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Melanism as a potential thermal benefit in eastern fox squirrels (*Sciurus niger*)

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ABSTRACT

Melanistic fox squirrels (*Sciurus niger*) have expanded westward and increased in frequency in the Omaha, Nebraska, and Council Bluffs, Iowa, metropolitan areas. The selective advantage of melanism is currently unknown, but thermal advantages have been hypothesized, especially in winter. No difference in metabolic response curves were measured between melanistic (black) and rufus (orange) fox squirrels. When exposed to sunny skies, both melanistic and rufus squirrels had higher surface (skin and fur) temperature as ambient temperatures increased. Melanistic squirrel surface temperatures did not differ when squirrels were exposed to sunny or cloudy skies. However, rufus individuals showed significantly lower increases in surface temperatures when under cloudy skies. During fall months, rufus individuals were about 1.5 times more active throughout the day than melanistic individuals. However, in winter, melanistic fox squirrels were approximately 30% more active in the mornings (before 13:00) compared to rufus squirrels. Pre-winter body condition was higher in melanistic (25.5 ± 1.8 g/cm) compared to rufus (20.30 ± 3.6 g/cm) fox squirrels; however, there were no significant differences between melanistic (22.8 ± 1.4 g/cm) and rufus (23.9 ± 0.8 g/cm) fox squirrel post-winter body condition. The results of this study indicate that melanistic fox squirrels may have a slight winter thermal advantage over rufus fox squirrels by maintaining higher skin temperatures.

KEYWORDS

Eastern Fox Squirrel, Melanism, *Sciurus niger*, Thermoregulation

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INTRODUCTION

Melanism is the concentration of black, or nearly black, pigment associated with the skin, feathers, or fur of animals (Caro 2005). Pigmentation in mammals often provides concealment on different substrates, providing an advantage to both predator and prey species. Prey species, including rock pocket mice (*Chaetodipus intermedius*; Nachman *et al.* 2003), deer mice (*Peromyscus maniculatus*; Linnen *et al.* 2009), and oldfield mice (*Peromyscus polionotus*; Protas & Patel 2008), adapt to match different substrates (sand, lava flows, etc.) to avoid detection from predators. These color changes result in increased fitness and spread throughout the population. Some nocturnal predator species, such as leopards, also benefit from dark coloration because it aids in concealment from prey and can more effectively ambush (Kawanishi *et al.* 2010; Schneider *et al.* 2012).

The genetics behind pigmentation are complex, with more than 150 genes affecting color and patterning (Protas &

Patel 2008, Hubbard *et al.* 2010); however, two genes constitute the majority of cases of melanism: melanocortin-1-receptor (MC1R) gene and the agouti signaling protein (ASIP) gene (McRobie *et al.* 2009; McRobie *et al.* 2014). Although melanism occurs from changes in either the MC1R or the ASIP gene, the effects of these genes are potentially pleiotropic and may have different functional effects on other physiological and behavioral characteristics (Keeler *et al.* 1968; 1970).

Two major hypotheses for the benefits of melanism dominate the literature: crypsis and thermoregulation. In the Tularosa Basin of New Mexico, color variation in three species of lizard correspond with crypsis from aerial predators, with blanched lizards found on light gypsum dunes and melanistic forms found on dark basalt rocks (Rosenblum 2005). Similarly, in fox squirrels inhabiting the Southeastern United States, melanism may be related to crypsis in forests that are exposed to periodic burning (Kiltie 1989, 1992a, 1992b). After a fire, the

substrate is blackened, giving an advantage to dark individuals that can blend in and avoid predation.

Thermal advantages for melanism have been especially noted among ectotherms with melanistic ectotherms reaching homeostasis faster (Rosenblum 2005; Sherbrooke *et al.* 1994), attaining higher body temperatures (Forsman 1995; Bittner *et al.* 2002; Clusella-Trullas *et al.* 2009) and have faster body warming (Tanaka 2005). Following the results of melanism on ectotherm thermoregulation, some studies on endotherms have concluded that darker fur tends to absorb greater amounts of solar energy than lighter fur (Armitage 2009; Fratto & Davis 2011). However, this may be spurious as most coloration studies use “painted” fur or feathers on mounted skin patches. When live animals, or unaltered patches of fur, were used, the connection between coat color and radiative heat gain disappeared (Walsberg & Wolf 1995). In live animals, this was almost certainly due to the use of piloerection, positional changes, and changes in blood flow to offset any absorption properties because of fur color (Walsberg & Wolf 1995).

Endotherms must maintain a constant body temperature in order to survive (Gillooly *et al.* 2001). To do this, their rate of metabolism varies to accommodate changes in the thermal environment (gain or lose heat to the environment), and maintaining a high metabolism can be a very energy-demanding process (McNab 2012). As a result, most endotherms have a range of environmental temperatures (the thermal neutral zone) where they are most comfortable and can maintain the lowest metabolic rate while maintaining their normal body temperature. However, when ambient temperatures are too cold or too hot (above or below the critical temperatures), endotherms must generate more heat or actively lose heat to maintain their constant body temperature. Either of these adjustments results in an increase in metabolic rate as the individual tries to adjust to the heat or cold. (McNab 2002). Actively thermoregulating decreases the energy available to other processes such as reproduction, foraging, and growth (Walsberg & Wolf 1995). By altering the amount of energy required for thermoregulation, microclimates can impact how much metabolic energy is available for reproduction, growth, and maintenance. This is especially true for small mammals, whose surface area-to-volume ratio promotes heat loss (Walsberg & Wolf 1995).

Within the tree squirrels of North America, melanism can be a prominent color variant for local populations, and several authors have noted that melanism increases in frequency in the northern portion of species’ ranges, where snow (white background) is common (Robertson 1973; Banfield 1974; Gustafson & VanDruff 1990). The contrast between black squirrels against the snow would seemingly make melanistic individuals prone to increased predation (Forsman 1995; Bittner *et al.* 2002); therefore, there must be some added benefit to melanism that would counteract a potential increase in predation. High frequencies of melanism are also reported in urban tree squirrel populations (Robertson 1973; Gustafson & VanDruff 1990), which may have fewer natural predators, thus eliminating the problem of increased conspicuousness.

The phenotypic expression of melanism in the fox squirrel can present itself in various ways. In the Southeastern United States, melanistic squirrels can present with patches of white on their ears and nose (Steele & Koprowski 2001; Kiltie 1992b). In Omaha and Council Bluffs, variations in fur color have been observed, ranging from the presence of both rufus and black fur throughout the body to a black body with a rufus tail or vice versa. Melanistic fox squirrels have been reported in Georgia, Mississippi (Kiltie 1989), and are the official mascot of Marysville, Kansas. Melanistic fox squirrels have been observed in the Omaha, Nebraska, and Council Bluffs, Iowa, metropolitan areas (referred to henceforth as Omaha) since the 1840s (Audubon 1843). Lueninghoener (1973) first censused fox squirrel coloration in the Omaha/Council Bluffs metropolitan area and found that melanistic fox squirrels were primarily found in Council Bluffs (45.2% of the population) and only along the Missouri River in Omaha. A more recent survey found that the density of melanistic individuals was still higher in Council Bluffs (50.8%) compared to Omaha (4.6–7.6%); however, melanistic squirrels were now observed throughout all of Omaha (Wilson 2013). However, the specific selective advantage that maintains or increases the proportion of melanistic individuals in this population remains unknown.

Several hypotheses, including crypsis and thermoregulation, can be made about the observed increase and persistence of melanistic fox squirrels in Omaha. Other hypotheses for the increase in melanistic squirrels in Omaha include favoritism by humans (they feed them more), a willingness to avoid running them over with vehicles, or random genetic drift. In this study, we focus on one hypothesis: a possible thermal advantage. According to the thermal melanism hypothesis, dark individuals have an advantage at lower temperatures. They heat up faster than lighter individuals because their dark fur absorbs solar radiation, whereas light fur reflects it. This external heat source can potentially lower reliance on metabolic heat production (Clusella-Trullas *et al.* 2008). However, it is also possible that absorbing solar radiation and warming the skin would increase the heat gradient between the skin and the air, making melanistic individuals lose heat to the environment faster (McNab 2002) resulting in a metabolic disadvantage at lower ambient temperatures.

We hypothesize that melanistic fox squirrels have a thermal advantage, especially in winter, over rufus fox squirrels through increased solar heat gain, and therefore higher surface temperatures. As fox squirrels do not hibernate, they must be active during the coldest months of the year, which may place a high energetic burden on them as they generate heat to offset cold ambient temperatures. As melanistic and rufus individuals are the same species, we hypothesize that there will be no difference in metabolic rates between the two color morphs. Increasing the amount of solar heat gain in the fall and winter could lower the demands for metabolic heat production and Lower energetic costs for thermoregulation. Increased solar heat gain in melanistic fox squirrels may allow melanistic individuals to increase their daily activity during colder ambient

temperatures (winter, early mornings, and evenings) and be able to maintain higher body conditions through winter.

1. METHODS

1.1. Study Sites

A combination of five sites were used in Omaha and consisted of a combination of municipal park and forested land. Park habitat consisted of large expanses of manicured grass with isolated trees and public utility equipment (playgrounds, benches, and sports fields). Forested sections consisted of some combination of maple (*Acer* spp.), northern red oak (*Quercus rubra*), white mulberry (*Morus alba*), cottonwood (*Populus deltoides*), hickory (*Carya* spp.), black walnut (*Juglans nigra*), buckeye (*Aesculus glabra*), green ash (*Fraxinus pennsylvanica*), American sycamore (*Platanus occidentalis*), and bur oak (*Quercus macrocarpa*).

1.2. Live Capture and Handling

Melanistic and rufus fox squirrels were captured in the fall and again in the following spring to obtain mean pre- and post-winter body mass. Pre-winter body mass was measured between September and October 2012 and post-winter body mass was measured between March and May 2013. Individuals were captured using live traps (Models 202 and 204, Tomahawk Live Trap Company, Tomahawk, Wisconsin) baited with corn and peanut butter or a mixture of sunflower seeds, nuts, and bread. Traps were placed in a line at 5-m intervals and checked every 1–1.5 h and were open only during daylight hours. Traps were closed at temperatures $>24^{\circ}\text{C}$ or $<7^{\circ}\text{C}$ to prevent thermal stress (Huggins & Gee 1995; Arenz 1997).

Captured squirrels were transferred to a handling bag (Arenz 1997) of a known mass and anesthetized with an intramuscular injection of Telazol[®] (100 mg/ml; Fort Dodge Animal Health, Fort Dodge, Iowa). After immobilization, individuals were removed from the handling bag and measurements of body length (tip of nose to base of tail, cm), tail length (anus to tip of tail, cm), and total body mass (difference between handling bag and handling with fox squirrel, g) were collected and given an individual ear tag. Subsequently, fox squirrels were returned to the site of capture and allowed to recover from anesthesia before being released.

1.3. Surface Temperature

Surface temperatures (combination of skin and fur) of melanistic and rufus fox squirrels were measured at different ambient temperatures (-17 to 37°C). Surface temperature was measured using a Raytek[®] infrared thermal gun (Fluke Company, Everett, Washington) with settings set to collect average temperatures ($^{\circ}\text{C}$) at an emissivity (ϵ) of 0.98. Surface temperatures were measured by placing the laser target on the midsection, or flank, of fox squirrels. Barnes et al. (2017) found that surface temperature measured with the infrared thermal gun was correlated with core body temperatures and that this method

is an effective, noninvasive method of measuring an animal's temperature. Ambient temperatures were measured simultaneously using a Springfield[®] digital thermometer (Taylor Precision Products, Inc., Las Cruces, New Mexico). All fieldwork was conducted under an approved animal care and using protocol (#11-088-11-EP) and met the guidelines recommended by the American Society of Mammalogists (Sikes et al. 2011).

1.4. Squirrel Activity

Activity was observed from just prior to sunrise to just after sunset (Geeslin 1970). Squirrel observations followed the focal animal technique of Altman and Shapiro (1974) with individual squirrels being monitored at 15-s intervals until individuals were no longer observable. Every 15 s, the squirrel's behavior was recorded as 1 of 27 behaviors. However, in this study, the exact behaviors were not of interest, only the amount of time all individuals were active during the day. Total observation hours were calculated for each hour of the day (05:00–20:00) giving a total number of observations a squirrel could potentially be seen (observation time). Similarly, the number of observation times that squirrels were active was summed for each hour (squirrel activity). Percent activity was then calculated as: (squirrel activity)/(observation time)*100 for each hour of the daytime observation period.

1.5. Squirrel Metabolism

Metabolic rates were calculated using oxygen consumption and carbon dioxide production while inside a Sable System Inc. (Las Vegas, NV) flow through respiratory metabolic chamber (Campbell & Hochachka 2000). This metabolic chamber was put into a dark environmental cabinet (PELT-5, Sable Systems) where ambient temperatures could be changed. Airflow was supplied to the squirrel at a rate of 2 L/min and monitored by a flow regulator (Mass Flow Systems). The airline leading to the metabolic chamber was attached to a copper coil inside the environmental cabinet to ensure that the temperature of the air entering the chamber was the same as the ambient temperature in the cabinet. A relative humidity meter (RH-300) and Foxbox O_2/CO_2 analyzer were used to obtain relative humidity, O_2 , CO_2 , and barometric pressure readings. All data throughout the metabolic trial were recorded to a computer file using a UI-2 Controller.

The metabolic rate of each squirrel was taken at eight different temperatures (0°C , 5°C , 10°C , 15°C , 20°C , 25°C , 30°C , and 35°C) with no more than one test per day per individual. At temperatures of 0°C and 5°C , dry ice was used to cool down the chamber. At 10°C , ice packs were used for the same purpose. At temperatures of 30°C and 35°C , Drierite was used to eliminate water vapor that could interfere with airflow.

Before each trial, the squirrel was given 1 hour to acclimate to the chamber and temperature. Each trial consisted of a 5-min baseline reading from ambient air, a 7-min reading from the chamber, another 3-min baseline reading, an additional 7-min chamber reading, and ended with a final 5-min baseline. Data were cleaned and analyzed using the program

EXPEDATA (Sable Systems, Las Vegas, NV). For each reading, percentage of use of O₂ and percentage of addition of CO₂ by the animal was calculated using baseline concentrations of O₂ and CO₂ obtained from ambient airflow.

1.6. Data Analysis

Relative body condition between rufus and melanistic individuals was assessed by comparing residuals obtained from a length × mass regression (Schulte-Hostedde *et al.* 2005). If no pattern exists between color morphs then individual residuals should be equally spread across the two color morphs. However, if one color morph has higher, or lower, residuals then we would infer that that color morph is in better, or worse, condition, respectively. Comparisons between melanistic and rufus body condition was analyzed using a two-tailed Student's t-test with a Bonferroni adjustment for multiple comparisons (SAS 2002) between color morphs (melanistic, rufus) and time (pre- and post-winter).

Differences in surface temperature were analyzed using general linear model (GLM) analysis in SAS with the solutions option to test for differences in slope and intercept. Differences between color morphs were tested for each solar category (sun, cloudy) as well as differences between solar categories within each color morph. Appropriate color morph (melanistic and rufus) was used as the main treatment with solar category. Differences in the percent activity of melanistic and rufus squirrels were analyzed by season and hour using the Mantel–Haenszel chi-square test to account for the repeated measures across hours of the day and study sites (SAS 2002).

The lower critical temperature (LCT) of the fox squirrel's thermal neutral zone was calculated using the segmented package in Program R. To determine whether the two color morphs had similar or different metabolic response curves, two generalized additive mixed models (GAMMs) were created using the gamm4 package in Program R. GAMMs were used because the results were not expected to be linear and metabolic rate needed to vary by individual. The first model was based on a shared curve; the second model consisted of separate curves distinguished by color, melanistic or rufus. In addition to the models, a t-test was performed on the residuals of the data to determine if there was a pattern present based on color. All data are reported as means ± standard error, and all differences were considered significant if $\alpha = 0.05$ level.

2. RESULTS

2.1. Capture of individuals

A total of 43 individuals (23 male and 20 female fox squirrels) were captured across both fall (4 male and 6 female melanistic; 7 male and 4 female rufus) and spring (7 male and 5 female melanistic; 5 male and 5 female rufus) for use in the body condition portion of this study. This resulted in a sample size of 21 individuals (11 male and 10 female fox squirrels) for fall and 22 individuals (12 male and 10 female fox squirrels) for spring.

Individual total body mass of captured individuals in the fall were 619.8 ± 56.4 g (female melanistic), 568.2 ± 33.0 g (female rufus), 809.0 ± 30.5 g (male melanistic), and 470.6 ± 50.8 g (male rufus). In spring, individual total body mass was 670.4 ± 51.8 g (female melanistic), 690.0 ± 33.0 g (female rufus), 683.0 ± 31.1 g (male melanistic), and 683.6 ± 40.2 g (male rufus).

2.2. Body Condition

Pre-winter body condition was higher in melanistic (25.5 ± 1.8 g) compared to rufus (20.3 ± 3.6 g) fox squirrels ($t_{19} = 4.32$, $P = 0.0004$; Fig. 1). However, post-winter body condition was not significantly different between melanistic (22.8 ± 1.4 g) and rufus (23.9 ± 0.8 g) fox squirrels ($t_{19} = 0.04$, $P = 0.97$). There was also a significant difference between pre- and post-winter body condition within rufus fox squirrels ($t_{20} = 4.85$, $P < 0.0001$) but not between pre- and post-winter body condition within melanistic fox squirrels ($t_{18} = -0.92$, $P = 0.37$).

2.3. Surface Temperature

Surface temperatures of both melanistic and rufus fox squirrels were positively correlated with ambient air temperatures (Fig. 2). However, surface temperatures of melanistic and rufus squirrels showed significant differences between the color morphs when under sun or cloudy sky conditions. For melanistic individuals, surface temperature in sun ($F_{1,26} = 34.61$, $P < 0.0001$) and cloudy ($F_{1,26} = 17.91$, $P = 0.0003$) conditions were both positively correlated with ambient temperatures (Fig. 2A). In addition, there was no significant difference in either intercept ($T_1 = 0.38$, $P = 0.7$) or slope ($T_1 = -0.68$, $P = 0.5$) for melanistic surface temperature in melanistic squirrels exposed to sun or cloudy skies. In fact, melanistic individuals in both sun and cloudy conditions had surface temperatures that were the same across all ambient temperatures measured. In contrast, surface temperatures for rufus individuals were positively correlated with ambient temperature in sun ($F_{1,45} = 100.5$, $P < 0.0001$) but not cloudy ($F_{1,29} = 1.26$, $P = 0.27$) conditions (Fig. 2B). Consequently, rufus individuals differed in their slope ($T_1 = 0.20$, $P < 0.0001$) and intercept ($T_1 = 3.43$, $P = 0.0001$) when under sun or cloudy conditions.

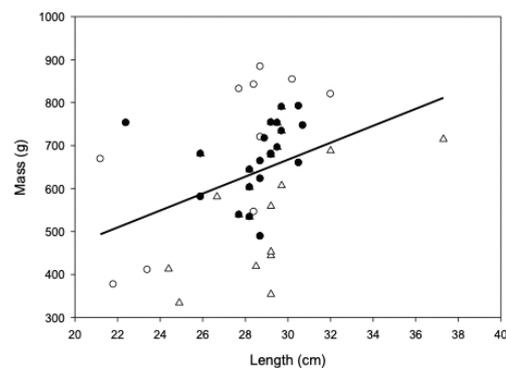


Figure 1. Figure 1. Pre- (open) and post-winter (solid) body condition (residuals of length vs. mass) in melanistic (circle) and rufus (triangle).

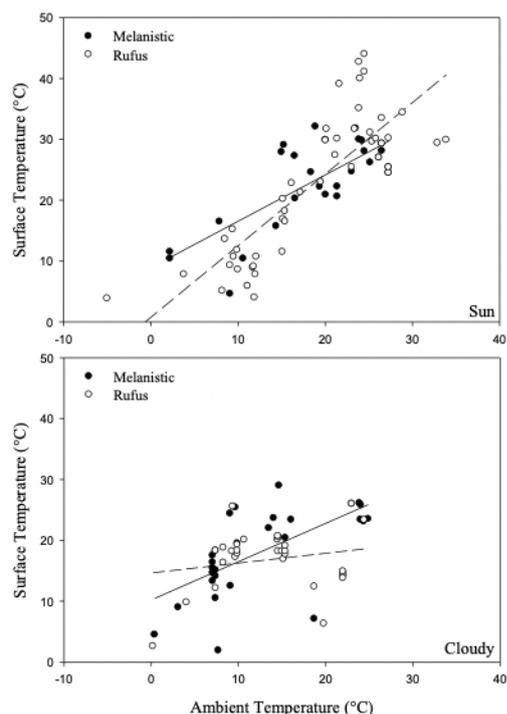


Figure 2. Surface (skin/fur) temperature for melanistic (black circles) and rufus (open circles) fox squirrels under sun and cloudy conditions.

When comparing squirrel coloration (melanistic vs. rufus) under the same sky condition, we found that, under sunny skies, surface temperatures of melanistic and rufus showed a similar response with surface temperatures increasing with increasing ambient temperatures. However, rufus squirrels had a significantly higher slope (1.17 rufus vs. 0.76 melanistic; $T_1 = -2.16$, $P = 0.03$), which resulted in melanistic individuals having a significantly higher intercept (8.89 melanistic vs. 0.73 rufus; $T_1 = 2.11$, $P = 0.04$). Under cloudy conditions, melanistic individuals maintained a similar response to ambient temperatures as was seen under sunny conditions; however, only the slope (0.63) was significantly different ($T_1 = 2.25$, $P = 0.03$) from rufus individuals. In fact, because there was no significant relationship between ambient temperature and surface temperature in rufus squirrels under cloudy skies (slope does not significantly differ from zero, see statistics above), only melanistic individuals had their surface temperatures respond to increased ambient temperatures under cloudy skies.

2.4. Squirrel Activity

Individual squirrels were active from just after sunrise to just before sunset (Fig. 3). Fox squirrels showed a bimodal pattern of activity (morning and afternoon) during spring and summer and a unimodal pattern of activity, occurring throughout mid-day (approximately 11:00–14:00), in fall and winter. Differences in the hourly activity level of rufus and melanistic squirrels were detected for fall ($\chi^2_1 = 37.4$, $P < 0.001$), winter ($\chi^2_1 = 6.74$, $P = 0.01$), and spring ($\chi^2_1 = 23.6$, $P < 0.001$) but not for summer

($\chi^2_1 = 0.7$, $P = 0.4$). During fall, rufus individuals were more active ($39.7\% \pm 6.3\%$) than melanistic ($18.5\% \pm 3.2\%$) individuals. However, during spring and winter, melanistic squirrels were more active ($10.5\% \pm 1.8\%$ and $42.5\% \pm 6.0\%$, respectively) than rufus ($8.7\% \pm 2.5\%$ and $29.2\% \pm 5.4\%$, respectively) individuals. Summer activity levels were not statistically different between rufus ($13.9 \pm 2.5\%$) and melanistic ($9.2 \pm 1.6\%$) individuals.

2.5. Squirrel Metabolism

The metabolic response curves of both color morphs of fox squirrel showed the classic response with respect to lower temperatures. Once below the thermal neutral zone, metabolic rate increased with decreasing ambient temperature (Fig. 4). The lower critical temperature was calculated to be 28.63°C (Fig. 4). An upper critical temperature could not be determined by the data in this study, meaning that the thermal neutral zone for fox squirrels ranges from 28.63°C to at least 35°C .

After data analysis, it can be concluded that the two color morphs of fox squirrels do not have significantly different metabolic response curves. This conclusion is supported by two measures, the first being the comparison of the two GAMM models. The intercept of the model with the shared curves, or model 1 (intercept = 1.43), was equal to the that of the model with the separate curves split by color, or model 2 (intercept = 1.43). In addition, the R^2 values of models 1 and 2 were very similar (0.705 vs. 0.702), indicating that the variability was similar between color morphs. However, model 1 (AIC = 12.41) had slightly more support than model 2 (AIC = 123.43). In addition, there was no significant difference ($t = 1.72$, $P = 0.087$) in the regression residuals for rufus or melanistic individuals on the metabolic curve.

3. DISCUSSION

In this study, pre-winter body condition was higher in melanistic fox squirrels, but following winter, rufus and melanistic squirrels had similar body conditions. These results show that rufus individuals decreased their body condition across winter, whereas melanistic individuals maintained the same body condition throughout winter. In addition, surface temperatures also showed a difference between rufus and melanistic individuals. Both color morphs increased their surface temperature when ambient temperatures increased; however, melanistic squirrels maintained the same surface temperature in both sunny and cloudy conditions, effectively equalizing cloudy and sunny conditions. In rufus squirrels, their surface temperatures increased in sunny conditions, but there was no relationship between ambient and surface temperature in cloudy conditions. All squirrels were diurnally active with morning and afternoon peaks in activity during spring and summer and a single peak in activity during the mid-day during fall and winter. Activity levels were higher in rufus individuals during fall, but higher in melanistic individuals during spring and winter. There was no difference between rufus and melanistic activity levels during summer months. Finally, there was no difference in the metabolic rate

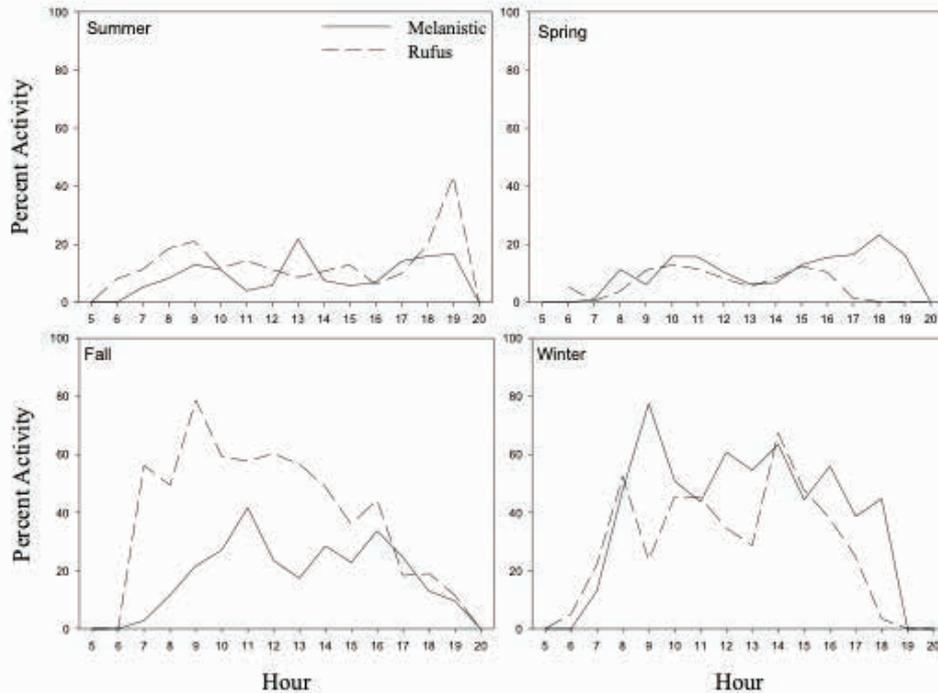


Figure 3. Hourly percent activity of melanistic (solid) and rufus (dashed) fox squirrels by season.

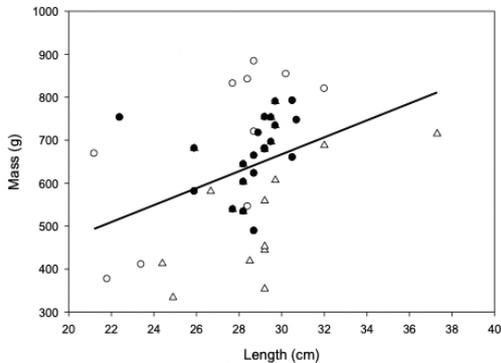


Figure 4. Rate of metabolism (black line) of rufus (open circles) and melanistic (closed circles) fox squirrels as a function of temperature with a lower critical temperature of 28.6°C.

of rufus and melanistic individuals indicating that internal heat production was equal between the color morphs.

In this study, there was a significant difference between melanistic and rufus fox squirrel pre-winter body condition, with melanistic fox squirrels having a greater pre-winter body condition than rufus fox squirrels. Eastern fox squirrels do not hibernate; therefore, they must accumulate enough body fat, or store enough food, to survive winter. In addition, fox squirrels must be active during the coldest months of the year, which may place a high energetic burden on them as they generate heat to offset cold ambient temperatures. Increasing the amount of solar heat gain in the fall and winter could lower the demands for metabolic heat production and allow fat reserves to remain at higher levels.

Similarly, solar heat gain could also allow melanistic individuals with higher surface temperatures to become active earlier in the day and remain active longer. In this study, we found that melanistic and rufus fox squirrels had similar levels of activity in summer but varied in spring, fall, and winter. During fall months, rufus individuals were about 1.5 times more active throughout the day than melanistic individuals. However, in spring and winter, melanistic fox squirrels were approximately 30% more active in the mornings (before 13:00) compared to rufus squirrels. This would give melanistic individuals more foraging time during the winter and would be especially important given our observation that there was no difference in metabolic heat production between the color morphs.

The results of this study provide evidence that melanistic squirrels receive some thermal benefit through the maintenance of higher surface temperatures compared to rufus fox squirrels. This thermal advantage may allow melanistic fox squirrels to forage both earlier and later into the day and possibly allow melanistic individuals to maintain a higher fat reserve throughout the winter. However, our sample size for body condition was too low to conclusively state whether this observation was completely valid. Additional research is needed to determine if there is a link between the thermal benefits of increased solar absorption that maintains higher surface temperatures, metabolism, and body condition. Specifically, our results for surface temperature conflate the complicated nature between the conduction of heat among the air, the outer layer of fur, the inner layer of fur, and the body core. Almost no studies have looked at this complete process, but several studies have investigated individual components. This study investigat-

ed the combined effect of solar heat gain on the outer surface of two color morphs in living fox squirrels, but more studies are needed to determine the specific heat transfer dynamics between the squirrels and their environment.

In this study, surface temperatures in melanistic fox squirrels increased with increasing ambient temperatures under both sun and cloud conditions. In fact, the rate of increase in surface temperature in melanistic individuals did not differ between sunny and cloudy conditions. Rufus fox squirrels, by comparison, showed higher surface temperatures with increasing ambient temperatures under sunny skies but did not have any connection between surface temperatures and ambient temperatures under cloudy conditions. In addition, under sunny skies, melanistic squirrels surface temperatures were lower than rufus surface temperatures at ambient temperatures $>20^{\circ}\text{C}$ but were lower than rufus surface temperatures at ambient temperatures $<20^{\circ}\text{C}$. This varied effect in the melanistic individuals may be the result of a combination of absorption properties of the fur and changes in the position of the fur or the underlying blood flow. Regardless of the mechanism, melanistic fox squirrels in Omaha appear to be able to adjust their thermodynamics more than the rufus individuals, which may give them an advantage at both cold and hot ambient temperatures.

The hypothesis that fur coloration, especially melanism, can affect the thermoregulation of endothermic individuals has been suggested for many years and has found both support (Hamilton & Heppner 1967) and refutation (Walsberg & Wolf 1995). Thermal benefits of melanism have been suggested to derive from direct effects of increased absorption of solar radiation (Ellis 1980) or lower heat conduction (Innes & Lavigne 1979). However, benefits may also be the result of indirect pleiotropic effects such as differences in heat conductivity (Geen & Johnson 2014), differences in hair structure (Fratto & Davis 2011), or alterations in behavior (Ibanez *et al.* 2016).

Heat transfer from an animal to the environment (or vice versa) can be split into two major pathways: (1) transfer of heat from the body core to the surface of the body and (2) transfer of heat from the body surface to the environment (Wooden & Walsberg 2000). How much solar radiation is absorbed by the body of the mammal is determined by the physical properties of the hair (i.e., coloration, position, and density; Walsberg & Wolf 1995). For example, if most of the solar radiation is absorbed by the outer layer of fur then most of that heat will be transferred to the surrounding air and not incorporated into the animal's heat budget. However, if the sunlight penetrates deeply into the fur coat then it will contribute to the heat load of the mammal (Walsberg *et al.* 1978; Grojean *et al.* 1980; Walsberg 1983). Walsberg (1990) showed this in golden-mantled ground squirrels, where populations living in the arid Arizona desert had lighter coat color that reflected more (29%) light compared to those living in colder, wetter Washington state whose darker fur color reflected much less (19%) light.

When photons enter the layer of fur that surrounds a mammal, it can be absorbed or scattered back into the atmo-

sphere, and the optical properties of the fur dictate the fate of the photon (Walsberg 1992). Animals can alter the solar absorptive properties of their fur coat and control the amount of solar heat gain. Increasing the depth of light penetration into the fur will increase the solar heat gain and reduce the heat loss to the environment (Walsberg *et al.* 1978). Specifically, depressed (flat) fur has higher reflectivity compared to erect fur, which allows solar radiation to penetrate deeper into the fur, increasing the solar heat gain (Cooper *et al.* 2003). In addition, many mammals have fur that varies in color (dark and light bands) along the shaft of the hair and have a fine inner layer of hair and a coarser outer layer (Walsberg 1988). Walsberg (1988) found that outer coat reflectivity was greater than that of the inner coat. This was largely due to the lower absorptivity and higher forward scattering of light by the hairs in the outer coat. In addition, changes in hair morphology often accompanies the summer and winter coats of most mammals and can result in a 16–22% reduction in the solar heat gain of summer compared to winter fur (Walsberg & Schmidt 1989).

Animal surfaces absorb 60–95% of the long-wave radiation that is intercepted; however, the actual amount of absorbed radiation is affected by the animal's position, its shape, and the physical properties of its fur (Walsberg 1988; Walsberg 1992; Walsberg & Wolf 1995). For example, if most of the solar radiation is absorbed by the outer layer of fur then most of that heat will be transferred to the surrounding air, but if the sunlight penetrates deeply into the fur coat then it will contribute to the heat load of the mammal (Walsberg *et al.* 1978; Grojean *et al.* 1980; Walsberg 1983).

Solar heat gain can be beneficial or detrimental, depending on the specific circumstances. For example, in colder ambient temperatures, solar radiation increases the heat load of a mammal such that it is the equivalent of increasing ambient temperature by 5–8°C (Walsberg & Wolf 1995). Although being warmer on cold days seems like a benefit, increasing the temperature gradient between the animal and the surrounding air may also increase the conduction of heat out of the animal (McNab 2002). Similarly, mammals in hot environments are at risk of hyperthermia and increasing the surface temperature of the animals can decrease the thermal gradient between the skin and air, thereby reducing heat absorption by the animal. Animals that can adjust the amount of solar heat gain would have an adaptive advantage, especially in heterothermic environments (Wooden & Walsberg 2000).

Solar heat gain in an animal is defined as the reduction in metabolic rate because of the presence of solar radiation and can result in a 19–42% reduction in metabolic rate (Walsberg & Wolf 1995). In mammals, the temperature gradient between the body ($\sim 37^{\circ}\text{C}$) and the air can range widely and the combination of wind speed and solar radiation can result in reducing this gradient by 22–67% (Walsberg & Wolf 1995). Solar radiation, alone, can reduce daily energy expenditure by 6–12% (Walsberg and Wolf 1995). As a result, energy savings from solar heat gain can effectively reduce the amount of metabolic energy required for thermoregulation, which can

impact how much metabolic energy is available for reproduction, growth, and maintenance. This is especially true for small mammals, whose surface area-to-volume ratio promotes heat loss (Walsberg & Wolf 1995).

Although the results of this study show that melanistic fox squirrels derive some thermal benefit that may allow them to be more active in the spring and winter, our results should be interpreted with caution. The mechanism that animals adjust their thermal environment is complex and involves numerous factors including metabolism (heat production), blood flow, and external heat transfer (conduction, convection,

evaporation, and solar radiation). The rate at which animals gain or lose heat from the environment is also dictated by skin and fur properties, which impact conductivity values. Measuring all of the factors involved in thermoregulation is a monumental task that has been attempted by very few researchers, most notably Walsberg and associates (see above discussion). In this study, we attempted to measure a small part of the overall process and demonstrate that melanistic individuals in the Omaha in Nebraska area derive some thermal benefit from melanism.

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