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Pelage color of red bats *Lasiurus borealis* varies with body size: An image analysis of museum specimens

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Abstract Mammalian pelage color can vary among individuals of many species, although this intraspecific variation is often overlooked by researchers, perhaps because of its sometimes subtle nature and difficulty in assessing it quantitatively. Thus, such variation is rarely studied in mammals, and this is especially true within the order Chiroptera, where there has been very little empirical research. We examined museum specimens of red bats (*Lasiurus borealis*, family Vespertilionidae) from Georgia, USA, to determine the extent of sexual dimorphism in pelage color and to explore possible associations between body size and pelage color. We photographed 54 specimens under uniform lighting, and used an image analysis program to measure pelage hue on the uropatagium region, which is fully furred in members of the genus *Lasiurus*. Statistical analyses of pelage hue scores showed males had significantly redder pelage than females when considered alone, but when examined together with effects of body size and collection year, sex was not significant, and collection year and body size were. More recent specimens tended to be less red than older specimens, which might indicate a wearing of the buffy tips of hairs from older specimens, and smaller bats of both sexes tended to be more red. These interesting findings are encouraging and we suggest that future explorations into intraspecific variation in pelage color of bats using this or similar approaches are warranted to clarify the significance of the patterns. This study also demonstrated that care must be taken in analyses of mammalian pelage color from older museum skins, or at least that researchers must take into account the age of the specimens [*Current Zoology* 56 (4): 401–405, 2010].

Key words Pelage color, Sexual dimorphism, Red bats, *Lasiurus borealis*, Image analysis

Mammalian fur, or pelage, shows a considerable degree of inter- and intraspecific color variation, yet this variation is rarely studied empirically (Caro, 2005). Research on pelage color of mammals has not undergone the same expansion of studies, ideas and techniques that has occurred in the past two decades within the field of avian plumage color (Hill, 2002; Hill and McGraw, 2006). To some degree, this may stem from the more brightly-colored plumage of birds, which may elicit more attention by researchers. In contrast, most mammalian species have coats that appear drab in color, and may range only in shades of brown to black. Nevertheless, pelage color usually varies to some degree across individuals of many mammalian species, often very subtly to the human eye, yet the biological reasons for this variation are not clear.

Although cross-species comparisons of mammalian pelage color have been performed to elucidate why species display particular color patterns (Caro and Stankowich, 2010; Caro, 2009), the few prior studies examining between-individual (intraspecific) variation in color have focused only on the degree of color-matching between fur and environment (Heth et al., 1988; Carraway and Verts, 2002). Furthermore, the measurement of pelage color in most studies of mammals has been largely performed using subjective matching of fur to Munsell Color Chips, which is a useful, but decades-old approach (McCarley, 1956). In recent years, image analysis, whereby digital photographs of specimens are examined using computer software, has increasingly been used to quantify minute variation in integument color across many animal species (Davis et al., 2007; Davis and Grayson, 2007; Kause et al., 2008; Sezer and Tekelioglu, 2009). This approach is easily adaptable to the study of mammalian pelage color, especially for small mammals such as rodents or bats, which can be readily photographed, and under standardized conditions.

The red bat (*Lasiurus borealis*, family Vespertilionidae) is a common insectivorous bat found in eastern North America, and is a species for which little is known of the significance of its pelage color. As the name implies, red bats have tan- to reddish-colored fur over most of their body (including their tail membrane),

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and males are known to be generally more red than females (Nowak, 1991; Whitaker and Hamilton, 1998) based on field observations. In fact, this species is one of only a few mammals for which sexual dimorphism in pelage color exists. The reasons for this sexual dimorphism are not known. Further, cursory observation of museum specimens indicates that there is a non-trivial degree of variation in the shade of red pelage among individuals (Davis, *pers. obs*). Here, we report the results of a descriptive study of pelage color of a collection of prepared red bat specimens that were examined using a photographic image analysis approach, and where possible differences in pelage color between sexes and in relation to body size were explored.

1 Materials and Methods

1.1 Measuring specimens

We obtained 54 prepared skins of red bats from the Georgia Museum of Natural History, in Athens, GA, USA. All specimens were collected in the state of Georgia between 1940 and 1993. All specimens were adults and 27 were females, while 27 were males (based on data provided with specimens). We recorded the total body size of each bat that was listed with the specimen (which was recorded before preparation). This is a standard measure of body size in bats and represents the length between the nose to the tip of the bony tail on the uropatagium. The bat skins had all been prepared in generally the same fashion, in that the wings were folded and the lower legs spread to display the uropatagium (Fig. 1). We photographed each specimen from above using a digital camera attached to a copystand, which maintained a standardized distance from all specimens. There was no flash used in the pictures; lighting was provided by two lamps above the copystand. The photography was performed in a room in the museum, and all specimens were photographed in the same session. These conditions served to minimize the variation in lighting between pictures. Moreover, the background color of the copystand was a neutral grey (Fig. 1), which served as a color standard for digitally adjusting the lighting of the images (below).

All images were imported into an image analysis program (FoveaPro, Reindeer Graphics, Inc.), that has been used before in similar applications (Davis et al., 2004; Davis et al., 2007; Davis and Grayson; 2007, Todd and Davis; 2007, Davis and Grayson, 2008; Davis, 2009). Given that each specimen was preserved so that the uropatagium was spread out and somewhat flattened, we selected this area to measure pelage color. The fur in this area also tended to be somewhat uniform (i.e. not matted or bent over, as sometimes happened on the dorsal surface). For each specimen, we drew a circle (on the image) over the uropatagium that filled this area (Fig. 1). We then ran a color measure routine within FoveaPro that returned the average hue, saturation and density scores of all pixels within the circle. At the picture resolution we used (8 megapixels) most circles contained over 50,000 pixels. For the purposes of this study we focused only on the hue scores, which we felt reflected the majority of the variation in ‘redness’ across the specimens (Fig. 1). Hue is measured in degrees, with 0 degrees being perfect red so that lower numbers indicate ‘redder’ pelage (Fig. 1). Finally, to ensure constancy of lighting conditions across all pictures, we adjusted the pelage hue scores based on measurements of the hue of the copystand background in each bat image. This ensured that any minor variation in lighting across pictures was standardized.

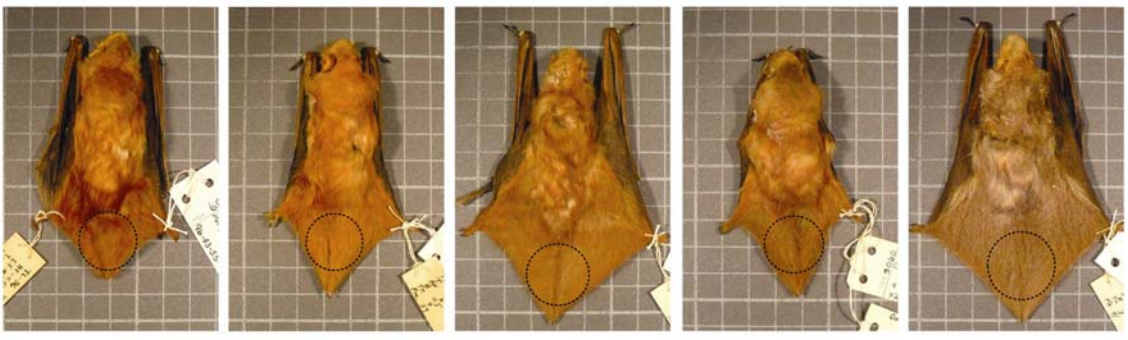

Fig. 1 Photographs of red bat specimens that show the range in pelage hue scores (indicated below specimen) observed in this study
Dashed circles indicate where pelage color was measured.

1.2 Repeatability of hue scores

A subset of the bat specimens (n=10) were photographed three times each, and in each case, the specimen was removed from the copystand and replaced before the next photograph. From these images, we collected data on pelage hue scores as outlined above, and examined the repeatability of the color scoring procedure following Lessells and Boag (1987). This exercise resulted in a repeatability score (i.e. intraclass correlation coefficient) of 0.95, which demonstrates that the color scoring system used here had 'very high repeatability' (Measey et al., 2003).

1.3 Data analysis

We used analysis-of-covariance to examine the variation in pelage hue (response variable) due to sex and body size (i.e. total length, included as a covariate) across the 54 red bat specimens. Because of the possibility that older skins may have faded over time (Armenta et al., 2008, Doucet and Hill, 2009), we included year of collection as another covariate in the model. We also included the interaction effect of sex × body size. Because data on body size was missing from 10 specimens, the total degrees of freedom in the final model with main effects only was 43. The analysis was conducted using Statistica 6.1 software (Statistica 2003) and significance was accepted when P<0.05.

2 Results

Across all specimens with body length data (n=44), the average total length of females (105.6 mm ± 8.1SD) was greater (two-sample t-test, t= -3.37, P=0.002) than in males (97.7 mm ± 7.4 SD). Pelage hue scores ranged from 22.3 (the reddest) to 33.4 (the least red) and displayed an approximately normal distribution (Fig. 2). As Fig. 2 shows, when considered alone, hue scores of males appeared to be lower (i.e., they were more red) than females, and a Student's t-test reflected this difference (t=–3.17, df=52, P =0.003). However, when the effects of sex, body size and specimen age were considered simultaneously in the ANCOVA model, sex was not significant ($F_{1, 39}$=0.01, P=0.941). In this model, the effect of specimen age (i.e. year of collection) was significant ($F_{1, 39}$=4.85, P=0.034), as was the effect of body size (total length; $F_{1, 39}$=9.48, P=0.004). For the specimen age effect, the relationship was positive (r=0.32, P =0.018), such that more recent specimens had *higher* hue scores, or were *less* red, than the older specimens. In terms of body size, smaller bats tended to have lower hue scores (i.e. were redder; Fig. 3). As the interaction of sex × body size was not significant in this model ($F_{1, 39}$=0.003, P=0.957), this relationship between size and color was similar for males and females. Collectively, these results demonstrate that while male red bats tend to be redder than females, this pattern is the result of males tending to be smaller than females, and small body size in this species is associated with redder pelage.

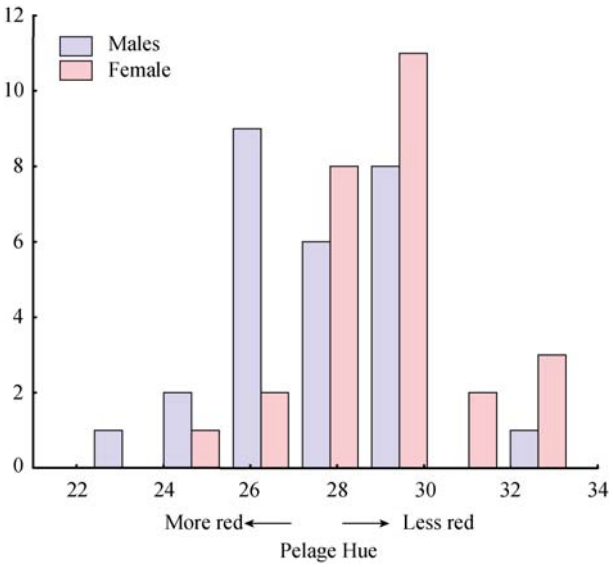

Fig. 2 Frequency-distribution of pelage hue scores for all male (n=27) and female (n=27) red bat specimens.

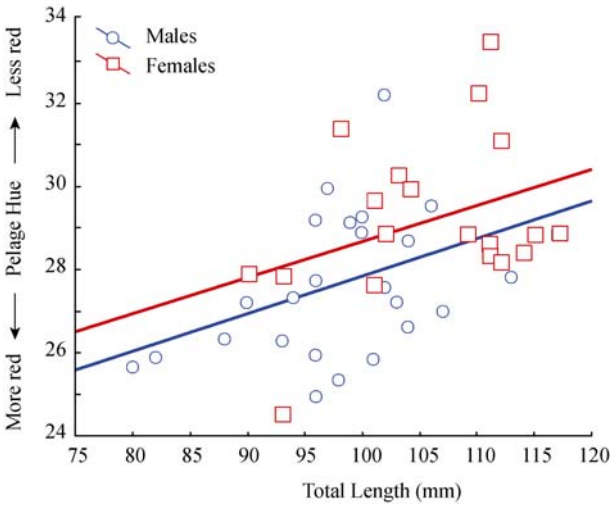

Fig. 3 Relationship between red bat size (total length, measured prior to skin preparation) and pelage hue

3 Discussion

Little is known about the biology of pelage in bats in general, or why individual bats, of any species, would vary in the shade of their pelage color. As such, this study was largely exploratory and descriptive in nature,

with the hope that it will stimulate further investigation. The first pattern of interest found was the overall difference between male and female pelage color, which was consistent with prior anecdotal observations of bat in the field (Whitaker and Hamilton, 1998). Here, the image analysis scoring of pelage hue demonstrated that males in this species tend to have more reddish pelage than do females (Fig. 2). Surprisingly however, this result appears to be an artifact of the smaller size of males, which given the relationship between size and pelage color in this species (smaller individuals tend to be redder; Fig. 3), means that males are the redder sex simply because they are smaller than females. This finding was indeed intriguing, although we offer little speculation into its cause until further study is undertaken by us or other investigators. One possible avenue to pursue for this work would be investigating the physiological or ecological fitness (using live specimens) of individuals across the range of pelage colors, to determine if pelage color is a visual signal to conspecifics of (or is a consequence of) optimal or suboptimal health. This could involve exploring the associations between pelage color and body condition scores or parasite loads (Lourenco and Palmeirim, 2007, Reckardt and Kerth, 2009), or estimation of survival and reproductive success of individuals through mark-recapture studies (e.g. Kerth and Melber, 2009).

A surprising result found here was the trend for newer specimens to be duller (i.e. less red) in color. In the analysis of pelage hue, we had included year of collection as a covariate only to account for possible effects of specimen *fading* over time, which is known to occur in avian specimens (Armenta et al., 2008). Since newer specimens tended to be duller in color than older specimens, it would appear that pelage color-fading was not an issue. It is not clear why older red bat specimens would be redder. Cursory examination of several older and newer specimens revealed no accumulation of dust on the specimens, nor oils on the fur (i.e. from human handling) that might influence the overall color. However, microscopic examination of hair fibers on the specimens revealed one possible explanation. Most red bat hair fibers have a red-colored shaft with a lighter-colored or buffy tip (Davis, *pers. obs.*), and in several older specimens we noted that many of these tips were worn off, probably as a result of routine museum handling over a long time period. If this occurred over most of the body, this could result in a redder coat color overall in the older specimens. To be sure of this point, more systematic and detailed morphological measurements of hair fibres would need to be obtained from older and newer specimens, which interestingly, can also be addressed using image analysis (Davis, in press). For now, this result emphasizes the importance of considering the specimen age in future investigations into mammalian pelage color.

Aside from early attempts to subjectively assess pelage variation across seasons (Constantine 1957, 1958), to our knowledge, this was the first study to quantitatively measure intraspecific variation in pelage color of a bat species using objective methods, and the intriguing results obtained suggest there is merit in doing so. Recent work examining individual variation in pelage color of other small mammals is also encouraging (Camargo et al., 2006; Lai et al., 2008), and emphasizes the growing interest in this line of research. In addition, studies of individual variation in the darkness of male lion manes, which convey information regarding the age, dominance and nutritional condition of males, should also be mentioned (e.g. West and Packer, 2002). However, results from the studies of lions may not be comparable to the current one because the degree of mane *darkness* in males is the result of variation in levels of eumelanin in the hair fibers, while in red bats the red hair color is likely attained via a mixing of eumelanin and pheomelanin, as in seen in other small mammal species (Singaravelan et al., 2010).

Finally, we note that there are many questions engendered by these results that could be addressed in future investigations. For example, why would smaller individuals be the reddest? Is there a trade-off between large size and pigment production? And why does this nocturnal mammal have red pelage in the first place? Is it simply a form of background color-matching to blend in with roost habitats (Fenton, 1992)? If so then why would there be variation in the degree of red that is associated with body size? With respect to other bat species, we also wonder if a similar relationship between size and pelage color exists in other bats? We expect that these and other questions will be addressed in the future, now that digital photographic technologies exist that can aid such research, and we hope that this paper provides a useful methodological and conceptual framework upon which to build.

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