

Colour phases of the eastern screech owl: a comparison of biomechanical variables of body contour feathers

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Summary

1. Melanin is a ubiquitous pigment in the animal kingdom and has been reported to increase biological tissue strength. Melanin is responsible for many observed cases of colour polymorphism, or existence of two or more distinct plumage colours in one species (regardless of age, sex, or mass). Researchers have postulated that the relative concentration of melanin pigments deposited in feathers may function to strengthen feathers but colour polymorphic species have been ignored. Here, we investigate whether different morphs of a polymorphic bird species exhibit differential plumage strength.
2. While colour polymorphism is widespread in birds, there is no consensus on the adaptive significance of polymorphism or the maintenance of clinal variation in colour morphs. The eastern screech owl (*Megascops asio*) is a polymorphic species; individuals exhibit rufous, intermediate, or grey coloration that is likely caused by relative amounts or concentration of black or rufous melanin subtype (eumelanin and pheomelanin respectively). This species exhibits clinal variation in morph prevalence; the rufous morph predominates in humid deciduous forests while the grey morph is more common in dry or cold environments.
3. Here, we test the hypothesis that differences in feather strength may select for darker plumage in drier climates. First, we use reflectance spectrometry to objectively verify differences in colour morphs of the eastern screech owl. Second, we measure mechanical variables and morphology of dorsal feather barbs.
4. While reflectance spectrometry objectively separated the three colour morphs, we found no significant differences in the mechanical variables of plumage morphs. These results fail to demonstrate differences in plumage strength among eastern screech owl colour variants.
5. This conclusion rejects the hypothesis that melanin subtype affects the strength of body contour feathers in the eastern screech owl and may not factor in the maintenance of the observed clinal variation.

Key-words: clinal variation, colour polymorphism, melanin, microscopy, strength

Introduction

Melanin is the oldest and most ubiquitous light absorbing pigment in the Animal Kingdom (Voitkevich 1966; Needham 1974) and is responsible for most black, grey, brown, and rufous animal coloration (Theron *et al.* 2001; Mundy & Kelly 2003; Mundy *et al.* 2003; McGraw 2006). Variation in melanin-based coloration is influenced by both melanin subtype (eumelanin = black or pheomelanin = rufous) and the relative and absolute amount of melanin pigments deposited into tissue (McGraw 2006). While quantitative analysis through liquid chromatography has been used to

understand how melanin subtype and pigment concentration contributes to intraspecific variation (McGraw, Safran & Wakamatsu 2005), the procedure is difficult and thus rarely emphasized (Ito & Fujita 1985; Ito 2003). The function of melanin deposition in biological tissues has been well studied and implicated in disease resistance (Hadley 1999), protection and strength (reviewed in Bortolotti 2006), crypsis (reviewed in Bortolotti 2006), and sexual signalling (reviewed in Jawor & Breitwisch 2003).

Melanin deposition in tissues has been proposed to protect and strengthen bird feathers (reviewed in Burtt 1986; Bortolotti 2006). The function of melanin in protection and strength has been studied with various mechanical and microbiological experiments designed to test the relative strength of

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differently coloured tissues. In the willow ptarmigan (*Lagopus lagopus* Linnaeus 1758) flight feathers have melanic and non-melanic areas (stripes). The melanic areas of willow ptarmigan flight feathers exhibit greater Vicker's hardness (the materials' ability to resist mechanical deformation) compared to the non-melanic areas (Bonser 1995). Burtt (1986) measured abrasion resistance by blowing powdered glass on flight feathers of species' in the family Parulidae and determined that melanized barbs fracture less than non-melanic barbs. In addition to mechanical studies, melanin presence has also been implicated in microbiological resistance. Goldstein *et al.* (2004) found that melanized feathers of the white leghorn chicken (*Gallus gallus* Linnaeus 1758) are more resistant to bacterial degradation than unmelanized feathers. Field observations have also provided support that melanin may increase tissue strength. For example, the flight and tail feathers of an albino great frigatebird (*Fregata minor* Gmelin 1789) (Schreiber *et al.* 2006) and an albino flight feather of a yellow-rumped warbler (*Dendroica coronata* Linnaeus 1766) (Barrowclough & Sibley 1980) exhibited greater feather wear than their pigmented counterparts.

While previous studies have reported that melanin pigmentation may increase cortical thickening (Voitkevich 1966; Burtt 1986; Goldstein *et al.* 2004) they fail to account for variables such as cross-sectional area, breaking force (F_{brk}), breaking stress (σ_{brk}), and breaking strain (ϵ_{brk}) in feathers used in strength or bacterial degradation experiments. Butler & Johnson (2004) were the first to compare the F_{brk} of white and melanized areas of osprey (*Pandion haliaetus* Linnaeus 1758) feathers. Although melanized barbs exhibited greater F_{brk} there was no significant difference in the σ_{brk} (F_{brk} divided by cross-sectional area) for unmelanized and melanized barbs. Moreover, all mechanical differences between melanized and unmelanized barbs disappeared when the researchers considered the morphological parameters of the breakage position on the feather barb. Butler & Johnson (2004) hypothesized that previous studies had measured the strength of feather fragments of different sizes and proposed that the relationship between relative pigmentation and strength was not adequately addressed. Although Butler & Johnson (2004) demonstrated no effect of melanin deposition on mechanical strength of differently pigmented feathers fragments, they and other previous researchers (Burtt 1986; Schreiber *et al.* 2006) have used flight feathers, which have been subjected to selective pressure for increased strength due to the stresses of flight (Corning & Biewener 1998). Additionally, past research has ignored melanin subtype (pheomelanins and eumelanins), which may have different molecular interactions as a consequence of differential amino acid incorporation during biochemical synthesis (Needham 1974). Differences in concentrations of melanin subtype may also affect feather strength. To date, there has been no study that has tested whether intraspecific colour differences, likely due to differential melanin subtype, influence the strength of body feathers of bird plumage.

Colour polymorphism is an extreme form of intraspecific variation in coloration, and occurs when two or more dis-

tinct colours exist in one species regardless of age, sex, or mass (reviewed in Roulin 2004). Colour polymorphism is present across many varieties of animals but is especially apparent in birds. In Aves, colour polymorphism is most common in owls and nightjars (33% of species) but is also present in 61% of the other orders and 3·5% of all avian species (Fowlie & Kruger 2003; Galeotti *et al.* 2003; Galeotti & Rubolini 2004). Within the Strigiformes, colour polymorphism has been associated with important ecological variation, such as clinal variation in eastern screech owls (*Megascops asio* Linnaeus 1758) (Gehlbach 1994) and tawny owls (*Strix aluco* Linnaeus 1758) (Galeotti & Cesaris 1996), adult survivorship in eastern screech owls (VanCamp & Henny 1975) and tawny owls (Brommer, Ahola & Karstinen 2005), and parasitism in tawny owls (Galeotti & Sacchi 2003). Despite this extensive literature, mechanisms contributing to the adaptive acquisition and maintenance of colour polymorphism are still incompletely understood (Lank 2002).

The eastern screech owl is a colour polymorphic species (Fig. 1) that exhibits a clinal variation in the relative proportion colour morphs (Gehlbach 1994). The species' range extends throughout much of North America; from Canada to Mexico, and from the east coast to the Rocky Mountains (Gehlbach 1995). Principle colour morphs observed in the eastern screech owl are rufous and grey and a low frequency of brown intermediates, a mix of grey and rufous pigments, also exists partially in Florida (Owen 1963a,b). Rufous morphs predominate in warm, humid areas of the range while grey morphs predominate in more dry dusty regions and also in colder northern climates. Variation in physiology (Mosher & Henny 1976; Dexter 1996) and behaviour (Dexter 1996) associated with particular morphs of the eastern screech owl has been proposed to explain this clinal variation (Gehlbach 1994); however, the persistence of differential colour morph proportion across the range has not been completely explained.

The purpose of this study was to investigate the hypothesis that clinal variation in colour morphs might be maintained by an increased ability of dark morphs to withstand the greater abrasive environment of dry, dusty, or windy climates. In accordance with this hypothesis, we predicted that feathers of darker morphs would be stronger than rufous or intermediate forms. First, we used reflectance spectrometry to objectively and quantitatively compare the coloration of each morph. Reflectance spectrometry also allowed us to compare the spectral curves of the eastern screech owl to that of other bird species coloured by eumelanin, pheomelanin, or some mixture of the two. Second, we tested the tensile strength (σ_{brk} , the amount of stress a material is able to withstand when being pulled lengthwise before ultimate failure occurs), toughness (T , the amount of mechanical energy a material can absorb before breakage), and Young's modulus (E , the stiffness of an elastic material) of dorsal feathers from differently colour morphs and quantified the morphological parameters of the breakage point with scanning electron microscopy.

Materials and methods

TEST OWLS AND COLOUR ANALYSIS

We obtained frozen eastern screech owls from the Blue Ridge Raptor Rehabilitation Clinic, Carolina Raptor Center, the American Museum of Natural History, Audubon Society of Florida, North Carolina Museum of Natural Sciences, and the Avian and Exotic Animal Clinic of Indianapolis. Owls with worn or damaged plumage were discarded from the current study. We removed approximately five barbs from the proximal pennaceous portion of the rachis of three dorsal tract feathers from the anterior region for each individual owl. Barbs were carefully selected as to obtain homogenously coloured barbs. We measured spectral reflectance of five whole feathers against a black background (Canson® Cat:#425 Stygian black) using an USB4000 spectrometer (range 250–880 nm; Ocean Optics, Dunedin, FL) with a bifurcated micron fibre-optic probe. The probe was held at 90°, 1 mm from the sample surface, and shielded from stray light by a rubber sheath. The reading area was illuminated with both UV (D-2000 deuterium bulb, Ocean Optics) and visible (tungsten-halogen) bulbs. All data were generated relative to a white standard (WS-1, Labsphere, North Sutton, NH).

Colour was expressed as percent reflectance per nm wavelength and this output was processed using ColoRv1.5 (R. Montgomerie copyright 2002). We generated spectral curves for each measurement location. Because many eastern screech owl plumages are mottled, each colour patch was measured 5 times and the spectral curves averaged. We summarized reflectance data by calculating a standard descriptor of melanin-based spectral data; red chroma (Siefferman & Hill 2003, 2005). Red chroma was calculated as the proportion of the total reflectance (R300–700) that is in the red part of the spectrum (R605–700). This is the first study to utilize spectrophotometric methods to classify birds with continuous colour polymorphism.

MECHANICAL TESTING

One researcher (EP) performed tensile strength tests of barbs from dorsal feathers of eastern screech owl colour variants under the supervision of Dr. Louis T. Germinario of the Physical Chemistry Research Laboratory of the Eastman Chemical Company; Kingsport, TN. Tests were conducted in the same lab in a short time period so humidity was constant. First, we glued barbs (Superglue®, Rancho Cucamonga, CA, USA) between two pieces of polyethylene and inserted them into brass film tension grips. We then extended the barbs at a controlled strain (preload force = 0·0010 newtons (N); initial displacement = 10·0 µm; ramp displacement = 100·0 µm min⁻¹) until breakage in a dynamic mechanical analyzer (DMA) Q800 V7.0 build 113 (Thermal Instruments, Trevose, PA). Barbs that broke within the grips were discarded. We measured σ_{brk} (F_{brk} divided by cross-sectional area) and ϵ_{brk} [breaking extension divided by original barb length (l_0)] using Thermal Analysis Q series and Universal Analysis 2000 data analysis software. T was determined by finding work to break (W_{brk}) (the integration of area under force-extension curve) and dividing by the cross-sectional area of the cortex (s_c) and the l_0 ($W_{\text{brk}} / s_c l_0$). Both T and E (the slope of the stress-strain curve between 0–1% strain) were calculated in Microsoft Excel (Microsoft Office 2007).

SCANNING ELECTRON MICROSCOPY

All microscopy work was conducted by EP at the William C. and Ruth Ann Dewel Microscopy Facility at Appalachian State University.

sity. First, we cut barbs close to the proximal portion of the breakage point and attached the barb to double sided tape adhered to a small mechanical nut. We then attached the nut to a stub with a double sided carbon disc, and sputter-coated with gold. We obtained the micrographs using a FEI Quanta 200 environmental scanning electron microscope (ESEM) (FEI Company, Hillsboro, OR). We measured height (H), width (W), and cross-sectional area of breakage points (barb s_o , s_c , and pith s_m) using AxioVision AC Release 4.3 software.

STATISTICAL ANALYSIS

We used Levene's tests to determine homogeneity of variances on the data. We compared the red chroma of the rufous, intermediate, and grey feathers using one-way ANOVAs and a subsequent least squares difference (LSD) tests in SAS 9.1 (SAS institute, Cary, NC; Zar 2005). We compared the tensile strength, T , and E of the rufous, intermediate, and grey feathers using a one-way ANOVA and a subsequent Duncan test (SAS institute; Zar 2005).

Results

The reflectance (Fig. 2) of grey owls was greater at lower wavelengths (*c.* 300–500 nm) than red and intermediate owls; however, the reflectance of red owls was greater at higher



Fig. 1. *Megascops asio*, the model organism for this study (Photo: Nina Fischesser).

wavelengths (*c.* 500–700 nm). Red chroma differed significantly between all three treatments (Fig. 3; $F = 13.68$, $P < 0.01$, d.f. = 2). Qualitative comparisons of the spectral curves of rufous and grey colour morphs of the eastern screech owl with other published literature from bird species [eastern bluebirds (*Sialia sialis* Linnaeus 1758), barn swallows (*Hirundo rustica* Linnaeus 1758)] in which melanin subtype and spectral reflectance have been quantified suggest that the rufous feathers are coloured by a greater abundance or concentration of pheomelanin pigments while grey morphs likely have a greater abundance or concentration of eumelanin pigments (McGraw *et al.* 2004a,b; McGraw, Safran & Wakamatsu 2005; McGraw 2006). Moreover, comparisons of the absorbance spectra of eumelanin and pheomelanin pigments suggest that eumelanins reflect less light overall while pheomelanin reflect more light in the longer wavelengths (McGraw 2006).

The current study was the first to utilize dorsal feather barbs, which have a mean H and W *c.* 2–3 times smaller than previously published reports that utilized primary flight feathers (Butler & Johnson 2004). Mean cross-sectional areas of the s_c and s_m were *c.* 5–6 times smaller than previously published reports (Butler & Johnson 2004), T was slightly lower than the average of 10.1 MJ m^{-3} reported by Butler & Johnson (2004), and strain (ϵ_{brk}) in the current study was slightly smaller than previously reported values of ~ 0.06 (Astbury & Woods 1934; Butler & Johnson 2004). The range of E was 48.09 – 332.66 MPa , which are in the range for other Young's modulus values reported for feather keratin (7.75–10.0 GPa, Purslow & Vincent 1978; 0.045–0.682 GPa, Macleod 1980; 1.78–2.78 GPa, Bonser & Purslow 1995). Figure 4 shows the breakage points from an eastern screech owl dorsal feather used in the current study compared with breakage points of flight feathers as reported in previous studies. The cross-section of primary feather barbs tend to be elongated with a thick cortex compared to body feathers which have a thinner cortex in proportion to the overall cross-section and are also more rounded.

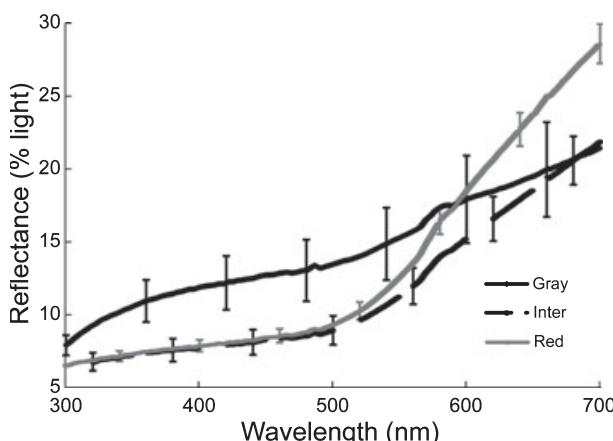


Fig. 2. Average reflectance spectra (with SEM. bars) of the pennaceous region of dorsal feathers of eastern screech owl (*Megascops asio*) colour variants.

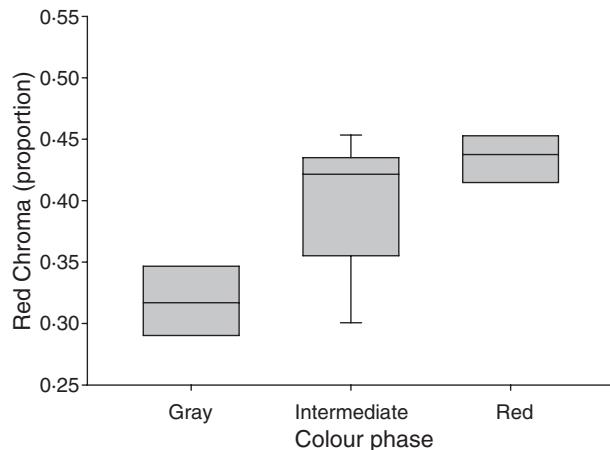


Fig. 3. Average red chroma (605–700 nm) of the pennaceous region of dorsal feathers of eastern screech owl (*Megascops asio*) colour variants. The lines in the box are the median, the upper and lower box borders are the 25th and 75th percentiles, and the upper and lower lines are the 10th and 95th percentiles.

We found no significant differences in mechanical variables, F_{brk} , σ_{brk} , T , E , and ϵ_{brk} of the feathers taken from the three colour morphs (Table 1, Fig. 5). We also found no significant differences in the morphological parameters of the breakage point of rufous, intermediate, and grey feathers (Table 1).

Discussion

We found that colour morphs as categorized by the human eye were verified by reflectance spectrometry. Moreover, qualitative comparisons of the spectral curves of the eastern screech owl with other published literature suggests that rufous feathers are coloured by a greater abundance of pheomelanin pigments while grey morphs likely have a greater abundance of eumelanin pigments. We found, however, no significant differences in the mechanical variables of dorsal feathers of eastern screech owl colour morphs. Although we hypothesized that the greater tensile strength of grey feathers could explain the relatively low frequency of rufous morphs in dry dusty parts of the eastern screech owls range, we found no support for this hypothesis. Because the microstructural anatomy of the feather was considered, the data suggest that melanin subtype does not influence differential feather strength.

We measured mechanical variables of contour feathers rather than flight feathers, and as a consequence, the mechanical values we obtained were much smaller than previous studies. This occurs, in part, because the feather cross-sections are much smaller in area compared to those of the flight feathers previously studied (Fig. 4). Feather morphology (e.g. nodal morphology, barb length, amount of pigmentation etc...) varies greatly, not only between bird species, but within individual plumage tracts and single feathers (Dove 1997; Stettenheim 2000). Because certain feather tracts of the plumage are involved in sustaining airfoils (remiges and

Table 1. Summary statistics from one way ANOVA of morphological measurements and mechanical variables of the breakage point of eastern screech owl (*Megascops asio*) colour variant dorsal feather barbs (Means \pm SEM, ϵ_{brk} values are proportional)

Variable	Colour			<i>F</i>	<i>P</i>
	Grey (<i>n</i> = 26)	Inter (<i>n</i> = 31)	Red (<i>n</i> = 50)		
F_{brk} (N)	0.09 \pm 0.01	0.10 \pm 0.01	0.10 \pm <0.01	0.50	0.61
D (mm)	0.36 \pm 0.02	0.38 \pm 0.02	0.42 \pm 0.02	2.66	0.07
l_o (mm)	9.16 \pm 0.29	8.51 \pm 0.26	9.40 \pm 0.25	2.96	0.06
s_o (μm^2)	925.39 \pm 72.92	939.00 \pm 71.58	1049.40 \pm 43.85	0.31	0.73
s_m (μm^2)	590.00 \pm 58.48	623.67 \pm 51.57	691.43 \pm 37.04	1.22	0.30
s_c (μm^2)	334.62 \pm 32.46	314.33 \pm 21.32	358.16 \pm 15.66	0.05	0.95
H (mm)	0.11 \pm 0.015	0.05 \pm 0.001	0.07 \pm 0.005	1.83	0.17
W (mm)	0.04 \pm 0.015	0.03 \pm 0.001	0.04 \pm 0.005	1.02	0.36
ϵ_{brk}	0.040 \pm 0.002	0.045 \pm 0.002	0.045 \pm 0.002	1.73	0.18
σ_{brk} (N mm $^{-2}$)	323.17 \pm 34.08	328.69 \pm 24.66	284.08 \pm 13.85	1.65	0.20
T (MJ m $^{-3}$)	7.52 \pm 0.82	8.12 \pm 0.75	7.86 \pm 0.60	0.14	0.87
E (MPa)	125.95 \pm 11.43	127.89 \pm 10.20	106.25 \pm 4.40	2.60	0.08

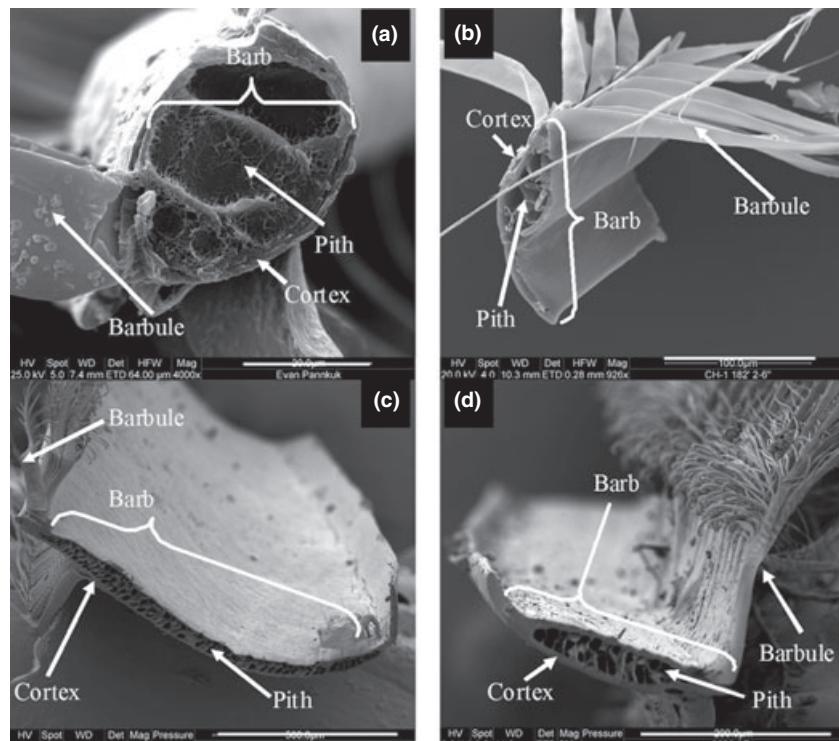


Fig. 4. SEM micrographs illustrating comparative cross-sectional morphology of feather barb breakage points. Primary barb cross-sections are removed at the intermediate section of the rachis and broken at the zone of long friction barbules [a-eastern screech owl (*Megascops asio*) dorsal barb used in study, scale bar = 20.0 μm ; b-eastern screech owl 9th Primary barb (not from current study), scale bar = 100.0 μm ; c-osprey (*Pandion haliaetus*) 9th Primary barb, scale bar = 500 μm ; d-great-horned owl (*Bubo virginianus* Hoy 1853) 9th Primary barb, scale bar = 200 μm].

retices) the effects of pigmentation on the total strength of keratinized tissue may be misleading because the development of keratin in flight vs. body feathers may have different evolutionary constraints. For this reason, we argue that the link between differential pigmentation and strength may be better investigated in contour feathers. Our data also demonstrate considerable variation in the location and morphology of feather breakage points (Fig. 4) and the effect of tissue size should be considered when correlating pigmentation and strength. Although positive co-variation between feather thickness and feather coloration may occur, our data suggest that the effect of melanin subtype on feather strength is negligible.

Many researchers have found that pigments, such as melanin, tend to reduce feather breakage (Burtt 1986;

Schreiber *et al.* 2006) or inhibit bacterial degradation (Burtt & Ichida 2004; Saranathan & Burtt 2007; Goldstein *et al.* 2004). While few studies have commented on cortical thickening due to melanin presence (Voitkevich 1966; Burtt 1986; Goldstein *et al.* 2004) no study experimentally correlated the microstructural size of the feathers with strength until Butler & Johnson (2004). Because the microstructural size of feathers varies considerably within a single bird and even within a single feather (Lucas & Stettenheim 1972), feather size should be considered in all studies that incorporate pigmentation with tissue strength. Although tensile strength tests may not mimic natural conditions as well as the abrasion experiments done by Burtt (1986), the logistics of obtaining cross-sectional areas after an abrasion experiment are daunting. The hypothesis that differential types of melanin pigmentation may

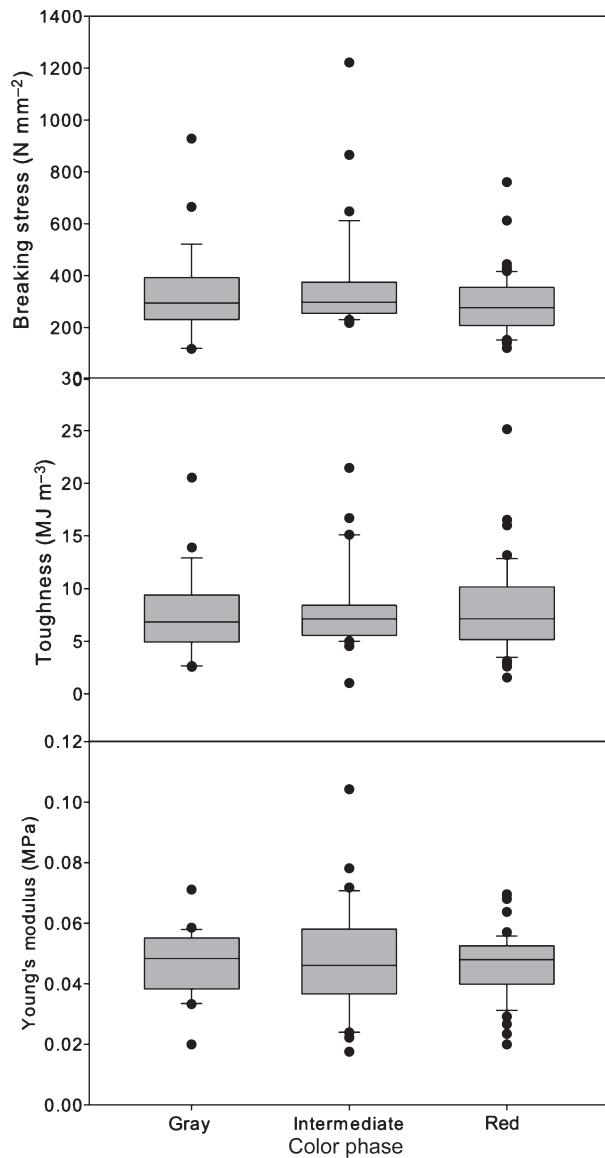


Fig. 5. (a) Breaking stress (N mm^{-2}), (b) toughness (MJ m^{-3}), and (c) Young's modulus (MPa) for eastern screech owl (*Megascops asio*) dorsal barbs (Mean \pm SEM, $n = 26$ for grey, $n = 31$ for intermediate, and $n = 40$ for red). The lines in the box are the median, the upper and lower box borders are the 25th and 75th percentiles, and the upper and lower lines are the 10th and 95th percentiles.

lead to differential strength has been rejected, but our data underscore the importance of quantifying morphological parameters of feathers in conjunction with biomechanical testing. The effect of melanin production on cortical thickening is an important question that requires further research.

This is the first study that has attempted to determine if melanin subtype could lead to differential strength of biological tissue. The present study rejects the hypothesis that melanin subtype affects the strength of body contour feathers in the eastern screech owl and should not be considered a factor in the maintenance of the observed clinal variation. Other hypotheses include adaptive responses, such as differing thermoregulation between the morphs (Mosher & Henny 1976; Dexter 1996) or the effect of landscape com-

position on crypsis (Gehlbach 1994, 1995). Moreover, it is also possible that pleiotropic interactions between melanin production and other aspects of the phenotype of eastern screech owls influence their clinal variation (Ducrest, Keller & Roulin 2008).

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List of symbols

Nomenclature: F_{brk} , Breaking force; s_o , Cross-sectional area of barb outside of cortex wall; s_c , Cross-sectional area of cortex; s_m , Cross-sectional area of pith; τ_{brk} , Breaking stress; l_0 , Original barb length; D , Displacement; ϵ_{brk} , Strain; H , Height; W , Width; W_{brk} , Work to break; T , Toughness; E , Young's modulus.

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