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Continent-wide variation in feather colour of a migratory songbird in relation to body condition and moulting locality

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Understanding the causes of variation in feather colour in free-living migratory birds has been challenging owing to our inability to track individuals during the moulting period when colours are acquired. Using stable-hydrogen isotopes to estimate moulting locality, we show that the carotenoid-based yellow–orange colour of American redstart (*Setophaga ruticilla*) tail feathers sampled on the wintering grounds in Central America and the Caribbean is related to the location where feathers were grown the previous season across North America. Males that moulted at southerly latitudes were more likely to grow yellowish feathers compared with males that moulted more orange–red feathers further north. Independent samples obtained on both the breeding and the wintering grounds showed that red chroma—an index of carotenoid content—was not related to the mean daily feather growth rate, suggesting that condition during moult did not influence feather colour. Thus, our results support the hypothesis that feather colour is influenced by ecological conditions at the locations where the birds moulted. We suggest that these colour signals may be influenced by geographical variation in diet related to the availability of carotenoids.

Keywords: migration; carotenoids; stable isotopes; feather growth

1. INTRODUCTION

Tissue colours, such as those on feathers, that are derived from ingested carotenoids are important signals of animal health and individual quality (Lozano 1994; Olson & Owens 1998). However, understanding the mechanisms that influence variation in feather colour in migratory birds has been challenging owing to the difficulty in tracking individuals during the moulting period when colour-producing carotenoids are incorporated into growing feathers. In this study, we use a novel approach to examine the causes of variation in feather colour in the American redstart (*Setophaga ruticilla*), a small (8 g) migratory songbird that breeds in North America and winters in the Caribbean and Central America. Conspicuous patches on their tail and flight feathers vary in colour from yellow to red–orange (Norris *et al.* 2004) and are produced from carotenoids acquired from their insectivorous diet (McGraw 2006). Redstarts conduct a complete moult once a year soon after breeding, typically just before fall migration (Sherry & Holmes 1997).

Recently, we showed that redstarts moulting at a breeding site in Ontario, Canada produced tail feathers that had higher red chroma values (a measure of colour saturation reflecting the type or concentration of carotenoids; Saks *et al.* 2003) than individuals breeding at the same site but moulted at more southerly locations during autumn migration (Norris *et al.* 2004). Here, we explore two hypotheses to explain this pattern. First, the 'condition hypothesis' proposes that dull-coloured tissues are produced because individuals are in relatively poor physiological condition (Hill & Montgomerie 1994). Since carotenoids have important roles in endocrine and immune systems, the prediction is that individuals in poor body condition should have fewer carotenoids available for tissue coloration (Olson & Owens 1998). To assess this hypothesis, we examined feather colour (red chroma) in relation to the daily growth rate of feathers from both a breeding site in Ontario and across the tropical wintering range. Feathers sampled in both of these periods were grown at the end of the previous summer, and thus indicate both colour and condition during moult. Second, the 'environmental constraints hypothesis' proposes that variation in feather colour arises primarily from differences in the type or the concentration of carotenoids consumed, which may vary geographically (Hill *et al.* 1994; McGraw 2006). To assess this hypothesis, we examined the relationship between red chroma and moulting latitude (estimated by stable-hydrogen isotopes [δD]) of tail feathers sampled throughout the wintering range. δD is incorporated into animal tissue from the local diet and can be used as a geographical tracer of the moulting locality because it reflects the latitudinal variation in δD from precipitation (Hobson & Wassenaar 1997).

2. MATERIAL AND METHODS

(a) *Feather sampling*

On the breeding grounds (May–June 2001–2004), we sampled tail feathers from male redstarts captured at the Queen's University Biological Station (44°34′ N, 76°19′ W) in southeastern Ontario, Canada. Individuals marked in *year x* were recaptured in *year x*+1 and a single tail feather (third retrix) was removed (Norris *et al.* 2004), thus providing an estimate of feather growth rate during the annual moult in *year x*. We also sampled a third retrix from after-hatch-year (AHY; second wintering season or older) and hatch-year (HY; first wintering season) males (n=122) at 12 locations throughout the wintering range (9.3°–32.2° N and 60.6°–105.7° W; figure 1*a*). We collected feathers in January–March 2001–2004, except from the Dominican Republic (1997) and Belize (1999).

(b) *Analysis of moulting region*

We used stable-hydrogen isotopes (δD) to estimate moult location. Stable-hydrogen isotope ratios ($^2H/^1H=R$) are expressed in δ notation (‰) where $\delta=[(R_{sample}/R_{standard})-1]\times 1000$ and $R_{standard}$ is the hydrogen isotope ratio of Vienna Standard Mean Ocean Water. For a detailed description of sample preparation and analysis, see Norris *et al.* (2006). δD values in feathers sampled on the wintering grounds (January–March) indicate moulting latitude the previous July–September. Norris *et al.* (2006) used a likelihood assignment method to estimate the moulting regions (figure 1*a*) of individuals that were sampled on the wintering grounds, based on

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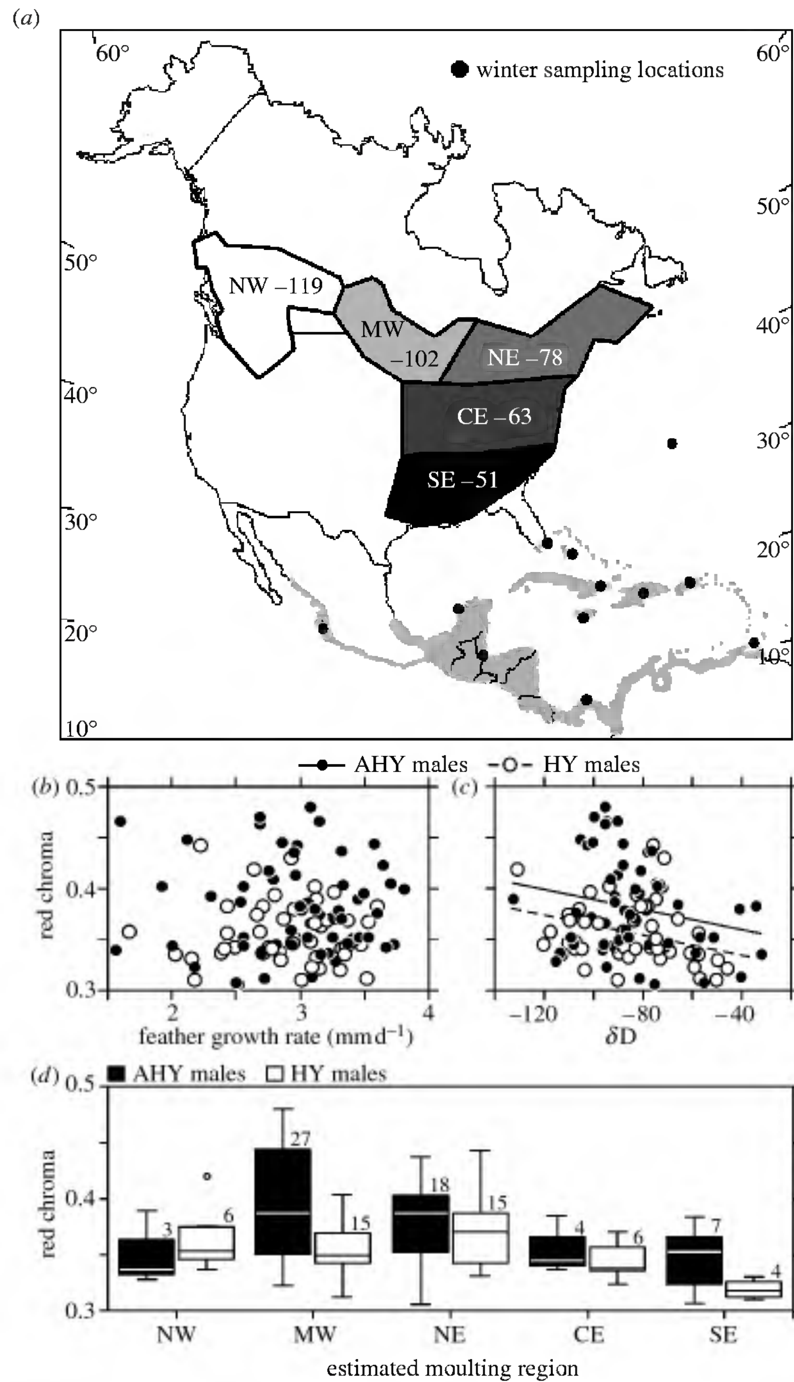

Figure 1. (*a*) American redstarts sampled on the tropical wintering grounds (winter range shaded light grey) were assigned to one of the five moulting regions (NW, northwest; MW, midwest; NE, northeast; CE, central east; SE, southeast) based on stable-hydrogen isotope (δD) values in their tail feathers and their relative breeding abundance (Norris *et al.* 2006). Mean predicted δD values based on growing-season precipitation are shown within each region. Projection is Lambert Zenithal Equal-Area. (*b*) No relation between red chroma and daily feather growth rate for either AHY (closed circle) ($n=67$) or HY (open circle) ($n=48$) males (same results when controlling for δD, see text). (*c*) Red chroma in relation to δD values in AHY (closed circle) ($n=59$) and HY males (closed circle) ($n=46$). Regression lines shown are from an ANCOVA with the interaction term removed (see text). (*d*) Box plots of red chroma values from the red–orange patch on the third rectrix of AHY (filled square) and HY males (open square) classified as moulting in one of the five regions shown in (*a*). Box plots show median, 10th, 25th, 75th and 90th percentiles by horizontal lines and all data points outside this range; sample sizes above each box.

expected δD values in precipitation and relative breeding abundance. We found no significant variation in δD values among years within localities (Norris *et al.* 2006), hence we pooled samples across years for each locality.

(c) *Feather colour analysis*

We measured reflectance across the bird-visible spectrum (320–700 nm) of five haphazardly chosen areas within each yellow–orange colour patch on each tail feather using an Ocean Optics USB2000 spectrometer connected to a PX-2 pulsed xenon light source (Norris *et al.* 2004). From each spectrum, we calculated *red chroma* as the proportion of total reflectance from the orange–red region of the spectrum (575–700 nm) and calculated the mean for each individual. Red chroma is a measure of spectral purity in that part of the spectrum (Montgomerie 2006) and is correlated with the amount of carotenoids deposited in the feather (Saks *et al.* 2003).

(d) *Feather growth rate analysis*

We digitally photographed each feather under a dissecting microscope with the feather illuminated at an oblique angle by a fibre-optic light source to reveal the faint growth bars that result from each day's growth (Hill & Montgomerie 1994). Using ImageJ (v. 1.35a; available at http://rsb.info.nih.gov/ij/), we then measured the total distance (parallel to the rachis) encompassed by three adjacent growth bars located about one-third of the distance from the feather tip. We also measured as many adjacent growth bars as possible on each feather. Images were enlarged 5× and the same part of each feather was measured to control for any differential growth during moult. To reduce measurement error, growth bars were measured once in each direction and an average was calculated. We used means from the maximum number of growth bars measured, assuming this to be more accurate as it is based on a larger sample. Our conclusions are the same for both measures.

(e) *Statistical analyses*

For wintering feathers, we used analyses of covariance (ANCOVA) with male age (AHY and HY) as a factor because redstarts have delayed plumage maturation, with HY males having more yellowish colours than AHY males. Non-significant interaction terms were removed when they did not significantly improve the fit of a model; when the interaction term was significant, we analysed age categories separately.

3. RESULTS AND DISCUSSION

For feathers sampled on the breeding grounds, mean daily feather growth rate of AHY males was not significantly related to δD-values ($r^2=0.17$, $p=0.47$, $n=23$). Moreover, based on expected δD values of AHY males breeding in Ontario (Norris *et al.* 2004), we found no significant difference in mean feather growth rates of birds that moulted on the breeding grounds versus those that moulted further south during migration (one-tailed t-test, $t_{21}=-0.79$, $p=0.22$).

Similarly, for AHY males sampled on the wintering grounds, there was no relationship between feather growth rate and δD values ($r=0.15$, $p=0.24$, $n=60$). There was however a negative relationship between feather growth rate and δD values for HY males ($r=-0.30$, $p=0.04$, $n=47$), suggesting that nestlings at higher latitudes grew feathers faster. Nonetheless, red chroma was not significantly related to the growth rate of feathers sampled on either the breeding ($r=0.22$, $p=0.33$, $n=23$) or the wintering grounds (ANCOVA, $R^2=0.14$, no significant interactions; growth rate effect, $F_{1,101}=0.13$, $p=0.72$; δD effect, $F_{1,101}=6.69$, $p=0.01$; age effect, $F_{1,101}=10.7$, $p=0.001$; figure 1*b*), suggesting that condition during moult did not influence carotenoid concentration of the feathers. We included moulting latitude as a factor in this analysis to control for local environmental effects that could confound the relationship between feather growth rate and colour.

Next, we examined whether feather colour was related to large-scale geographical differences in moulting location (figure 1*a*). Previously, we reported a significant negative correlation between red chroma and δD-values in feathers sampled on the breeding grounds (Norris *et al.* 2004). For males sampled throughout the wintering range, there was a similar negative relationship between red chroma and δD values (ANCOVA, $R^2=0.14$, no significant interactions; δD effect, $F_{1,102}=6.7$, $p=0.01$, *standardized* $\beta=-0.24$; age effect, $F_{1,102}=10.7$, $p=0.002$; figure 1*c*), providing additional evidence that moulting location influences feather colour. Similarly, red chroma varied significantly among regions (one-way ANOVA, $R^2=0.22$, no interaction term; moulting region effect, $F_{4,99}=4.2$, $p=0.003$; figure 1*d*), with males moulting in the southeast (SE) region having significantly lower red chroma than two (MW and NE) of the three northern regions ($p<0.05$; Tukey's *post hoc* tests).

Our results thus support the idea that feather colour is influenced by geographical variation in moulting location. We provide independent evidence, from males sampled thousands of kilometres apart in different periods of the annual cycle (winter and summer), that feathers with low red chroma were most likely grown at low latitudes in the southeastern US. We suggest two possible reasons for this pattern. First, redstarts moulting at lower latitudes may consume insects with lower carotenoid concentrations. There are no data available to test this idea, and to the best of our knowledge there is no information on geographical variation in carotenoids in avian diets. Anecdotal evidence suggests that redstarts may consume small amounts of carotenoid-rich fruits during moult (Sherry & Holmes 1997), which could also vary geographically. Second, the absorption, conversion or acquisition of carotenoids may be influenced by parasite loads (Brawner *et al.* 2000) and, if the incidence and virulence of avian parasites vary among populations of migratory birds (Piersma 1997), then this could also influence geographical variation in colour. If parasites influence colour in redstarts, however, we would have expected to see a relationship between red chroma and feather growth rate, since birds with high parasite loads should also be in relatively poor condition (Hill & Montgomerie 1994).

Since our results suggest that geographical variation in feather colour may arise through ecological differences between regions, the colonization of new areas could lead to differences in the colour of carotenoid-based tissues. If changes in male colour result in a modification or adjustment in female choice, then reproductive isolation could also be influenced by environmentally induced phenotypic traits (e.g. Boughman 2001). We suggest future studies to examine the patterns and mechanisms of geographical variation in dietary carotenoids to help address this interesting question.

We demonstrate here how the combination of novel analytical techniques applied to a single feather can reveal unique insights into avian ecology. Many migratory birds are difficult to track during the secretive moulting period, hence the use of stable isotopes provides a method to estimate their moulting locality no matter where or when they are sampled. When combined with advanced colour measurement techniques (Andersson & Prager 2006), our approach can be used to rapidly assess relationships between moulting latitude and feather colour in a variety of species with different life-history strategies and geographical ranges.

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