

Effects of urbanization on the evolution of coat color in eastern gray squirrels
(Sciurus carolinensis)

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Honors in

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Table of Contents

	Page
Table of Contents.....	2
Abstract.....	3
Introduction.....	4
Methods.....	8
Experimental design and photography.....	8
Measuring detection time: online game.....	10
Objective analysis of luminance.....	12
Statistical analyses.....	13
Results.....	15
Discussion.....	16
Tables.....	21
Figures.....	23
Appendix A.....	31
Acknowledgements.....	34
References.....	35

Abstract

Urbanization causes many ecological changes that can act as strong selection pressures on organisms and lead to evolutionary change. There are two common, genetically-based, color morphs of the eastern gray squirrel (*Sciurus carolinensis*), gray and melanic (black). Prior to European settlement, the melanic morph dominated rural forests, but today are most prevalent in cities. The objective of this study was to investigate possible causes of natural selection in urban and rural areas (secondary and old growth forests) that could explain the decline of the melanic morph in rural forests and its abundance in cities. Human detection time of each morph in secondary forests, old growth forests and on roads was measured using a web based-hidden object game, and the degree of luminance match to immediate tree and road backgrounds was quantified for each morph using just noticeable difference values. Participants took longer to detect the gray morph in secondary forests and marginally longer to detect the melanic morph in old growth forests, whereas on roads the melanic morph was easiest to detect. Melanics had the greatest luminance contrast to immediate tree and road backgrounds in all three environments, suggesting their more conspicuous appearance on trees and roads than the gray morph. My study suggests that urbanization and the change in forest structure in rural areas, from secondary to old growth forests, affects the way crypsis functions for the eastern gray squirrel, as well as causes divergent natural selection for melanism along urbanization gradients.

Introduction

Urban areas are the most rapidly expanding type of land cover worldwide, with more than half of the human population living in cities (Johnson et al. 2015, Ratcliffe et al. 2016). In the United States, the percentage of people living in cities has increased from 46% to 81% over the last century (United Nations 2018). Urbanization causes significant environmental change, such as increased air and ground surface temperatures, altered hydrology and biogeochemical cycles, increased noise and light pollution and increased atmospheric concentrations of greenhouse gases (CO₂, CH₄, and O₃) (Grimm et al. 2008). Cities lack natural vegetation and are dominated by impervious surfaces such as roads, sidewalks and driveways, leading to habitat fragmentation and contamination of local streams, rivers and lakes by runoff (Grimm et al. 2008, Donihue and Lambert 2014). Urbanization also alters species composition, introducing nonnative species and affecting native species richness (Grimm et al. 2008, McKinney 2008).

The dramatic environmental changes associated with urbanization tend to cause population declines of native plants and animals (McKinney 2008). For example, urban areas have only 8% of the density of native birds and 25% of the density of native plants compared to rural areas (Aronson et al. 2014). Yet some species do persist in cities, and these organisms often show phenotypic variation between populations in urban and rural areas (Alberti et al 2017). This raises the question of how urbanization can cause phenotypic differences between urban and rural populations.

The ecological changes associated with urbanization can act as strong selection pressures on organisms, impacting an individual's survival and reproduction, and ultimately lead to adaptive trait evolution (Johnson and Munshi-South 2017). Urban areas

have many novel environmental features that can cause novel selection. For example, impervious surfaces are a novel selection pressure on Puerto Rican tropical lizards (*Anolis cristatellus*) in urban areas. Urban lizard populations have evolved longer limbs and more sub-digital lamellae, allowing them to run faster on artificial substrates and better survive in urban areas, as compared to rural lizards with shorter limbs and less sub-digital lamellae (Winchell et al. 2014, Winchell et al. 2018). Some selection pressures can also become relaxed or weaker in cities (Lahti et al. 2009), such as predation due to predators using novel food subsidies (e.g., trash) and thus reducing predation pressure on prey (Fischer et al. 2012). For instance, in cities natural selection is relaxed on plumage color in Northern Cardinals (*Cardinalis cardinalis*), whereas in rural areas selection on plumage color persists as individual reproductive success declines with plumage brightness (Rodewald et al. 2011).

My research objective was to investigate the effects of urbanization on the evolution of coat color in the eastern gray squirrel (*Sciurus carolinensis*; hereafter “gray squirrel”), a tree squirrel common to forests and cities in the eastern United States and southeastern Canada (Koprowski et al. 2016). There are two common color morphs, gray and black (melanic). Melanism in gray squirrels is a simple Mendelian trait coded by a 24-bp deletion at the melanocortin-1 receptor gene (*Mclr*), which regulates production of eumelanin and pheomelanin (McRobie et al. 2009). Expression of two wild-type alleles ($E^+ E^+$) at *MclR* give rise to the gray phenotype (suppressed eumelanin production), two mutant alleles ($E^B E^B$) produces a jet black phenotype (eumelanin overproduction), and one of each allele ($E^+ E^B$) produces a black-brownish phenotype (intermediate eumelanin

production; McRobie et al. 2009). The 24-bp deletion in gray squirrels has a phylogenetic origin in fox squirrels (*Sciurus niger*), another tree squirrel species (McRobie et al. 2019).

Although the proximate genetic mechanisms and phylogenetic origin of melanism have been widely studied in gray squirrels, less is known about its adaptive function. Prior to European settlement in the 18th century, the melanic morph was more common than the gray morph in rural forests at northern latitudes (Schorger 1949, Robertson 1973). Melanics have greater thermogenic capacities and experience lower rates of heat loss in cold conditions, therefore they likely have a thermal advantage in cold climates, which may explain their historical abundance in northern parts of the species range (Ducharme et al. 1989). Following European settlement and agricultural expansion, the melanic morph declined in rural forests, and today they predominate mainly in cities (Gibbs et al. 2019). The change in forest structure from old growth to secondary forests (re-grown forests as a result of deforestation after European settlement) and changes in environmental conditions as a result of urbanization may have changed selection pressures on coat color.

Old growth forests that once dominated the landscape have high levels of structural complexity, including extensive vertical stratification and horizontal heterogeneity of vegetation, which create deep patches of shade (Spies and Franklin 1996, Franklin and Van Pelt 2004). Late successional confers with darker bark (e.g., hemlocks) persist in old growth forests, contributing to the overall darker appearance (Franklin and Van Pelt 2004, Thompson et al. 2013a). But during European settlement, cities grew and placed immense resource demands on surrounding rural forests, leading to the clearing of old growth forests. Forests have regrown in the northeastern United

States, but these secondary forests differ greatly in composition and structure from old growth forests (Thompson et al. 2013b). Secondary forests are typically even-aged stands with little vertical stratification of vegetation (less shading) and have trees with lighter bark, producing a lighter appearance compared to darker old growth forests (Franklin and Van Pelt 2004). The lighter appearance of secondary forests may be advantageous to the gray morph, as they would better match their background than melanics and be less likely to be attacked by predators, which is the dominant source of mortality for tree squirrels in rural forests (e.g., Bowers and Breland 1996, McCleery et al. 2008). Alternatively, the melanic morph may have been selected for in old growth forests due to a stronger match to a darker background than the gray morph.

A major predator of squirrels are human hunters. For instance, 197,172 gray squirrels were “harvested” during one hunting season (2017-2018) in one state alone (Illinois) (Williams et al. 2017). But in cities, for safety reasons, hunting is not allowed, consequently eliminating a major predator. Further, predation pressure from natural predators is also relaxed in cities and is likely replaced by vehicular collision as the most common cause of mortality for tree squirrels (McCleery et al. 2008). Although roads can be an important source of mortality and thus natural selection (e.g., cliff swallows; Brown and Bomberger Brown 2013), studies of road mortality often lack an evolutionary perspective (Brady and Richardson 2017). Animal color might be an important trait that affects how conspicuous animals appear on roads, which would affect the amount of time drivers have to slow down and avoid hitting them. Most drivers try to avoid hitting animals on roads, but often do not see them until it is too late and are unable to avoid a collision (Beckmann and Shine 2012). The darker coat of the melanic morph may be

advantageous in cities with extensive road networks as melanics would better stand out on roads making them easier to spot by drivers and less likely to experience vehicular collisions.

To better understand the role of urbanization in driving the evolution of coat color in eastern gray squirrels, I compared background matching and visual detection of melanic and gray morphs in old growth forests, secondary forests, and on roads. In order to do so, I measured the time it took human predators to visually detect each morph in each environment using an online hidden object game. Additionally, I quantified background matching by objectively measuring differences in luminance between each morph and their immediate background in each of the three environments.

Methods

Experimental design and photography

I took photos of squirrels in three secondary forests (Hanley Preserve, Seneca Falls, NY; Finger Lakes National Forest, Burdett, NY; Curry Woods, Trumansburg, NY) and two old growth forests (Smith Woods, Trumansburg, NY; Fischer Old Growth Forest, Ithaca, NY). Photos of squirrels on roads were taken in Geneva, NY. All photos were taken during the growing season in September 2019. Forest photos are of two taxidermic models, one gray and one melanic, in climbing poses on the base of a tree trunk (Fig. 1), whereas road photos are of two taxidermic models in running poses on the road (Fig. 2). Taxidermic models were standardized for size between morphs.

Photos were taken at 20 locations in secondary forests, 21 locations in old growth forests, and 20 locations on roads. Two photos were taken at each location: a photo at

close proximity for objective analysis of background matching and a more distant photo for measuring detection time of squirrels by humans. All photos were taken with the ProCamera application on an iPhone 8. Photos for objective analysis were taken as RAW files (DNG) with a gray standard (X-Rite ColorChecker Passport) to control for lighting conditions, enabling objective measurement of luminance (Troscianko and Stevens 2015). Distance photos for measuring detection time were taken in JPEG format. In all scenes, a tripod was used to stabilize the camera, and all photos were taken with remote shutter release. In forests, photos were taken on overcast or partly cloudy days to ensure minimal variation in lighting on tree trunks where squirrels were photographed. On roads, all photos were taken under sunny conditions.

In forests, a taxidermic model of each morph and the gray standard were affixed to the base of a tree trunk (Fig. 1A). The gray standard was placed 1.5 m above the ground, and morphs were placed 34 cm above and below the standard. Photos for objective analysis were taken 80 cm away from the tree with the tripod pointing away from the sun and centered on the standard. The camera was orientated vertically, parallel with the tree trunk. For camera settings, an ISO of 100 was used and remained consistent across the entire study. Focus, white balance, and shutter speed were set to automatic. This process was repeated a second time, where I switched the position of the morphs, to either above or below the gray standard.

To take forest photographs for the online game, both morphs and the gray standard remained on the tree in the same orientation as objective photos, but the tripod was moved back to a randomly-selected distance 10-20 m from the tree (Fig. 1C). The orientation of the camera was changed to a landscape view. When photographing, I tilted

the camera up in order to include the upper canopy and the squirrels in the same photo. Photos were taken from varying perspectives (i.e., not always centered on the squirrels) so that the squirrels would not be in identical positions between scenes. The camera settings were identical to those used for objective photographs.

Photographs for objective analysis of background matching on roads were taken approximately 88 cm above both models and the gray standard placed on the road in direct sunlight (Fig. 2A). Two photos were taken, switching the position of each morph on either side of the gray standard. For the online game, photographs of squirrels were taken in the middle of the road from a driver's perspective. I randomly assigned the orientation (facing left or right) and distance of squirrels from the camera (10-50 m; Fig 2B, C). Each squirrel was photographed separately, but in identical positions on the road.

Measuring detection time: online game

I created an online game to measure human detection time of taxidermic squirrel models on trees in secondary forests and old growth forests, and on roads. The online game was available to the general public over the internet. The game presented participants with a sequence of 36 images of squirrel models on trees and on roads, and participants were asked to detect (by clicking) the squirrel in each image.

I created forest distance images for each scene by using Adobe Photoshop to crop each squirrel from the base of the tree (Fig. 1C) to identical, unobstructed positions higher on the tree (Fig. 1D, E). I ensured the size of each morph within a scene had approximately the same number of pixels, and I flipped the orientation of one squirrel so that both squirrels were looking in identical directions. Separate images were created for each morph for each scene. Each image was cropped to 3800x1900 pixels, which

excluded the bottom of the photo where the original squirrel models and gray standard were located (Fig. 1D, E). The cropped region was the same for images of each scene to maintain identical positions of each morph. The resolution was adjusted to 1900x950 pixels with bilinear interpolation to minimize the size of each file and ensure rapid display online.

The online game was developed as an R Shiny application and deployed to <https://bcosentino.shinyapps.io/squirrelfinder/>. Data from the online game was collected during a 3-month period from November 21, 2019 to February 19, 2020. Before playing, participants were asked if they have played before (yes or no). Each participant was presented with 36 images. Each image included a single squirrel in a secondary forest, old growth forest, or road, and participants were asked to find the squirrel as fast as possible. I recorded whether the user located the squirrel within 15 sec, and if so, the time to click on the squirrel. I separated the sequence of 36 images into two phases. The first six photos constituted a “learning phase” to ensure participants had a balanced experience with each squirrel morph and environment (i.e., two photos of each environment and three photos of each squirrel morph). The remaining 30 photos were considered the “testing phase”. The testing phase consisted of 5 scenes each from secondary forests, old growth forests, and roads. I used a paired design when displaying photos in the game such that each scene was displayed separately with gray and melanic squirrel morphs (i.e., 3 environments x 5 scenes x 2 morphs = 30 photos). I employed a blocked design such that each sequence of three images included one scene of each environment, and photos of the same scene were never displayed back-to-back.

Objective analysis of luminance

I used the ‘multispectral image calibration and analysis toolbox’ (micaToolbox) in ImageJ to quantify the luminance match of each squirrel morph to their immediate background (tree or road; Troscianko and Stevens 2015a). I created one calibrated multispectral image for each scene. Photos were calibrated using the gray standard reflectance values, which normalized and scaled pixel values to known reflectance values of the standard. The calibrated multispectral images were displayed as linear color images and converted to a cone-catch model representing human vision. Human vision is the correct model for drivers on roads and a realistic model for predators of squirrels in forests (e.g., human hunters and other mammalian predators). A human cone catch model was created in micaToolbox using the chart-based cone-catch model, which combined standard reflectance values of the gray standard, human receptor outputs (CIE 1931 400-700 nanometers), and natural lighting illuminance (D65 400-700 nanometers). Finally, photos were converted to a CIELAB 32Bit color space which included a luminance channel (“L”) from 0 (black) to 100 (white).

In order to measure the average luminance of each morph and the immediate background (tree or road), I created regions of interest (ROIs) for each squirrel and the tree or road using ImageJ. For forests, I created ROIs outlining each squirrel and the tree, excluding areas where fur or bark overlapped the immediate edge of the squirrels and the tree (Fig. 1B). For roads, squirrel ROIs were created by tracing the dorsal trunk and tail of each squirrel, making sure that no road was included in either ROI (Fig. 2A). ROIs of the road background were created by merging two smaller ROIs in the photo, one ROI

above the gray standard (1025x600 pixels) and the other ROI between the squirrels (400x570 pixels), to standardize the size and location of road ROIs across all scenes.

I estimated just noticeable difference (JND) values to represent the difference in luminance between each squirrel and its immediate background (tree or road). Average luminance was calculated for each ROI using the “Pattern Colour and Luminance Measurement” tool in micaToolbox. Luminance bands were set to 2. Average luminance results were used to estimate JNDs between each squirrel and the tree or road, using the “Luminance JND Difference Calculator” tool in micaToolbox. A Weber fraction of 0.05 was used, a commonly used Weber fraction for human vision (Troscianko and Stevens 2015b).

Statistical analyses

Analyses of detection data were based on the testing phase for 239 unique participants who had not played the game before. The response variables included detection success (i.e., whether or not a participant found the squirrel) and detection time for each of the 30 images displayed during the testing phase to each participant. Detection times were log-transformed to maintain normality of errors. The experiment was a paired design to enable comparison of detection success and time between morphs within scenes, but exploratory analyses revealed a strong carryover effect of user experience on detection times (Appendix A). Detection times were consistently greater for the initial squirrel morph displayed in a scene, and this carryover effect was not washed out by the number of images displayed between morphs in a given scene (range = 2–29). Because user experience with a scene created a consistent source of bias, all

further analyses were based on only the first image displayed for each scene (i.e., a between-scenes design based on $n = 15$ scenes per participant).

I used linear mixed models to test how detection success and detection time were related to environment (old growth forest, secondary forest, road) and squirrel morph. Environment, morph, and the interaction between environment and morph were included as fixed effects in each model. I used a binomial error distribution for the model of detection success and a Gaussian error distribution for the model of log-transformed detection time. Each model included random effects of participant and scene identity. The models were fit using the *lme4* package (Bates et al. 2015) in R (R Core Team 2019), and I used *lmerTest* (Kuznetsova et al. 2017) for significance testing of the fixed effects with Satterthwaite's degrees of freedom. I used planned contrasts to compare detection probabilities (i.e., odds ratios) and detection times between morphs within each environment. I applied a Bonferroni correction method for three contrasts to maintain a comparison-wide type I error rate of 0.05. I used the *emmeans* package (Lenth 2020) to conduct contrasts.

For objective analysis of luminance, I used a linear mixed model to test how luminance JNDs were related to environment and squirrel morph. Environment, morph, and the interaction between environment and morph were included as fixed effects in the model. The model included the random effect of scene identity, and the model was fit using the *lme4* package (Bates et al. 2015). I used planned contrasts to compare JNDs between melanic and gray squirrels (melanic-gray) in all three environments. I used the Satterthwaite method for degrees of freedom, and I applied a Bonferroni correction method for three contrasts to maintain a comparison-wide type I error of 0.05

Results

There was a significant interaction effect between environment and morph on the probability that participants found the squirrel in the online game (Likelihood ratio test; $\chi^2 = 114.55$, $df = 2$, $P < 0.001$). Participants were more likely to find the melanic than gray morph in secondary forests (log odds ratio = 1.67, $SE = 0.22$, $z = 7.60$, $P < 0.001$) and on roads (log odds ratio = 3.32, $SE = 0.37$, $z = 8.89$, $P < 0.001$), whereas detection probability was greater for the gray than melanic morph in old growth forests (log odds ratio = -0.50, $SE = 0.21$, $z = -2.42$, $P = 0.047$) (Figs. 3, 4).

There was a significant interaction effect between environment and morph on detection time (Table 1). Participants took longer to find the gray than melanic morph in secondary forests (difference = -0.45 sec, $SE = 0.14$, $t = -3.31$, $df = 68.3$, $P = 0.0044$) and on roads (difference = -0.96 sec, $SE = 0.12$, $t = 8.89$, $df = 66.2$, $P < 0.0001$) (Figs. 5, 6). Conversely, in old growth forests there was no significant difference in detection time for melanic and gray morphs (difference = 0.22 sec, $SE = 0.12$, $t = 1.92$, $df = 68.8$, $P = 0.179$) (Figs. 5, 6).

There was a significant effect of morph on luminance JNDs of squirrels to their immediate backgrounds, but there was no effect of environment, or morph by environment interaction (Table 2). Mean luminance JND was greater for melanic than gray squirrels in secondary forests (difference = 3.05, $SE = 0.28$, $t = 11.03$, $df = 58$, $P < 0.0001$), old growth forests (difference = 2.24, $SE = 0.26$, $t = 8.70$, $df = 58$, $P < 0.0001$) and on roads (difference = 2.97, $SE = 0.27$, $t = 11.02$, $df = 58$, $P < 0.0001$), indicating melanics had consistently greater contrast against their tree and road backgrounds (Figs. 7, 8).

Discussion

My results suggest that urbanization and associated land use changes in adjacent rural areas changes the way camouflage functions for gray and melanic morphs of eastern gray squirrels. The historical change in forest structure from old growth to secondary forests in rural areas has likely made the gray morph less conspicuous than melanics to predators. My results show that the gray morph more closely matched tree backgrounds for luminance and is more difficult to detect by predators in secondary forests. Conversely, the gray morph was detected more quickly by predators in old growth forests despite having a closer luminance match to the tree background than melanics. In urban areas where vehicular collision is likely the most common source of mortality for squirrels (McCleery et al. 2008), my results suggest melanics may have a survival advantage on roads. Melanics had greater contrast to the road background for luminance and were easier to detect by drivers compared to the gray morph.

Animals that appear conspicuous in a given environment are more likely to be attacked by predators, as seen in studies of grasshoppers (Karpestam et al. 2013), rock pocket mice (Hoekstra et al. 2004), deer mice (Linnen et al. 2013) and old-field mice (Vignieri et al. 2010). For instance, predation rates are higher for darker old-field mice (*Peromyscus polionotus*) on white sand beaches but are lower on dark soil beaches, whereas for light colored mice predation rates are higher on dark soils and lower on white sands (Vignieri et al. 2010). Thus, animal color is incredibly important for crypsis and avoidance of predators. My results provide another example of the importance of animal color for crypsis, highlighting how urbanization and associated changes in rural land use alter the degree of camouflage for color morphs of gray squirrels.

Prior to European settlement, melanics were most common in rural forests at northern latitudes of the species range (Schorger 1949, Robertson 1973). Melanics have a thermal advantage in cold climates (Ducharme et al. 1989), which has been thought to explain their presence in northern rural forests, but my results suggest that melanics were also more cryptic in rural old growth forests, as participants were more likely to detect the gray morph and marginally faster at detecting the gray morph in old growth forests. Thus, urbanization and the change in land use in rural areas has changed how crypsis functions for each morph. After European settlement in the 18th century, the majority of forests in eastern United States were cleared and logged, and in the mid-1800s many of these lands were abandoned as agriculture shifted to the Midwest, allowing for forests to regenerate naturally (secondary forests) (Brown et al. 1999, Hall et al. 2003). My results indicate that the gray morph has a closer luminance match to the immediate tree background and is harder to detect in secondary forests, therefore suggesting that the change in forest structure as human densities increased in eastern North America is advantageous to the gray morph against predation.

Curiously I found that participants were more likely to detect the gray than melanic morph in old growth forests despite the gray morph having a closer luminance match to the immediate tree background. These results suggest that melanics may get their marginal cryptic advantage from a different mechanism, one that does not rely on immediate background matching to trees. Crypsis does not only depend on the relationship between an individual's coloration and the immediate background, but also on visual characteristics in the broader background (Merilaita 2003). Backgrounds with increasing visually complexity make it harder for predators to detect prey as they are

unable to obtain necessary visual information (e.g., color, lightness and edge distributions; Merilaita 2003), as seen in experiments with blue tits (*Cyanistes caeruleus*; Dimitrova and Merilaita 2009, Dimitrova and Merilaita 2011). Not only do old growth forests have darker trees and deeper patches of shade as compared to secondary forests, they are also more structurally complex by extensive vertical stratification and horizontal heterogeneity, and as a result they appear more visually complex. Thus, the mechanism of crypsis for melanics may not be due to immediate background matching to conifers with darker bark, but rather the overall complexity of the broader visual background. But the key question that remains, is whether or not detection in visually complex backgrounds is color dependent and if so, are some colors more likely to be detected (i.e., the gray morph in old growth forests). Strong matching to the immediate tree background is likely more important in visually simple environments, like secondary forests, which may explain why melanics are detected more quickly in secondary forests. Further studies are needed to understand the relative importance of different mechanisms of crypsis for squirrel color morphs in old growth and secondary forests.

In cities, where vehicular collision is the most common source of mortality, my results suggest that melanics had greater contrast to the road background than the gray morph, and as a result were easier to detect. Roads place intensive selection pressures on organisms (Brady and Richardson 2017), typically on morphology (grasses; Kiang 1982 and cliff swallows; Brown and Bomberger Brown 2013), behavior (increase in vocal pitch of birds; Slabbekoorn and Peet 2003, movement behaviors in snakes; Andrews and Gibbons 2005 and decline in vocal activity in frogs; Lengagne 2008) and physiological traits (increased metal tolerance in plants adjacent to roads; Briggs 1972, Wu and

Antonovics 1976, Atkins et al. 1982), but a study has yet to link animal color to road mortality. My results suggest that the strong visual contrast of melanic squirrels on roads may contribute to their persistence in cities. Melanics appear more conspicuous on roads and are easier to detect by drivers, as compared to the gray morph who closely matched the road background. Since drivers detected melanics quicker than the gray morph, drivers may be more likely to avoid hitting melanics, and as a result melanics are less likely to experience vehicular collisions.

Overall, my study suggests the change in forest structure in rural areas and the presence of extensive road networks in urban areas causes divergent natural selection for melanism along urbanization gradients. The cryptic gray morph is likely favored in rural secondary forests where predation is common, whereas the strong contrast of melanics on roads may give melanics a selective advantage in cities. Divergent selection may explain why we see clines in melanism decreasing from urban to rural areas today (Gibbs et al. 2019). However, additional experimental evidence is needed to measure natural selection on coat coloration more directly. A clear next step would be to measure predation rates on each color morph in old growth and secondary forests while controlling for traits other than coat color (e.g., clay plasticine models; Vignieri et al. 2010, Linnen et al. 2013, Farallo and Forstner 2012, Nafus et al. 2015). On roads, an additional step would be to test whether the gray morph is overrepresented among road killed squirrels compared to its background prevalence in urban areas.

It is important to recognize that selection on animal color may not be as direct as I have assumed in this study. For example, many studies have shown behavioral and physiological differences between color morphs, such as differences in mating calls and

mean diving depths (Western Grebes; Nuechterlein 1981), aggressive behaviors toward intruders (tree lizards; Hover 1985) and antipredator defense mechanisms (red-backed salamander; Otaibi et al. 2017). In this study, I assumed that melanic and gray morphs behave in similar ways on roads and in rural areas, which may not be the case. For example, my data suggest melanics should have a survival advantage on roads as they stand out better to drivers, but that may not be the case if the gray morph is more likely than melanics to behave in ways to mitigate the risk of being hit by vehicles (e.g., move faster across roads, avoid roads, or make use of corridors like electric lines to cross roads). Further study is needed to understand how behavioral differences along with the cypsis patterns I found might contribute to fitness differences between squirrel morphs in urban and rural environments.

Tables

Table 1. ANOVA table for the linear mixed model of detection times in the online game. Satterthwaite's method was used to estimate the degrees of freedom.

Fixed effect	Sum of squares	Mean square	Group df	Error df	F	<i>P</i>
Environment	3.18	1.59	2	56.22	5.60	0.006
Morph	17.13	17.13	1	2757.71	60.25	<0.001
Environment*Morph	28.10	14.05	2	2750.24	49.43	<0.001

Table 2. ANOVA table for the linear mixed model of luminance JNDs for objective analysis of background matching. Satterthwaite's method was used to estimate the degrees of freedom.

Fixed effect	Sum of squares	Mean square	Group df	Error df	F	<i>P</i>
Environment	2.40	1.20	2	58	1.57	0.22
Morph	222.71	221.71	1	58	290.62	<0.001
Environment*Morph	4.11	2.06	2	58	2.69	0.08

Figures



Figure 1. Example images taken in a forest scene showing (a) close-up image for objective analysis of background matching, (b) ROIs created in ImageJ for objective analysis (blue outline = melanic ROI, green outline = gray ROI, white = tree ROI), (c) distant image for detection analysis in the online game, and (d, e) final distant images of each morph presented to participants in the online game.

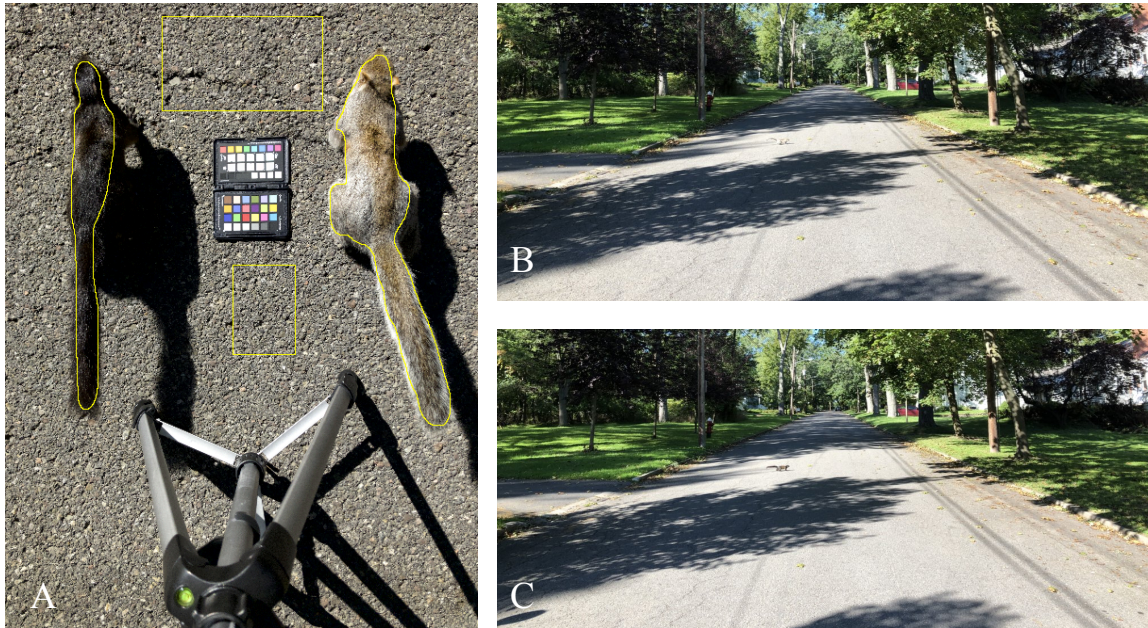


Figure 2. Example images taken in a road scene showing (a) close-up image, with ROIs for morphs and the road, used for objective analysis of background matching, and (b, c) distant images of each morph presented to participants in the online game

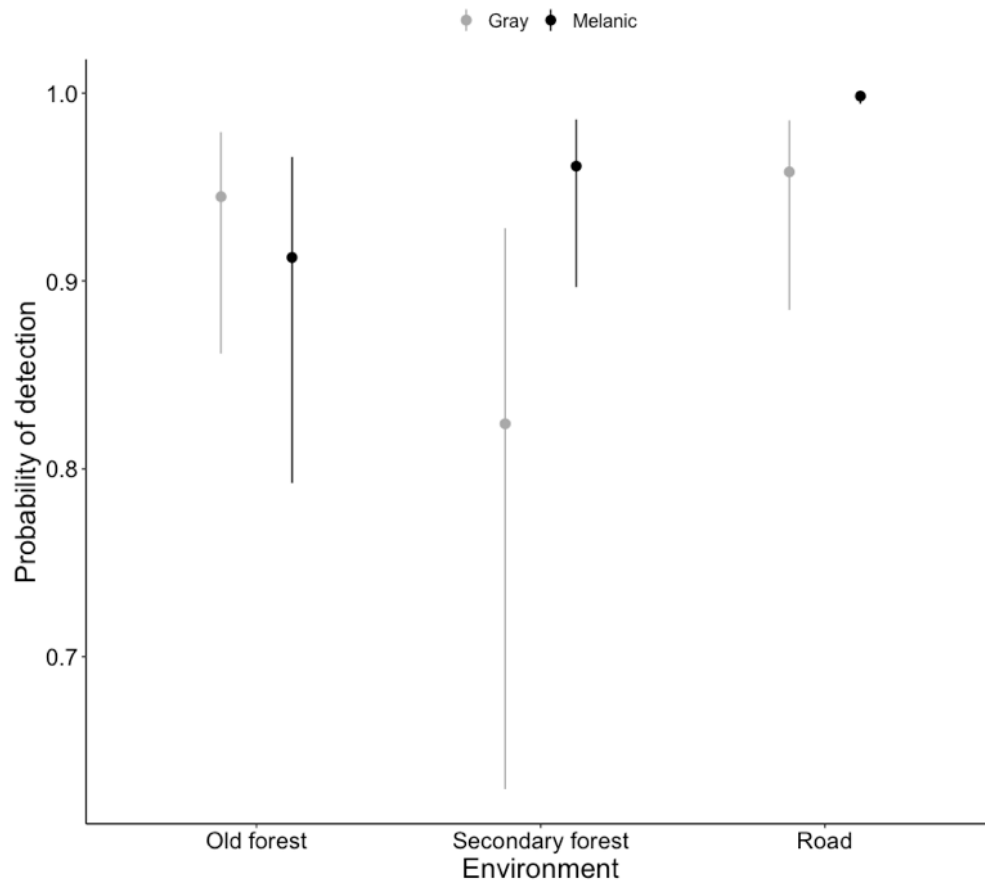


Figure 3. Probability of detection of gray (gray circles) and melanic squirrels (black circles) by participants in old growth forests, secondary forests and on roads. Error bars represent 95% confidence intervals.

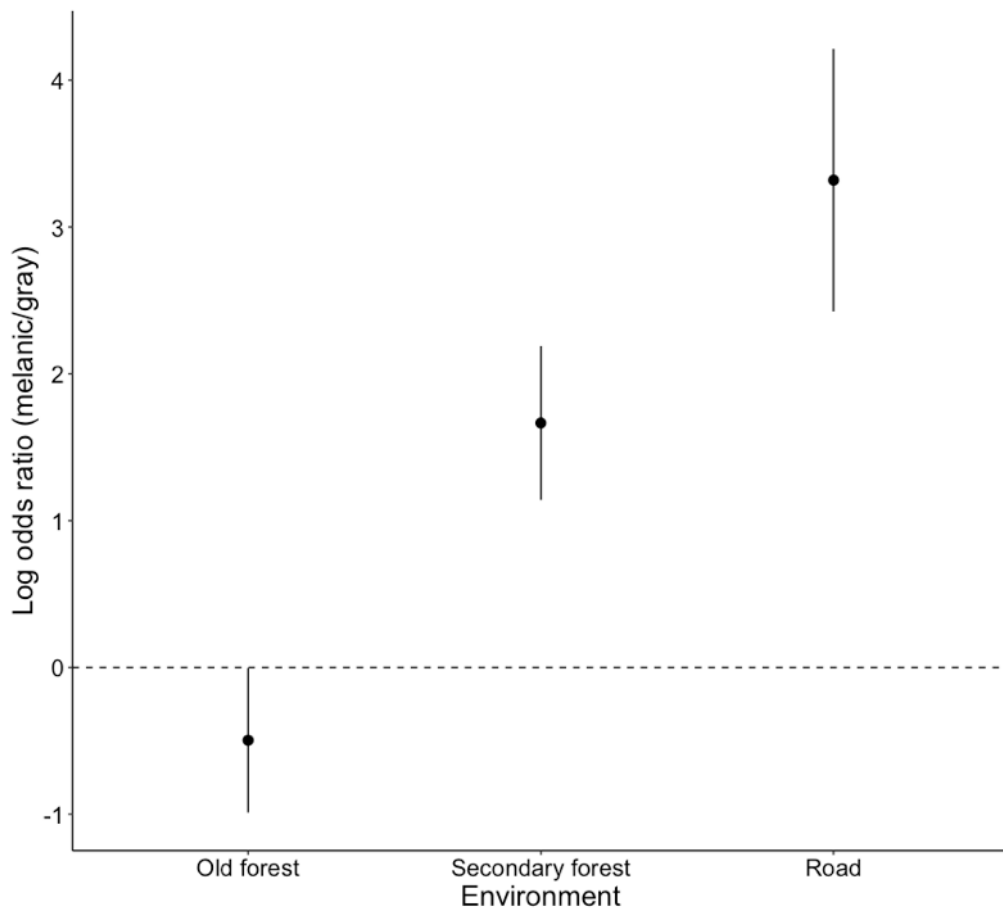


Figure 4. Log odds ratios for the probability of detection (melanic/gray). Positive values indicate a greater likelihood of finding melanic squirrels than gray squirrels, and negative values indicate a greater likelihood of finding gray squirrels than melanic squirrels. Error bars represent 95% confidence intervals.

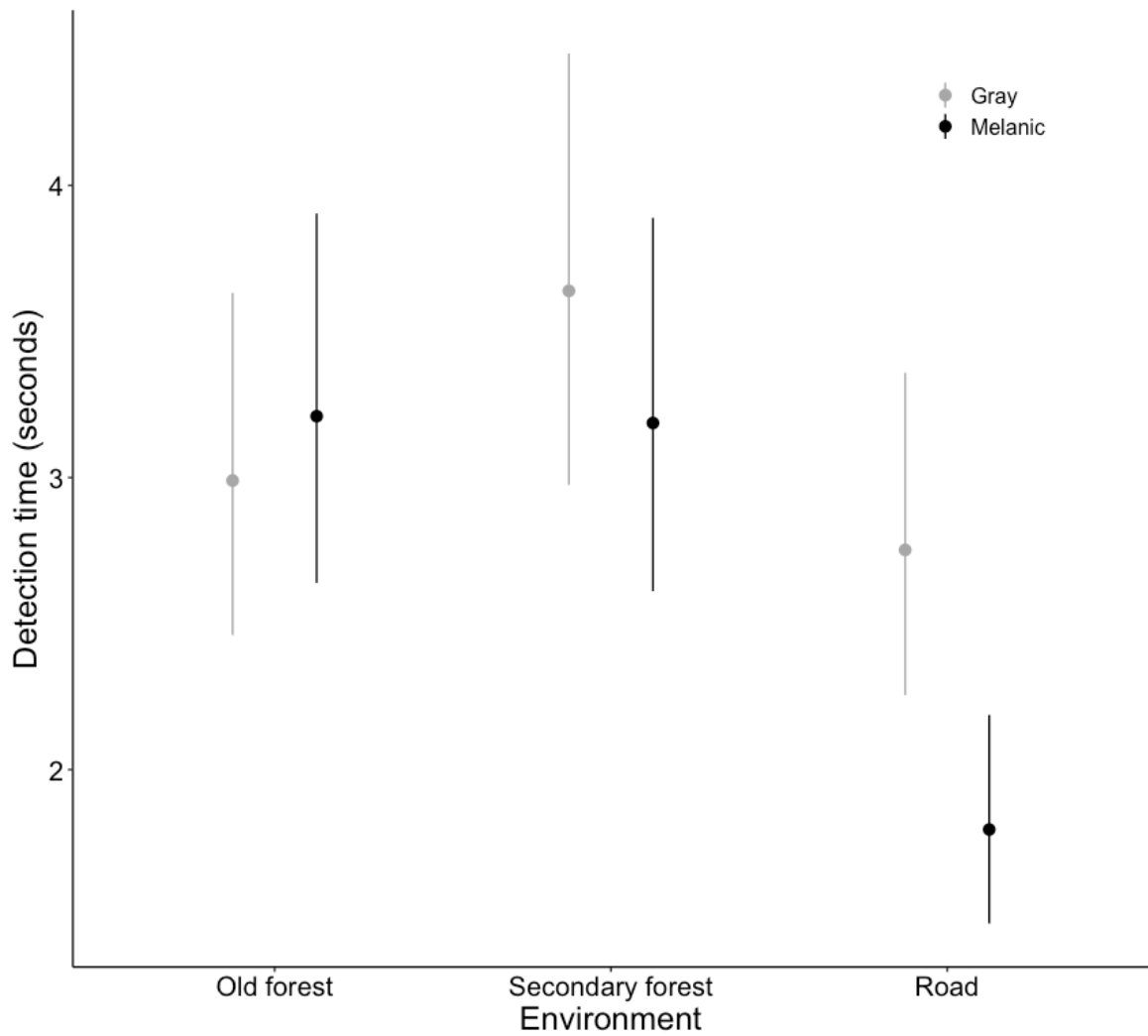


Figure 5. Mean detection time of gray (gray circles) and melanic squirrels (black circles) by participants in old growth forests, secondary forests and on roads. Error bars represent 95% confidence intervals.

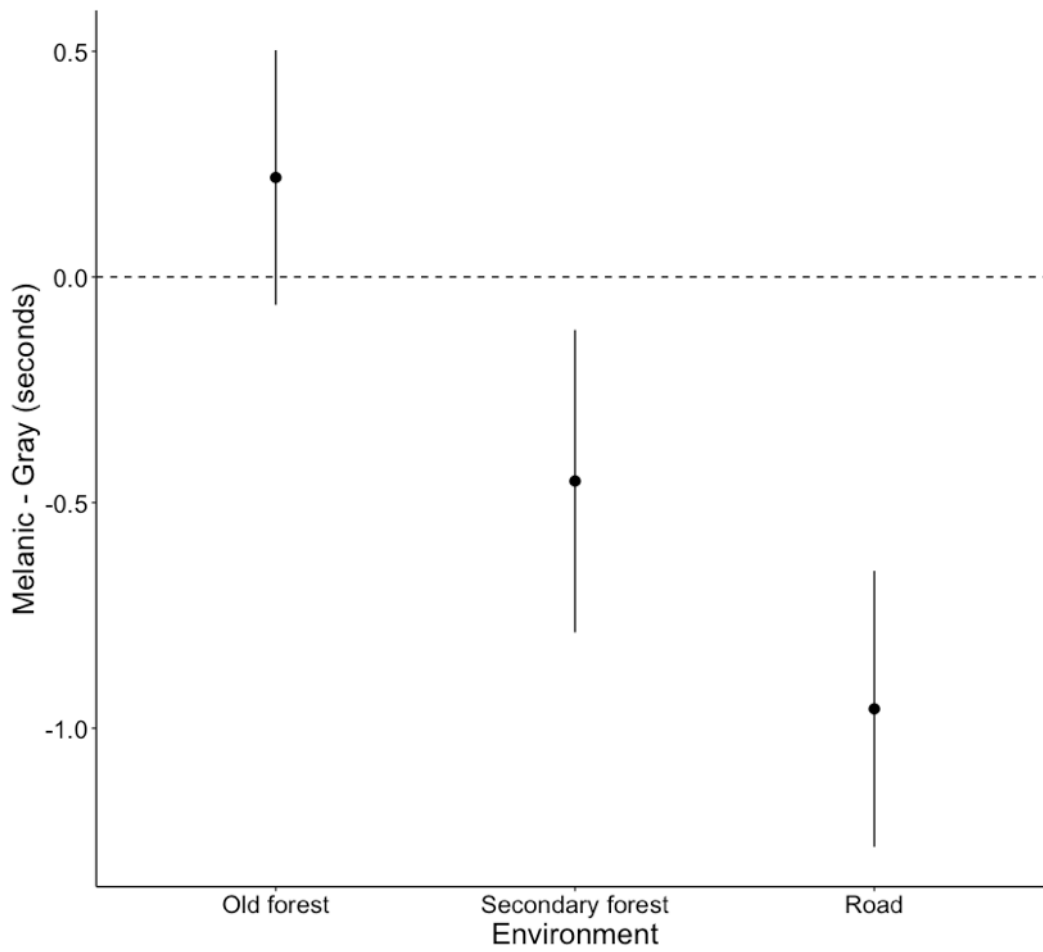


Figure 6. Difference mean detection times (melanic – gray) in old growth forests, secondary forests and on roads. Positive values indicate longer detection times for melanic than gray squirrels, and negative values indicate longer detection times for gray squirrels than melanic squirrels. Error bars represent 95% confidence intervals.

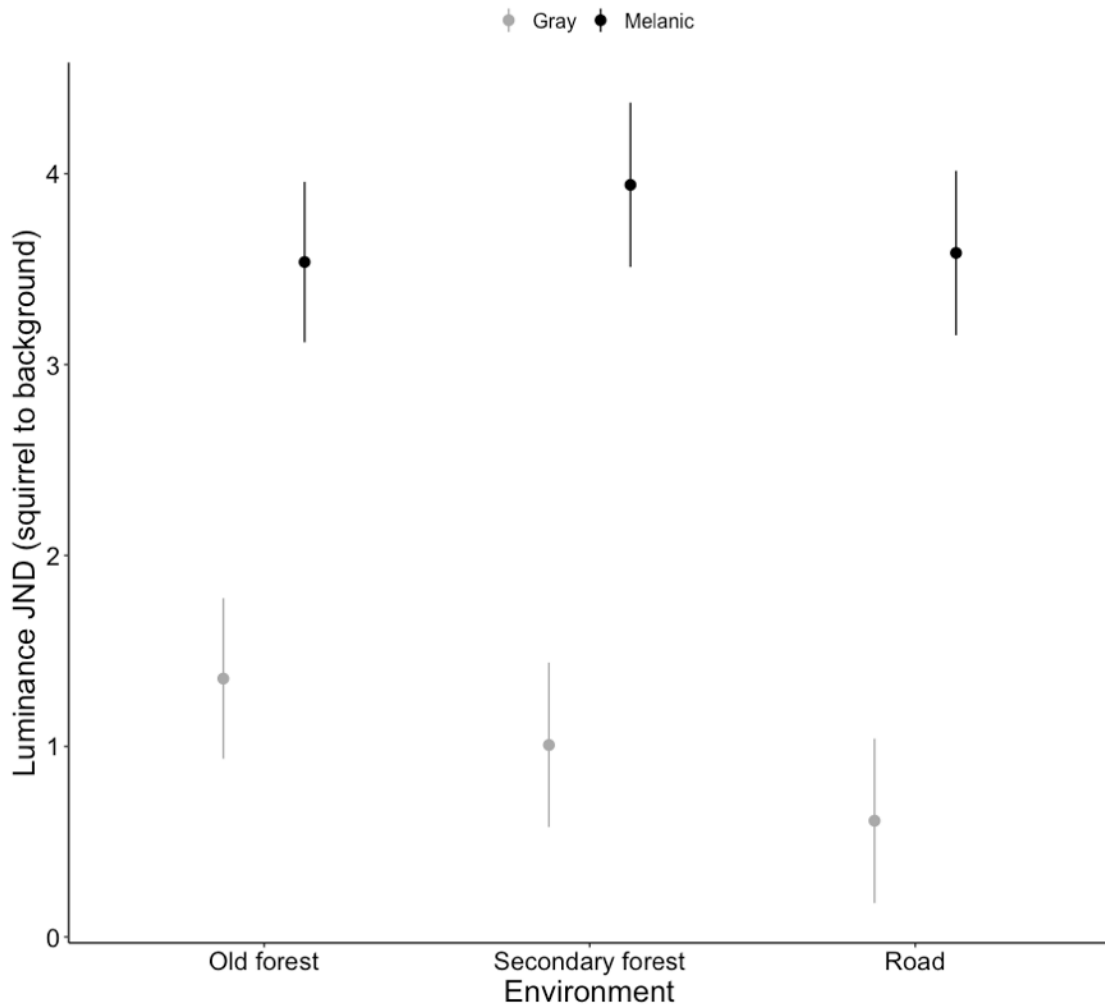


Figure 7. Mean luminance just noticeable differences (JNDs) of gray (gray circles) and melanic squirrels (black circles) to their immediate backgrounds in old growth forests, secondary forests and on roads. Smaller luminance JND values represent better background matching, whereas larger values indicate less background matching. Error bars represent 95% confidence intervals.

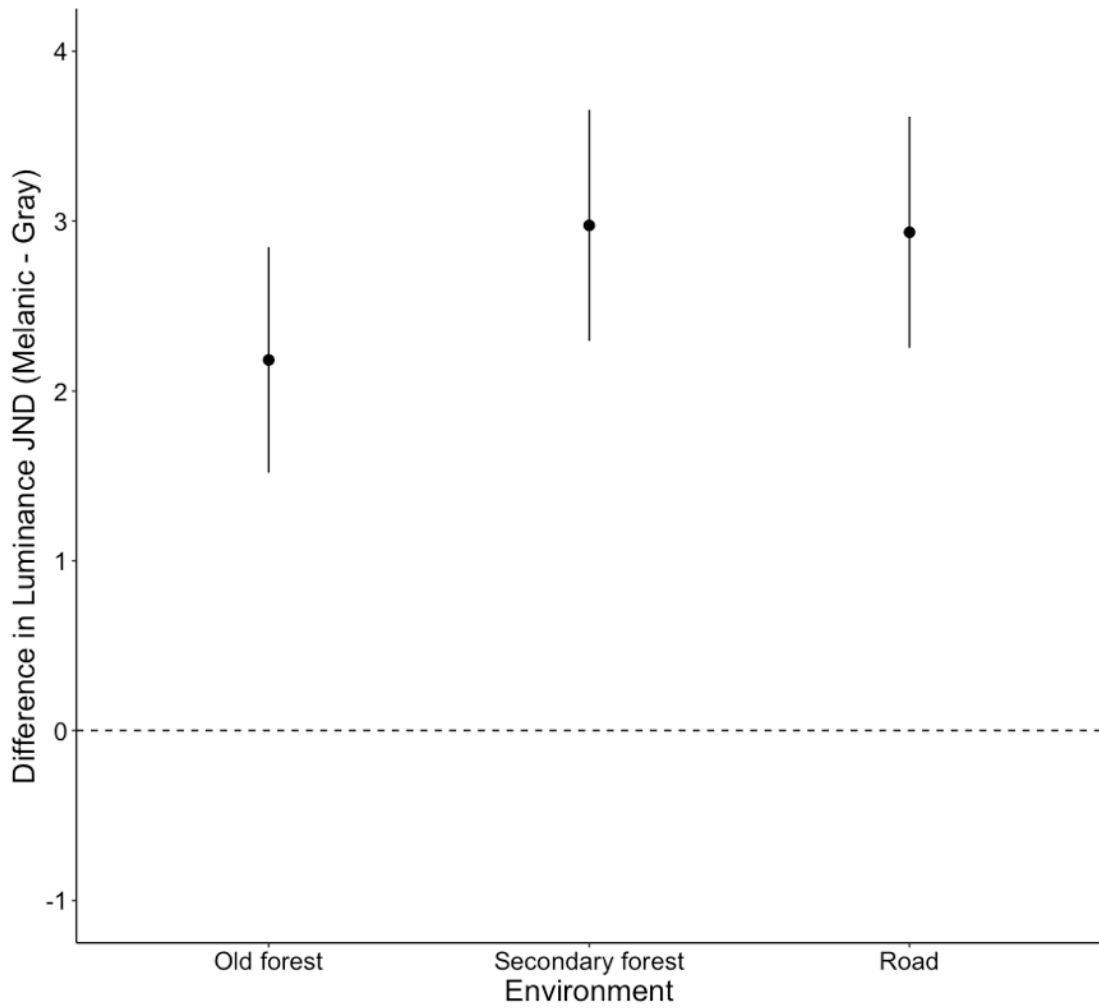


Figure 8. Difference mean luminance just noticeable differences (JNDs) (melanic – gray) to immediate backgrounds in old growth forests, secondary forests and on roads. Positive values indicate better immediate background matching of gray squirrels rather than melanic squirrels. Error bars represent 95% confidence intervals

Appendix A

I used a linear mixed model to test how the difference in time to find melanic and gray squirrels paired by scene (melanic – gray) was related to environment, the morph initially presented to the participant (melanic first or gray first), and the number of images shown between morphs in each scene. The initial morph presented was included to test for a carryover effect, and I included the number of images between morphs to determine whether the carryover effect was washed out by a large number of images presented between morphs in a scene. The model included a three-way interaction term between environment, initial morph, and images between morphs. Participant and scene identity were included as random effects, and I used a Gaussian error distribution. The only significant term in the model was the effect of initial morph (Table A1), and detection times were consistently longer for the morph initially presented to the participant within a scene (Fig. A1).

Table A1. ANOVA table for the linear mixed model of difference in detection time between morphs paired by scene (melanic – gray) with fixed effects of environment, initial morph presented, images between morphs, and their interactions.

Fixed effect	Sum of squares	Mean square	Group df	Error df	F	<i>P</i>
Environment	36.45	18.22	2	96.11	2.62	0.078
Initial morph	152.15	152.15	1	2617.29	21.85	<0.001
Images between	0.29	0.29	1	2621.48	0.04	0.839
Environment*Initial	8.47	4.23	2	2629.75	0.61	0.545
Environment*Between	26.29	13.15	2	2618.13	1.89	0.152
Initial*Between	2.46	2.46	1	2622.21	0.35	0.552
Environment*Initial*Between	23.06	11.53	2	2628/27	1.66	0.191

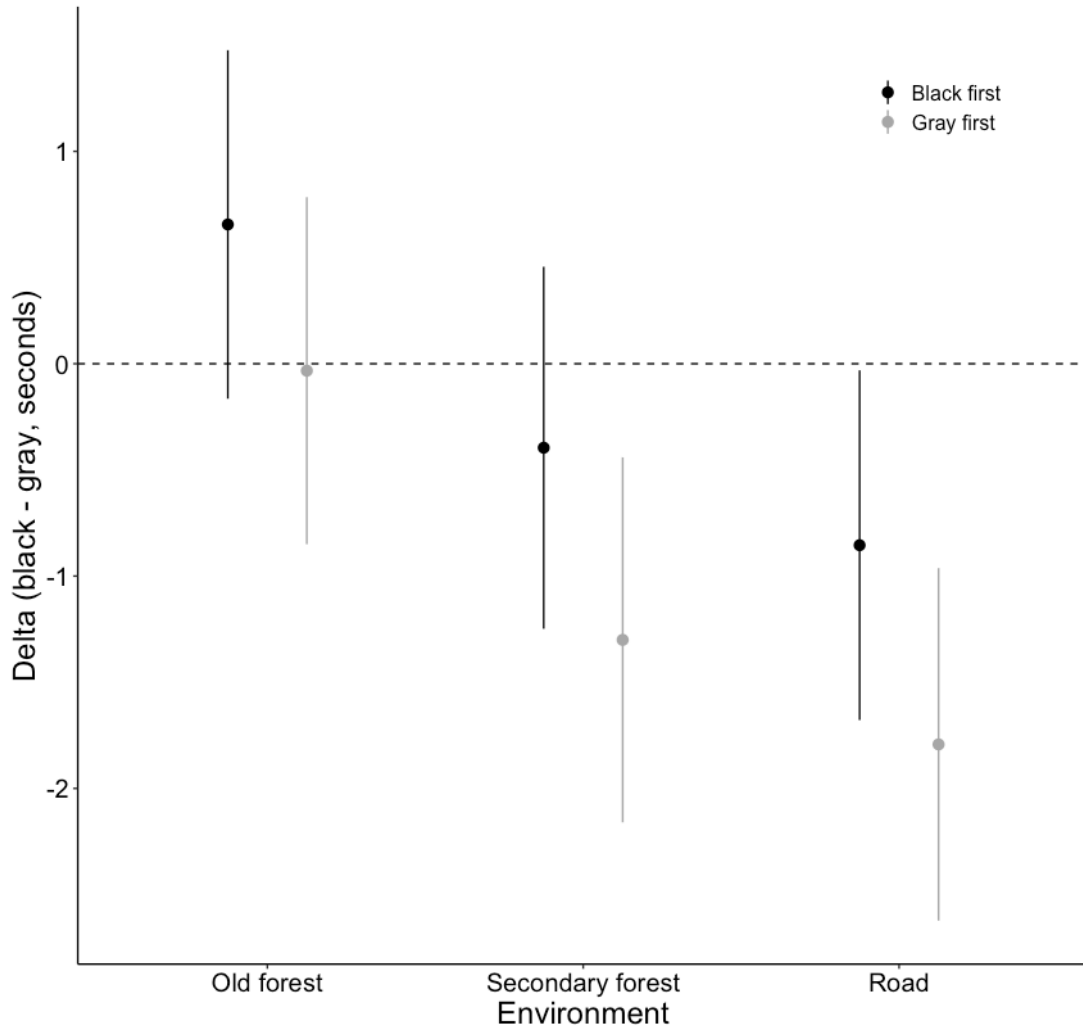


Figure A1. Mean difference in detection time (melanic – gray) when the melanic (black circles) or gray squirrel (gray circles) was presented first within scenes. Positive delta values suggest that detection time was longer for melanic squirrels, whereas negative delta values suggest that detection time was longer for gray squirrels. Error bars represent 95% confidence intervals.

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