site (Urban) | -1.89 | 1.12 | -1.68 | 0.09 |
| | Distance to tree | -0.09 | 0.32 | -0.27 | 0.78 |
| | Sex (Male) | 0.17 | 0.55 | 0.31 | 0.76 |
| | Julian Date | 0.47 | 0.35 | 1.34 | 0.18 |
| | Initial Distance | 0.23 | 0.30 | 0.76 | 0.45 |
| Latency to resume behavior | Intercept (Less urban, Female) | 4.48 | 0.67 | 6.70 | 0.002 |
| | Site (Urban) | -1.87 | 0.93 | 1.99 | 0.11 |

| | | | | |
|---|---|---|---|---|
| Distance to tree | -0.07 | 0.13 | -0.57 | 0.57 |
| Sex (Male) | 0.06 | 0.23 | 0.25 | 0.80 |
| Julian Date | 0.07 | 0.14 | 0.52 | 0.61 |
| Initial Distance | 0.002 | 0.12 | 0.02 | 0.98 |

**Table 4.** Associations between latency to resume behavior following the trial ("Latency") and first alert distance (FAD), flight initiation distance (FID), and probability of escaping the human observer by climbing a tree during the trial ("Went up tree?") in fox squirrels at urban and less urban sites that were not exposed to any playbacks. A random effect for site identity was included in the model or FAD ($\sigma^2 = 0.02$), FID ($\sigma^2 = 0.08$), and probability of climbing a tree ($\sigma^2 = 1.03$). FAD, FID, and latency were ln+1 transformed. Results are from 77 trials from six sites.

| Response Variable | Variable | *b* | SE | *t* or *z* | *P* |
|---|---|---|---|---|---|
| First alert distance (FAD) | Intercept (Urban, Female) | 2.42 | 0.10 | 24.2 | 0.0004 |
| | Site (Less urban) | 0.27 | 0.14 | 1.91 | 0.16 |
| | Latency | 0.25 | 0.08 | 3.07 | 0.005 |
| | Went up tree? | -0.10 | 0.07 | -1.39 | 0.17 |
| | Site (Less urban) x Latency | -0.23 | 0.12 | -2.01 | 0.053 |
| | Site (Less urban) x Went up tree? | 0.13 | 0.09 | 1.41 | 0.16 |
| Flight initiation distance (FID) | Intercept (Urban, Female) | 1.94 | 0.18 | 11.0 | 0.0005 |
| | Site (Less urban) | 0.63 | 0.25 | 2.54 | 0.067 |
| | Latency | 0.16 | 0.08 | 1.86 | 0.067 |
| | Went up tree? | -0.08 | 0.07 | -1.18 | 0.24 |
| | Site (Less urban) x Latency | -0.14 | 0.12 | -1.17 | 0.24 |
| | Site (Less urban) x Went up tree? | 0.06 | 0.09 | 0.70 | 0.48 |
| Latency to resume behavior | Intercept (Urban, Female) | 2.77 | 0.61 | 4.56 | 0.01 |
| | Site (Less urban) | 1.61 | 0.86 | 1.88 | 0.13 |
| | Went up tree? | 0.43 | 0.21 | 2.08 | 0.041 |
| | Site (Less urban) x Went up tree? | 0.01 | 0.26 | 0.05 | 0.96 |

**Table 4.** Effects of acoustic playbacks (control, hawk, dog) on first alert distance (FAD), flight initiation distance (FID), probability of escaping human observer by climbing a tree, and latency to resume behavior following the trial for fox squirrels observed at urban or less urban sites. Reference (intercept) was "less urban" for site, "control playback" for treatment, and "female" for sex. A random effect for site identity was included in the model or FAD ($\sigma^2 = 6.5$), FID ($\sigma^2 = 4.9$), probability of climbing a tree ($\sigma^2 = 0.07$), and latency ($\sigma^2 = 0.37$). Latency was ln+1 transformed. Results are from 94 trials from six study sites.

| Response Variable | Variable | *b* | SE | *t* or *z* | *P* |
|---|---|---|---|---|---|
| First alert distance (FAD) | Intercept (Less urban, Female, Control) | 14.94 | 0.89 | 16.71 | <0.0001 |
| | Site (Urban) | -2.32 | 1.33 | -1.74 | 0.11 |
| | Dog playbacks | 0.51 | 0.90 | 0.57 | 0.57 |
| | Hawk playbacks | -0.29 | 0.98 | -0.30 | 0.76 |
| | Distance to tree | 0.36 | 0.29 | 1.23 | 0.22 |
| | Sex (Male) | 0.29 | 0.58 | 0.51 | 0.61 |
| | Julian Date | -0.10 | 0.28 | -0.34 | 0.73 |
| | Initial Distance | 3.20 | 0.29 | 10.84 | <0.0001 |
| | Site (Urban) x Dog playbacks | 0.07 | 1.35 | 0.05 | 0.96 |
| | Site (Urban) x Hawk playbacks | 4.29 | 1.49 | 2.86 | 0.005 |
| Flight initiation distance (FID) | Intercept (Less urban, Female, Control) | 12.3 | 0.95 | 7.31 | <0.0001 |
| | Site (Urban) | -1.64 | 1.39 | -1.18 | 0.27 |
| | Dog playbacks | 1.89 | 0.79 | 2.40 | 0.018 |
| | Hawk playbacks | 1.21 | 0.85 | 1.42 | 0.16 |
| | Distance to tree | 0.08 | 0.26 | 0.30 | 0.76 |
| | Sex (Male) | 0.01 | 0.50 | 0.01 | 0.99 |
| | Julian Date | -0.39 | 0.25 | -1.59 | 0.11 |
| | Initial Distance | 2.99 | 0.26 | 11.51 | <0.0001 |
| | Site (Urban) x Dog playbacks | -1.52 | 1.18 | -1.29 | 0.20 |

| | | | | | |
|---|---|---|---|---|---|
| | Site (Urban) x Hawk playbacks | 2.53 | 1.30 | 1.94 | 0.056 |
| Probability of climbing a tree | Intercept (Less urban, Female, Control) | -0.20 | 0.62 | -0.33 | 0.74 |
| | Site (Urban) | -0.61 | 0.92 | -0.66 | 0.51 |
| | Dog playbacks | 1.14 | 0.82 | 1.39 | 0.16 |
| | Hawk playbacks | 1.26 | 0.90 | 1.40 | 0.16 |
| | Distance to tree | -0.64 | 0.27 | -2.34 | 0.02 |
| | Sex (Male) | 0.61 | 0.55 | 1.10 | 0.27 |
| | Julian Date | -0.26 | 0.27 | -0.97 | 0.33 |
| | Initial Distance | 0.01 | 0.27 | 0.04 | 0.96 |
| | Site (Urban) x Dog playbacks | -1.52 | 1.20 | -1.27 | 0.20 |
| | Site (Urban) x Hawk playbacks | 0.18 | 1.34 | 0.14 | 0.89 |
| Latency to resume behavior | Intercept (Less urban, Female, Control) | 3.83 | 0.39 | 9.69 | 0.0001 |
| | Site (Urban) | -0.60 | 0.57 | -1.05 | 0.33 |
| | Dog playbacks | 1.14 | 0.23 | 4.91 | <0.0001 |
| | Hawk playbacks | 1.71 | 0.25 | 6.76 | <0.0001 |
| | Distance to tree | 0.03 | 0.07 | 0.39 | 0.70 |
| | Sex (Male) | 0.24 | 0.15 | 1.61 | 0.11 |
| | Julian Date | 0.07 | 0.07 | -0.91 | 0.36 |
| | Initial Distance | 0.06 | 0.08 | 0.82 | 0.41 |
| | Site (Urban) x Dog playbacks | 0.03 | 0.35 | 0.10 | 0.92 |
| | Site (Urban) x Hawk playbacks | 0.22 | 0.39 | 0.57 | 0.57 |

**Figures and Figure Legends**

**Figure 1.** Variation in A) vigilance (first alert distance: FAD), B) anti-predator behavior (flight initiation distance: FID), C) probability of escaping up a tree during the trial, and D) latency to resume behavior following the trial among fox squirrels at urban (n = 38 trials) and less urban (n = 39) sites in trials where squirrels were not exposed to any playbacks. Squirrels in urban areas had significantly shorter FID compared to those in the less urban sites, but there were no other significant differences (Table 2). Upper and lower hinges correspond to first and third quartile, respectively. Upper/lower whiskers extend from the hinge to the highest/lowest value that is within 1.5x the interquartile range. Solid horizontal line shows median.

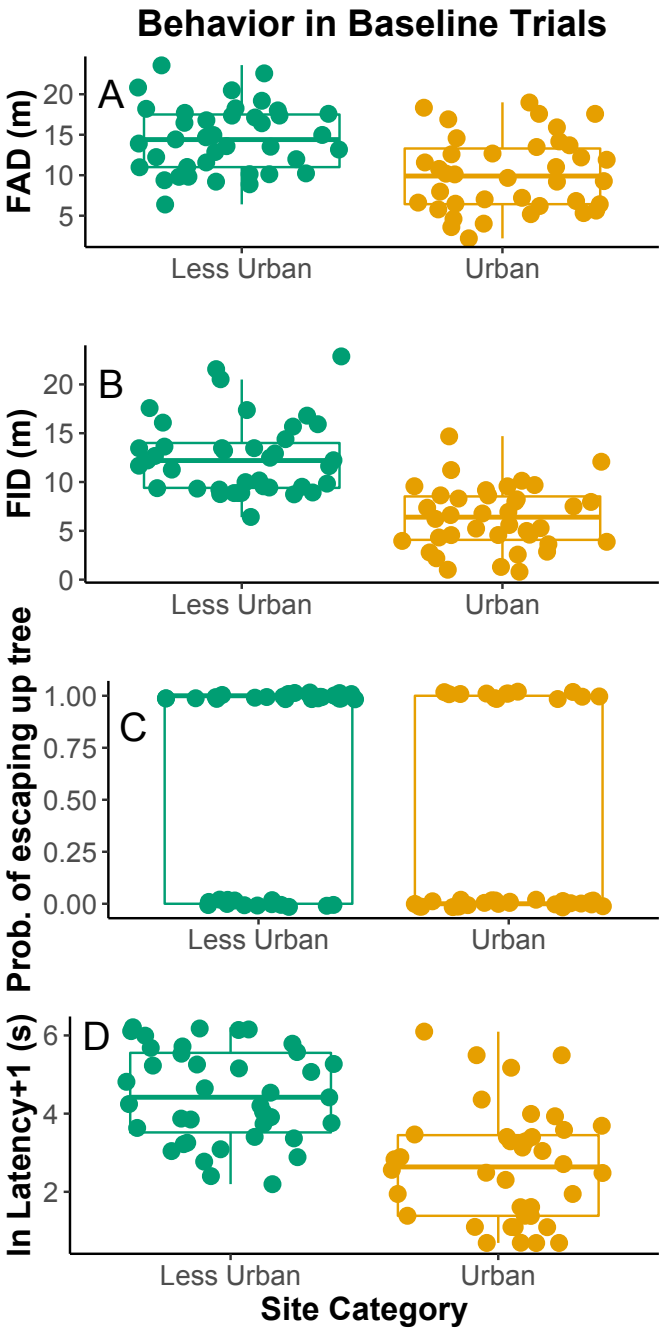


**Figure 2.** Associations between the latency of fox squirrels in urban and less urban study areas to resume typical behavior after a disturbance trial with A) first alert distance (FAD), B) flight initiation distance (FID), and C) probability of escaping the human observer by climbing up a tree. FAD and latency to resume typical behavior were significantly positively associated in urban squirrels but not those in less urban areas. In squirrels in both urban and less urban areas, those that had a higher FID or climbed a tree also took longer to resume typical behavior. differed between urban and less urban populations of squirrels. Note that latency is ln+1 transformed.

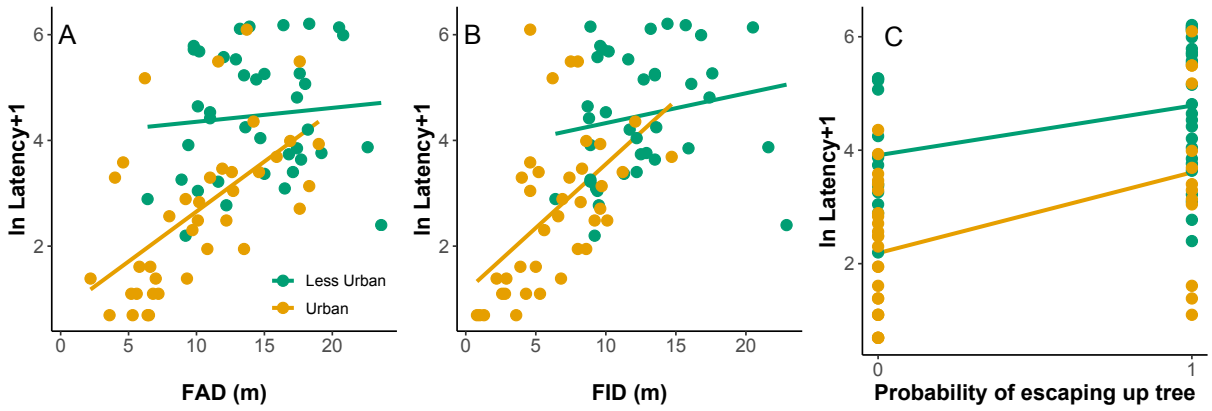
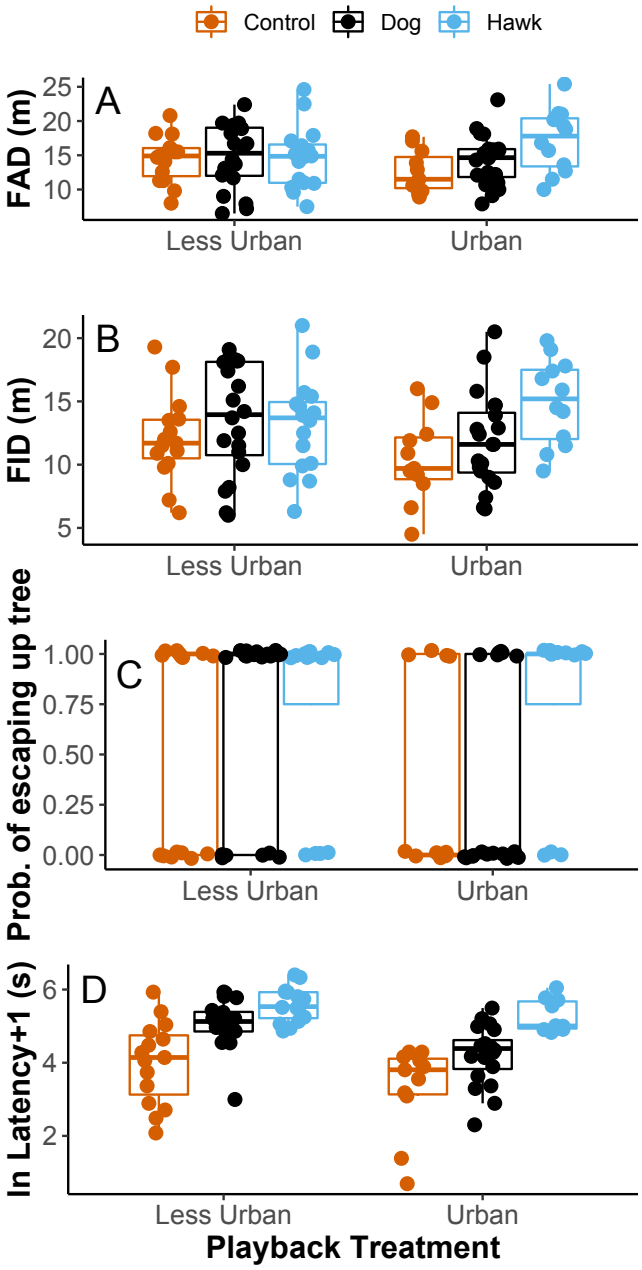


**Figure 3.** Effects of human observer approaching a squirrel while broadcasting one of three playback treatments (control playback, hawk or dog vocalizations) on A) first alert distance (FAD), B) flight initiation distance (FID), C) probability of escaping up a tree during the trial, and D) latency to resume typical behavior following the trial. Trials were conducted at less urban (n = 15 control, 20 dog, 16 hawk) and urban (n = 11 control, 20 dog, 12 hawk) sites. Results shown in Table 4. Upper and lower hinges correspond to first and third quartile, respectively. Upper/lower whiskers extend from the hinge to the highest/lowest value that is within 1.5x the interquartile range. Solid horizontal line shows median.



**Appendix I**

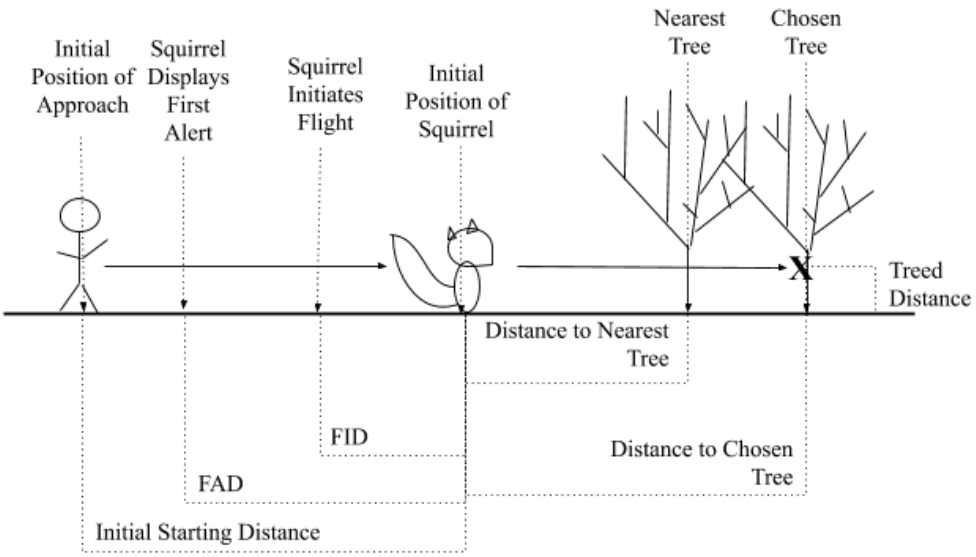



**Figure S1.** Overview of trial protocol. A marker was placed at the initial position of the observer when they initially identified a squirrel for a trial. The distance between observer initial position and the squirrel's initial position is the initial distance. From this position, the observer walked at a slow, steady, and constant pace in a direct line towards the squirrel. The observer then dropped a marker to represent FAD when the squirrel first displayed "alert" behavior (freezing with eye contact). An additional marker was dropped when the squirrel fled from its initial position to represent FID. Another marker was placed at an estimate of the squirrel's initial position. In playback manipulation trials, acoustic stimuli were broadcast when the observer started approaching the squirrel and stopped playing the stimuli when the squirrel fled. Distance from the squirrel's initial position to the nearest tree was recorded along with how high in the tree the squirrel took refuge. If the squirrel took refuge in a tree other than the one nearest, then distance to the chosen tree was recorded. Once the squirrel took refuge latency to resume normal activity (foraging or traveling off tree) was recorded. Trials ended when squirrels resumed normal activity or if latency exceeded 10 min.