

Age-related change in carotenoid-based plumage of the American Redstart (*Setophaga ruticilla*)

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Abstract Plumage colouration serves a variety of functions for birds, including conspecific signalling, crypsis, and predator–prey interactions. Though much research has been conducted on colour changes in species with delayed plumage maturation, where birds do not exhibit definitive adult plumage until their second breeding season or later, relatively few studies have examined how plumage colour changes once definitive adult plumage has been attained. In this study on male and female American Redstarts (*Setophaga ruticilla*), which exhibit male delayed plumage maturation, we used reflectance spectrometry to evaluate carotenoid-based tail colour changes over 11 breeding seasons, examining both within-individual and population-level changes. At a population level, males in their first breeding season in definitive adult plumage had a feather hue that was more orange-shifted than birds in their second year in adult plumage, and marginally, but not significantly, more orange-shifted than birds in their third year in adult plumage. Within-individual analysis of males recaptured in

subsequent seasons also revealed a shift away from orange towards a more yellow feather hue as individuals aged. Within individuals, red chroma was highest for males in their second year in adult plumage, but it showed no population-level effects. At the population level, female redstarts in their first breeding season displayed plumage with a higher red chroma and a lower brightness than birds in their second breeding season, potentially as a result of differences in the timing and conditions of moult (first-year bird tail feathers were grown in the nest). For adult males and females, there was no difference in plumage colouration between birds that returned to the study site compared to those that failed to return. Together, our results suggest that within-individual change rather than differential survival best explains our findings. We suggest that studies examining age-related colour changes are critical for understanding the evolution of complex signalling systems, such as that of American Redstarts.

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Zusammenfassung

Altersabhängige Änderung im karotinoid-gefärbten Gefieder des Schnäpperwaldsängers

Die Gefiederfärbung bei Vögeln dient verschiedensten Funktionen, darunter einer Signalfunktion für Artgenossen, der Tarnung und der Interaktion zwischen Räuber und Beute. Während es viele Untersuchungen gibt über den Farbwechsel bei Arten mit verzögerter Gefieder-Reifung, wenn Vögel ihr endgültiges Adult-Gefieder nicht vor der zweiten Brutzeit oder später aufweisen, gibt es relativ wenige Studien, die untersuchten, wie sich die

Gefiederfärbung ändert, nachdem das endgültige Adult-Gefieder entwickelt ist. In dieser Studie über männliche und weibliche Schnäpperwaldsänger (*Setophaga ruticilla*), bei denen die Männchen eine verzögerte Gefieder-Reifung aufweisen, setzten wir die Reflexions-Photospektrometrie ein, um die Farbveränderung der karotinoid-gefärbten Schwanzfedern über 11 Brutzeiten zu untersuchen, und zwar sowohl intra-individuelle als auch populationsweite Veränderungen. Auf Populationsebene hatten Männchen in ihrer ersten Brutsaison im Adult-Gefieder einen Farbton der Federn, der mehr Richtung orange ging als Vögel im zweiten Jahr und etwas, aber nicht signifikant mehr Richtung orange gehend als Vögel im dritten Jahr des Adult-Gefieders. Intra-individuelle Analysen an wiedergefangenen Männchen in aufeinanderfolgenden Jahren zeigte mit zunehmendem Alter der Tiere auch eine Verschiebung des Farbtons der Federn weg von orange mehr Richtung gelb. Innerhalb der Individuen war die Farbintensität für rot am höchsten für Männchen im zweiten Jahr des Adult-Gefieders, zeigte aber keinen populationsweiten Effekt. Populationsweit zeigten weibliche Schnäpperwaldsänger in der ersten Brutsaison ein Gefieder mit höherer Farbintensität und geringerer Helligkeit für rot, verglichen mit Vögeln in ihrer zweiten Brutsaison, möglicherweise als ein Ergebnis von Zeitpunkt und äußeren Bedingungen der Mauser (die erstjährigen Schwanzfedern wachsen noch im Nest). Für sowohl Männchen als auch Weibchen gab es keinen Unterschied in der Gefiederfärbung zwischen Vögeln, die zum Untersuchungsort zurückkehrten und solchen, die nicht zurückkehrten. Insgesamt legen unsere Ergebnisse nahe, dass intra-individuelle Veränderungen unsere Beobachtungen besser erklären als eine Abhängigkeit von Überlebensraten. Wir regen an, dass die Untersuchung von altersabhängigen Farbveränderungen entscheidend sind für das Verständnis der Evolution komplexer Signalsysteme, wie das des Schnäpperwaldsängers.

Introduction

Birds are some of the most colourful animals on earth, often exhibiting brilliant plumage that functions in a wide variety of contexts, from intraspecific signalling in mate attraction (reviewed in Hill 2010) and dominance interactions (Smith 1979; reviewed in Santos et al. 2011) to interspecific functions such as prey-flushing (Mumme et al. 2006; Mumme 2014) or toxicity signalling (Dumbacher and Fleischer 2001). In addition, different patterns and colours can be used for crypsis and to avoid predation (Slagsvold et al. 1995). Plumage colour functions in a variety of contexts, and individuals should benefit from producing and maintaining optimal plumage expression.

Carotenoid-based plumage in particular has received considerable attention because these pigments can only be obtained through diet (Hill and Montgomerie 1994), and their expression is generally thought to be limited by individual condition (Brush 1990; Hill 1992, 2011; Hill and Montgomerie 1994; Hill et al. 2002; Fitze et al. 2003; but see Prum 2010). They are thus often considered honest signals of individual condition and quality (Hill and Montgomerie 1994; reviewed in Olsen and Owens 1998; Hill et al. 2002; but see Prum 2010). Because plumage colouration is often closely linked to individual fitness (Hill 2006), changes in plumage colouration over an individual's life would be expected to affect lifetime reproductive success.

Many species exhibit delayed plumage maturation, whereby individuals do not reach their definitive adult plumage until their second or later year (Hawkins et al. 2012). In such cases, individuals with subadult plumage generally experience significantly lower reproductive success (via both social and extrapair mating success; Hawkins et al. 2012). However, within-individual changes in plumage colouration once definitive adult plumage has been obtained remain relatively poorly studied, in large part due to the need for long-term studies of individually marked and repeatedly recaptured birds.

Two nonexclusive hypotheses have been developed to explain directional changes in definitive plumage colouration: within-individual changes with age (Hill 1996; Delhey and Kempenaers 2006), and colour-biased survival (reviewed in Jennisons et al. 2001; Delhey and Kempenaers 2006). Most of the studies examining these hypotheses have been conducted on species that display structurally based plumage colouration, such as Blue Tits (*Parus caeruleus*; Delhey and Kempenaers 2006), Western Bluebirds (*Sialia mexicana*; Budden and Dickinson 2009), Eastern Bluebirds (*Sialia sialis*; Siefferman and Hill 2005), and Tree Swallows (*Tachycineta bicolor*; Bitton and Dawson 2008; Bitton et al. 2008).

Male and female Blue Tits both appear to experience an increase in ornamentation with age; older birds were observed to display more chromatic (higher spectral purity) crown plumage with higher brightness and UV reflectance (Delhey and Kempenaers 2006). In Western Bluebirds, males also became brighter with age and displayed larger areas of blue plumage (Budden and Dickinson 2009), while Eastern Bluebirds increased in rump brightness (Siefferman and Hill 2005). For both Blue Tits and Western Bluebirds, these colour changes occurred within individuals as they aged, supporting the age-related colour change hypothesis (Delhey and Kempenaers 2006; Budden and Dickinson 2009). Similarly, male Tree Swallows also exhibited within-individual increases in brightness with age (based on consecutive recaptures; Bitton and Dawson

2008). At the population level, older male Tree Swallows maximally reflected light at shorter wavelengths, resulting in more intense blue plumage than that of younger males. However, individuals did not experience increases in blue intensity in consecutive seasons; rather, variation in hue was related to the probability of returning to the study site. Individuals with less intensely blue plumage had lower survival or lower site fidelity than individuals with bluer plumage (Bitton and Dawson 2008). Tree Swallows thus serve as an example of how both within-individual age-based colour change and colour-biased survival can occur within the same species.

Studies that have investigated age-related colour change in species with carotenoid-based plumage have produced conflicting results. Val et al. (2010) found that as Great Tits aged, both males and females increased in ornamentation, growing breast feathers that were more green-shifted and more saturated than those of younger birds. This colour change was attributed to within-individual increases in foraging ability with age. Similarly, Grunst et al. (2014) found that Yellow Warbler (*Setophaga petechia*) males increased in carotenoid saturation with age; interestingly, the condition dependence of carotenoid saturation was both age and sex dependent. Within-individual colour change was observed in male Red Bishops (*Euplectes orix*; Edler and Friedl 2012). Males displayed the brightest, most carotenoid-saturated plumage midway through their lives (3–5 years old), displaying significantly duller plumage both earlier and later in their lives. The authors suggest that these birds may exhibit differential allocation of carotenoids during different life stages, with the oldest males preferentially using carotenoids to maintain their immune function (Edler and Friedl 2012). Conversely, European Serins (*Serinus serinus*) were not found to undergo any within-individual changes in colour, but on average older birds were brighter and more saturated in colour than younger birds, suggesting the possibility of colour-biased survival in this species (Pagani-Núñez and Senar 2012).

We studied a breeding population of American Redstarts (*Setophaga ruticilla*) in southeastern Ontario over 11 seasons (2001–2011), which provided an ideal opportunity to study both within-individual and population-level changes in carotenoid-based plumage colour. In our population of American Redstarts, plumage colouration appears to be an important visual signal that is associated with both inter- and intrasexual interactions (Table 1; see “Methods” and “Study species”).

Given the importance of plumage colouration in American Redstarts for both inter- and intrasexual signalling, the number of colour variables examined, and the complex patterns observed in previous studies (e.g., higher flank “redness” in males was associated with a greater probability of securing paternity, but a lower probability of

siring extrapair offspring; Reudink et al. 2009a; Table 1), our goal was to use both within-individual and population-level analyses to better understand patterns of colour change with age. Specifically, our aims were to quantify changes that occur once individuals have attained definitive adult plumage by (1) examining whether these changes occur at the individual or population level, (2) examining whether changes in plumage colouration at the population level are driven by differences in return rates, and (3) determining whether patterns of colour change are consistent between males and females.

Methods

Study species

American Redstarts are small (8 g), sexually dichromatic, long-distance migratory songbirds. American Redstarts exhibit delayed plumage maturation in males, with first-year males exhibiting female-like grey and yellow plumage until the end of their first breeding season, at which time they undergo their single post-breeding moult into jet-black plumage with brilliant orange colouration on their tails, wings and flanks (Pyle 1997). American Redstarts are insectivores whose common prey items include Homoptera, Diptera, Coleoptera, Lepidoptera, and Hymenoptera species, from which they obtain carotenoids (Robinson and Holmes 1982). The bright orange colouration in adult males appears to be produced by deposition of the pigments canthaxanthin and canary xanthophylls A and B (McGraw et al. 2005), which are metabolically converted from the precursors beta-carotene and lutein, respectively. However, other pigments such as α -doradoxanthin and adonirubin may also be present (J. Hudon, pers. comm.). Though the pigments that produce the yellow colouration in females and first-year males have not yet been reported, it is likely primarily due to lutein and its derivatives canary xanthophylls A and B. Little is known about how the carotenoid-based colour exhibited by American Redstarts changes once definitive plumage has been attained. Previous research on a small number of male redstarts in adult plumage ($n = 17$) captured in subsequent seasons suggested that tail feather redness (hue/chroma) decreased from one year to the next (Reudink et al. 2009a). Also, Osmond et al. (2013) recently reported that females in their second breeding season or later exhibited tail feathers with higher brightness than females in their first breeding season.

Previous work on the potential signalling function of American Redstart plumage colouration (Table 1) revealed that tail feather brightness may function as a dominance signal in male–male interactions on the breeding grounds, as males with brighter tail feathers were more likely to be

Table 1 Significant correlates of plumage colouration reported in previous studies of a breeding population of American Redstarts in southeastern Ontario

	Sex	Plumage variable	Correlated variable
^a Reudink et al. (2009b) ^b Tonra et al. (2014) ^c Germain et al. (2012) ^d Reudink et al. (2009a) ^e Reudink et al. (2015) ^f Germain et al. (2010) ^g Osmond et al. (2013) ^h Reudink et al. (2009a, b), Germain et al. (2010) and Osmond et al. (2013) applied principal components analysis (PCA) to the shape of the curve to generate values of “redness”, which are highly correlated with values of red chroma and hue used by Germain et al. (2012) and employed in this study	Male	Tail brightness	Winter habitat quality (+) ^a (no relationship) ^b First egg date (−) ^c Probability of polygyny (+) ^d Wing length (+) ^a July rainfall (−) ^c
		Tail “redness” ^h (hue/chroma)	First egg date (−) ^c Probability of siring extrapair offspring (−) ^d July rainfall (+) ^c Age (−) ^d
		Flank brightness	Male parental care (+) ^f Age (+) ^d
		Flank “redness” (hue/chroma)	Probability of securing paternity (+) ^d Proportion of within-pair offspring (+) ^d Probability of siring extra-pair offspring (−) ^d
	Female	Tail brightness	Female parental care (−) ^g Male parental care (−) ^g Age (first-year vs. adult) (+) ^g Fledging success (−) ^g July rainfall (−) ^c Age (+) ^d
		Tail “redness” (hue/chroma)	July rainfall (+) ^c
		Flank brightness	No observed relationships
		Flank “redness” (hue/chroma)	Male provisioning (−) ^g Male flank brightness (+) ^g

polygynous (Reudink et al. 2009a) and hold large or multiple territories. Flank colouration, on the other hand, has been implicated in intersexual signalling, as male flank colouration was associated with both the probability of retaining paternity and siring extrapair offspring (Reudink et al. 2009a) and with offspring provisioning rate (Germain et al. 2010). For females, individuals with brighter tails took fewer provisioning trips, and first-year females with brighter tails fledged fewer offspring (Osmond et al. 2013). Tail colouration may also serve an important signalling function on the wintering grounds. Male redstarts with brighter tail feathers were more likely to occupy high-quality habitat (Reudink et al. 2009b; but see Tonra et al. 2014) in winter grounds. Recent work has also demonstrated that across age and sex classes, tail colour was strongly associated with weather conditions during moult the previous year; birds arrived with higher red chroma and lower brightness following years with high July rainfall (Reudink et al. 2015).

Feather collection

Field work was conducted during each breeding season from 2001 to 2011 at the Queen’s University Biological

Station in southeastern Ontario, Canada (44°34′N, 76°19′W). American Redstarts have relatively high site fidelity to our study area. For example, 26 % of males (34 % of adult males) and 16 % of females on average each year from 2002 to 2011 were banded in a previous year (Ratcliffe and Marra, unpublished data), resulting in many individuals being captured in multiple years. The study area was dominated by deciduous trees, including Sugar Maple (*Acer saccharum*) and Eastern Hophornbeam (*Ostrya virginiana*; see Germain et al. 2010). Both male and female redstarts were captured in mist nets using song playback and decoys to simulate territory intrusion (see Reudink et al. 2009a). Females were captured less frequently than males, and in some years were not sampled at all (see Table 2).

Each individual was sexed and then aged as either first-year or adult following Pyle (1997). For males, this was based on the presence of adult plumage, and for females it was based on feather wear. Each individual was then given a unique band number, and a single rectrix (R3) was removed for colour analysis. A bird was classified as adult1 if it was either captured as a first-year bird the previous year or was captured for the first time as an adult. A bird was classified as adult2 if it was initially banded and

Table 2 The number of redstarts captured in each year of the study for which feathers were used for colour analysis

Capture year	Females			Males		
	First-year	Adult1	Adult2	Adult1	Adult2	Adult3
2001	0	0	0	3	0	0
2002	0	0	0	10	3	0
2003	0	0	2	1	12	3
2004	17	19	4	20	7	7
2005	9	12	3	25	7	2
2006	18	9	1	24	7	3
2007	14	13	2	37	6	4
2008	0	0	0	17	3	1
2009	7	9	2	14	7	3
2010	0	0	0	35	0	1
2011	0	0	0	11	0	0
Total	65	62	14	197	52	24

subsequently recaptured in its second occurrence as an adult, and so on. Note that this approach assumes that adult birds occurring for the first time at our study site were adult1, and thus some older individuals may have been categorised as being younger than they were. However, because site fidelity of adult males is high, we suggest that the impact of misclassification was relatively minor. If anything, misclassification of older individuals as younger would weaken any patterns of colour change over time, such as those we observed (see “Results”).

Colour analysis

Plumage was quantified by measuring reflectance across the avian visual range (300–700 nm) using an Ocean Optics JAZ spectrometer with a xenon light source. Light was transmitted through a fibre optic probe which was held in a nonreflective probe holder to consistently measure the feather from a set distance of 5.9 mm and at a 90° angle. To standardise the reflectance measurements, dark (sealed cylinder of Colorline #142 Ebony paper) and white (white standard, Ocean Optics 2012) standards were used between different feathers to set a baseline for the amount of light reflected. Each feather was mounted on low-reflectance black paper (Colorline #142 Ebony) and ten measurements were taken from the yellow-orange patch of each rectrix. Feathers with insufficient carotenoid colour, where the coloured area was too small to accurately measure, were not included in the colour analysis.

Reflectance curves were analysed using the RCLR v.28 colour analysis program (Montgomerie 2008). We first performed smoothing using a LOESS function to eliminate local peaks and noise in the curves, and then calculated several colour variables from smoothed curves (Montgomerie 2006). We examined three different colour variables: brightness, red chroma, and hue (see Montgomerie

2008). Brightness (mean $R_{300-700}$) was measured as the mean amount of light reflected by the feather across all wavelengths (300–700 nm). Red chroma ($R_{605-700}/R_{300-700}$) was a measure of spectral purity of the light reflected in the orange-red segment of the spectrum. Hue ($\arctan \left(\frac{[(R_{415-510}-R_{320-415})/R_{320-700}]}{[(R_{575-700}-R_{415-575})/R_{320-700}]} \right)$) was measured as a ratio of the light reflected by different segments in the spectrum, and provides information on the dominant wavelength of light reflected (i.e. the colour the feather appears; Saks et al. 2003). High hue values are representative of a shift towards shorter, yellower wavelengths, while low hue values represent a red shift. We then averaged values from the ten measurements from each individual to obtain a single brightness, red chroma, and hue score for each individual. Colour analysis methods and calculations follow previous work by our group on American Redstarts (Reudink et al. 2009a; Osmond et al. 2013; Tonra et al. 2014).

Statistical analyses

We first wished to determine whether older individuals differed in plumage colouration in comparison to younger individuals at a population level. Because of delayed plumage maturation in males, we analysed males and females separately, and for males we only considered adult individuals of different age classes (i.e. once definitive plumage had been attained), whereas for females we also considered first-year individuals, as they do not exhibit any apparent delayed plumage maturation. We used ANOVA to determine whether colour variables differed among age classes, and we used Tukey post-hoc tests where appropriate to determine which specific age classes differed in colour. We standardised colour variables for each sex by setting the mean to 0 and standard deviation to 1 within

each year. The reason for standardisation was that previous work showed significant yearly variation in colour variables in relation to weather conditions during feather growth (Reudink et al. 2015). Thus, by using standardised colour in each year, we were able to ask whether different age classes showed, on average, significantly different plumage colouration, while controlling for environmental effects during feather growth.

Given population-level differences among age classes in plumage colouration, we next wished to determine whether individuals changed plumage colouration as they aged. Our sample of returning females with colour measurements in at least 2 years was very small ($n = 2$), and so this analysis was only performed for males ($n = 26$). We used a linear mixed-effects model with year and age as fixed effects and individual as a random effect. For this analysis, we used nonstandardised colour variables, thus allowing us to ask whether individual age had an effect on plumage colouration after accounting for yearly and individual variation. We again used Tukey post-hoc tests where appropriate to examine differences among specific age classes.

Population-level differences in plumage among age classes could also be explained by differences in survival among differently coloured individuals. Our dataset was not complete enough for a full survival analysis with respect to colour, as this would require colour measurements for each individual in each year that they were present. Unfortunately, these data were only available for ten males and for no females. In contrast, we had adequate samples of colour measurements for adult1 individuals that either did or did not return to our study site in at least one subsequent year ($n = 197$ males with 48 returning, and 62 females with 16 returning). We used t tests to compare mean standardised colour between adult1 males and females that returned to our study site and those that did not return, making the assumption that the majority of birds that did not return had perished. While this is likely to be true for most males, based on re-sightings and on evidence from stable isotopes (see above; Lemon et al. 1996; Hobson et al. 2004; Studds et al. 2008), results for females should be interpreted with caution due to lower site fidelity in females (Lemon et al. 1996).

All analyses were performed in R version 3.0.1 (R Development Core Team 2013), and we used the packages lme4 and lmerTest for mixed-effects modelling. Prior to analysis, we used Mahalanobis distance outlier analysis to identify and eliminate multivariate outliers ($n = 50$), as analysis of spectral curves from reflectance spectrometry can occasionally result in unrealistic values (Montgomerie 2006). However, nearly all results remain unchanged (unless otherwise indicated) when outliers were included. Not all colour variables for each sex were normally distributed,

but results were equivalent when nonparametric tests were used, and so we show results for parametric tests only. Data are presented as mean \pm SE.

Results

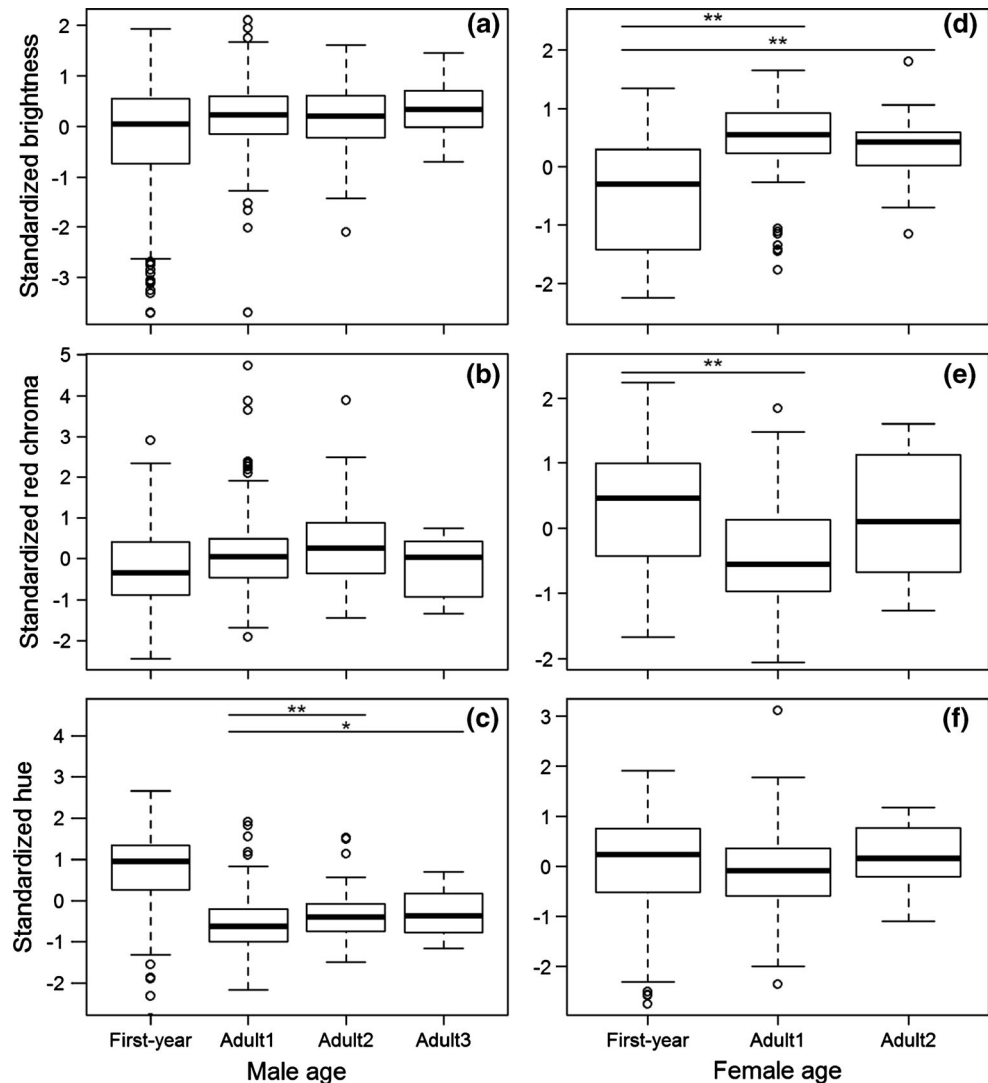
Population-level colouration

For adult males in definitive plumage, our sample included colour variables for 197 adult1, 52 adult2, and 24 adult3 individuals; adult4, adult5, and adult6 males were excluded from the analysis due to small sample sizes ($n = 2, 3$, and 1, respectively). For females, our sample included 65 first-year, 62 adult1, and 14 adult2 individuals; one adult3 individual was excluded (Table 2).

We found no difference in standardised male plumage brightness ($F_{2,270} = 0.71$, $p = 0.49$) or red chroma ($F_{2,270} = 2.56$, $p = 0.08$) among age classes (brightness: adult1 = -0.01 ± 0.07 , adult2 = -0.06 ± 0.15 , adult3 = 0.22 ± 0.17 ; chroma: adult1 = -0.01 ± 0.07 , adult2 = 0.2 ± 0.15 , adult3 = -0.34 ± 0.14), although the relationship approached significance for red chroma. Age classes differed significantly in plumage hue ($F_{2,270} = 6.14$, $p = 0.002$), and post-hoc tests revealed that adult1 males (-0.13 ± 0.07) differed significantly from adult2 males (0.33 ± 0.15 ; Tukey's HSD 95 % CI = 0.1, 0.81, $p = 0.008$), which exhibited a more yellow-shifted hue than adult1 males. Adult1 males were marginally, but not significantly, different from adult3 males (0.34 ± 0.15 ; Tukey's HSD 95 % CI = $-0.03, 0.95$, $p = 0.07$), which again exhibited a more yellow-shifted hue than adult1 males. Adult2 and adult3 males did not differ (Tukey's HSD 95 % CI = $-0.55, 0.57$, $p = 0.99$) (Fig. 1a–c).

For females, age classes differed significantly in standardised plumage brightness (first-year = -0.48 ± 0.12 , adult1 = 0.42 ± 0.1 , adult2 = 0.31 ± 0.2 ; $F_{2,138} = 17.01$, $p < 0.001$) and red chroma (first-year = 0.33 ± 0.12 , adult1 = -0.37 ± 0.11 , adult2 = 0.14 ± 0.26 ; $F_{2,138} = 9.13$, $p < 0.001$), but not in hue (first-year = 0.001 ± 0.14 , adult1 = -0.08 ± 0.11 , adult2 = 0.15 ± 0.17 ; $F_{2,138} = 0.34$, $p = 0.71$). Post-hoc tests revealed that first-year females had significantly lower plumage brightness than adult1 (Tukey's HSD 95 % CI = $-1.27, -0.52$, $p < 0.001$) and adult2 (Tukey's HSD 95 % CI = $-1.41, -0.16$, $p = 0.009$) females and exhibited higher red chroma than adult1 females (Tukey's HSD 95 % CI = $0.31, 1.1$, $p < 0.001$). Adult1 and adult2 females did not differ in brightness (Tukey's HSD 95 % CI = $-0.74, 0.51$, $p = 0.91$) nor in red chroma (Tukey's HSD 95 % CI = $-0.15, 1.16$, $p = 0.16$), and first-year females did not differ from adult2 females in red chroma (Tukey's HSD 95 % CI = $-0.46, 0.85$, $p = 0.76$) (Fig. 1d–f).

Fig. 1 Population-level variation in standardised plumage colouration across age classes in American Redstart males (a–c) and females (d–f). Shown are the median, interquartile range (IQR), and outliers ($>1.5 \times \text{IQR}$). Significance codes: ** $p \leq 0.01$, * $p \leq 0.1$. First-year males are included for comparison but were not included in the analyses (see text)



Within-individual change in colouration

We used a dataset of 26 males with plumage measurements in at least 2 years (2.5 ± 0.1 years) to evaluate individual changes in colour variables over time. For plumage brightness, only year had a significant effect on brightness values ($F_{9,50} = 3.73$, $p = 0.001$); age did not (adult1 = 0.23 ± 0.008 , adult2 = 0.23 ± 0.008 , adult3 = 0.24 ± 0.008 ; $F_{2,47} = 0.66$, $p = 0.52$). For red chroma and hue, both year (chroma: $F_{9,51} = 8.13$, $p < 0.001$; hue: $F_{9,52} = 25.1$, $p < 0.001$) and age (chroma: adult1 = 0.28 ± 0.003 , adult2 = 0.29 ± 0.003 , adult3 = 0.28 ± 0.003 , $F_{2,52} = 9.79$, $p < 0.001$; hue: adult1 = 0.24 ± 0.03 , adult2 = 0.33 ± 0.02 , adult3 = 0.33 ± 0.02 , $F_{2,50} = 8.43$, $p < 0.001$) had significant effects on colour values. Post-hoc tests revealed a significant increase in red chroma between ages adult1 and adult2 (Tukey's HSD 95 % CI = 0.005, 0.01, $p = 0.01$) and a decrease between

adult2 and adult3 (Tukey's HSD 95 % CI = -0.006 , -0.01 , $p = 0.001$), but no difference between adult1 and adult3 (Tukey's HSD 95 % CI = -0.003 , 0.004 , $p = 0.99$). For hue, males increased significantly from adult1 to adult2 (Tukey's HSD 95 % CI = 0.07, 0.12, $p = 0.003$) and from adult1 to adult3 (Tukey's HSD 95 % CI = 0.06, 0.12, $p = 0.011$), but not from adult2 to adult3 (Tukey's HSD 95 % CI = -0.02 , 0.03 , $p = 0.98$). These patterns are generally similar to those for population-level means (Fig. 1). Returning males showed the lowest hue values (most orange-shifted) in their first occurrence with adult plumage and tended to have increased values as they aged, whereas red chroma values were highest for males in their second occurrence as adults (Fig. 2). Note, however, that if outliers are included in the within-individual analysis, red chroma is no longer significantly influenced by male age ($F_{2,60} = 0.18$, $p = 0.84$), a result more consistent with the lack of significant differences among ages in the population-level analysis (Fig. 1).

Colouration and return rates

Standardised plumage red chroma (return = -0.12 ± 0.09 , no return = -0.05 ± 0.07 ; $t_{195} = -0.58$, $p = 0.56$) and hue (return = -0.23 ± 0.11 , no return = -0.13 ± 0.07 ; $t_{195} = -0.26$, $p = 0.79$) values of adult1 males that subsequently returned to our study site did not differ from those of males that did not return. Similarly, standardised plumage brightness (return = -0.14 ± 0.26 , no return = 0.14 ± 0.13 ; $t_{60} = -1.22$, $p = 0.23$) and red chroma (return = -0.26 ± 0.25 , no return = 0.004 ± 0.14 ; $t_{60} = -0.87$, $p = 0.39$) did not differ between adult1 females that returned and those that did not return. Thus, we did not find any evidence that population-

level colour variation among age classes could be explained by differential survival (or return rates).

Discussion

Ornamental plumage colouration can serve a variety of signalling functions, yet how those signals change as individuals age remains poorly understood. In this study, we demonstrate that American Redstart males experienced significant changes in plumage colouration once definitive plumage was attained at both the population and individual levels. At the population level, females did not exhibit any changes in hue, and limited recaptures precluded within-individual analyses (Fig. 1f). In contrast, males in their first occurrence in adult plumage (adult1) displayed the most orange-shifted plumage hue, while older birds (adult2, adult3) displayed more yellow-shifted plumage (Fig. 1c). A similar pattern was also observed within individual males, where adult1 males exhibited the most orange-shifted hue, which was significantly lower in hue values than when they aged to adult2 and adult3 (though there was no significant change from adult2 to adult3) (Fig. 2b). Red chroma exhibited a different pattern, with adult2 males exhibiting the highest red chroma values, though this was evident within individuals and only approached significance at the population level (Figs. 1b, 2a). For females, adult1 birds exhibited significantly lower red chroma than first-year birds (Fig. 1d). Perhaps surprisingly, we did not observe any changes in male tail brightness (Fig. 1a), which has been previously implicated in sexual (Reudink et al. 2009a) and social (Reudink et al. 2009b) signalling. In contrast, females increased in brightness from first-year to adult1 and adult2 (Fig. 1d). Changes in female colour from first-year to adult were reported previously and were suggested to be due to differences in the timing and conditions of moult, as first-year females retain tail feathers that were originally grown in the nest, sometimes over a month prior to the moult of adult females (Osmond et al. 2013). We suggest that these results support the age-related colour change hypothesis, as changes in colour were apparent at both the individual and population levels, but were not associated with return rate.

Because carotenoids can only be obtained through the diet, carotenoid-based colouration is often seen as a measure of individual condition or quality: birds that have access to high-quality, carotenoid-rich foods have more carotenoids available to deposit into plumage or other integuments (Simpson 1983; Hill and Montgomerie 1994). In many species, individuals become more efficient at foraging with age (Morrison et al. 1978; Gochfeld and Burger 1984; Val et al. 2010), which could result in plumage that is more enriched with carotenoid pigments in older individuals (cf. Grunst et al. 2014).

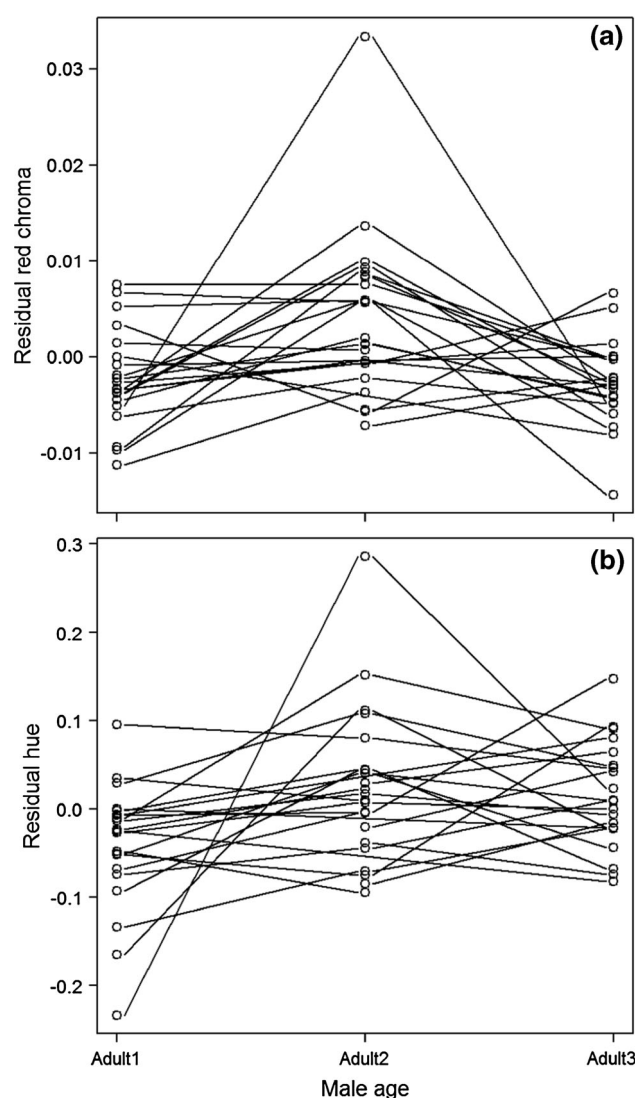


Fig. 2 Individual changes in plumage **a** red chroma and **b** hue in male American Redstarts. Residual values were taken from mixed-effects models including year as a fixed effect and individual as a random effect

Our results are perplexing on this front: the longitudinal analysis showed males exhibited the highest red chroma values in their second occurrence in adult plumage (this pattern was also apparent at the population level, though it only approached significance). High red chroma values should reflect high carotenoid contents (Saks et al. 2003). However, older (adult2 and adult3) individuals had more yellow-shifted hue than adult1 individuals at both the individual and population levels. A hue shift from orange to yellow could indicate a reduced carotenoid content (Saks et al. 2003); however, it might also result from differences in the types of carotenoids deposited in feathers. For example, a shift towards a yellow hue could occur if older redstarts deposit a higher proportion of yellow pigments, such as lutein and its derivatives canary xanthophyll A and B, and lower amounts of red pigments, such as canthaxanthin or α -doradoxanthin (Andersson et al. 2007). Analysis of feather carotenoid content and composition will be critical for understanding the proximate basis of age-related colour change. In addition, for species that modify carotenoids prior to deposition into feathers, recent work points to the importance of biochemical and cellular processes in regulating plumage colouration. For example, Hill and Johnson's (2012) vitamin A redox hypothesis points to the importance of enzymes such as β -carotene ketolase, which catalyses the oxidation of yellow carotenoids into red carotenoids, as this enzyme activity is sensitive to vitamin A availability and redox systems. As a result, colouration in species that use modified carotenoids should be directly linked to physiological state at the time of moult. If senescence in older males disrupts cellular and biochemical activities regulating carotenoid modification, the pattern of a shift towards yellow colouration may result.

Regardless of the mechanism, our finding that older male redstarts exhibited more yellow-shifted plumage at both the population and individual levels contrasts with previous work on other species. Pagani-Núñez and Senar (2012) found that individual European Serins did not exhibit changes in colour but, at the population level, older males were brighter and more chromatic than younger males. This change was attributed to selection pressures that favoured the survival of individuals with brighter plumage (Pagani-Núñez and Senar 2012). Indeed, differential survival or return rates are one potential explanation for population-level changes in plumage colouration; however, we did not detect any differences in hue or red chroma between adult1 males that returned to the study site and those that failed to return, suggesting that within-individual changes rather than differential survival is the best explanation for our results.

One potential explanation for the yellow shift in plumage colouration is a trade-off between plumage colour

production and reproduction, if a shift towards yellow reflects reduced signal quality. Though sexually mature, first-year males are much less successful in attracting a mate than adult males; pairing success rates in previous studies range from 40 to 71 % compared to 71–100 % success in adult males (Sturm 1945; reviewed in Procter-Gray and Holmes 1981). In our population, only ~55 % of first-year males paired and only 25 of the 220 first-year males (11 %) monitored from 2001 to 2011 produced fledglings (Ratcliffe and Marra, unpublished data). This pattern of reduced reproductive success in first-year males is common in species with delayed plumage maturation (Hawkins et al. 2012). Thus, the more orange-shifted plumage of adult1 birds could arise because, as they were first-year males, they may have had more time and energy available to invest in growing orange-hued feathers. Because they do not have offspring to care for, unsuccessful males (i.e. the vast majority of first-year males) may begin moulting earlier than successfully breeding males, providing them with a longer total period of time to complete the moult before the onset of migration (e.g. Hill and Montgomerie 1994; Serra et al. 2007). Unfortunately, this explanation fails to explain why adult2 males exhibited feathers with the highest red chroma. One possibility is that American Redstarts exhibit a similar pattern to Red Bishops, where the most colourful plumage is displayed midway through their lives, which Edler and Friedl (2012) suggested may reflect differential allocation of carotenoids during different life stages. Future studies examining how our calculated colour variables of brightness, hue, and red chroma reflect underlying carotenoid content are clearly required to understand this complex system. For females, differences in moult timing and conditions may also explain differences observed between first-year and adult individuals (see above). Although we were unable to conduct our analysis at the individual level for females, we found no evidence for differential return rates in association with female colour, suggesting that individual changes in female colour might also occur.

Taken together, our results may help explain some of the complexity and seemingly conflicting results from previous studies on our population of American Redstarts (Table 1). For example, Reudink et al. (2009a) reported that extrapair sires had lower tail "redness" (a more yellow-shifted hue, lower red chroma) than the males they cuckolded. This seemingly confusing result could be explained by a female preference for older males, a pattern common among birds (reviewed in Brooks and Kemp 2001). Understanding how individuals change in colour as they age may be crucial to understanding the targets of female choice and the dynamics of intrasexual signalling. In other words, age-related variation in plumage colouration could easily obscure patterns of mate choice or status signalling if this variation

is not taken into account. Similarly, if ornamentation increases with age, female preference for older males or a higher dominance status of older males could be erroneously interpreted as inter- or intrasexual selection for more colourful males.

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