

Faculty of Science

# **AGE-RELATED CHANGE IN CAROTENOID-BASED PLUMAGE OF THE AMERICAN REDSTART (*SETOPHAGA RUTICILLA*)**

2013 | KRISTEN MARINI

**B.Sc. Honours thesis**



**AGE-RELATED CHANGE IN CAROTENOID-BASED PLUMAGE OF THE  
AMERICAN REDSTART (*SETOPHAGA RUTICILLA*)**

by

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## **ABSTRACT**

Plumage colouration serves a variety of functions for birds, including signalling conspecifics, crypsis, and predator-prey interactions. Though much research has been conducted on colour change in species which exhibit delayed plumage maturation, where birds do not exhibit definitive adult plumage until their second breeding season or later, relatively few studies have examined how colour changes once definitive adult plumage has been attained. Studies that have focused on changes in definitive plumage colour have proposed two non-mutually exclusive hypotheses to explain colour change: age-related colour change occurs within individuals, and colour biased survival of more or less colourful individuals. Few studies have examined how carotenoid-based plumage colour changes, and those that have found conflicting results as to whether age-related colour change is occurring. In this study I examined the carotenoid-based plumage colour of the American redstarts, a migratory passerine that exhibits delayed plumage maturation where males obtain definitive plumage after their second year. Using reflectance spectrometry on feathers collected over a ten year period from a breeding population in Ontario, Canada, I quantified the colour characteristics of each feather and compared the intermediate (juvenile) colour to the definitive plumage colour, and tracked how the definitive adult plumage colour changed over time. As expected, due to delayed plumage maturation, male redstarts had definitive plumage that was significantly more orange and saturated in carotenoid chroma than their intermediate plumage. Additionally, males were most orange-shifted (reflected light at the longest wavelengths) during their first breeding season with definitive plumage, and became significantly more yellow-shifted (reflected light at shorter wavelengths) in the subsequent season. Female redstarts displayed significantly brighter and more orange-shifted plumage during their second breeding season compared to their first, but did not undergo any further colour change in subsequent years. The results of this study support the hypothesis that age-related colour change occurs within individuals, however I cannot rule out the possibility that differential survival also acts at the population level. Plumage colour is a complex trait and further research is needed to understand the relationship between change and reproductive effort.

Thesis supervisor: Dr. Matthew Reudink

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## TABLE OF CONTENTS

<b>ABSTRACT.....</b>	<b>ii</b>
<b>ACKNOWLEDGMENTS.....</b>	<b>iii</b>
<b>TABLE OF CONTENTS.....</b>	<b>iv</b>
<b>LIST OF FIGURES.....</b>	<b>v</b>
<b>LIST OF TABLES.....</b>	<b>vi</b>
<b>INTRODUCTION.....</b>	<b>1</b>
TYPES OF COLOUR .....	1
FUNCTIONS OF COLOUR .....	2
CHANGES IN COLOUR .....	3
COLOUR AND THE AMERICAN REDSTART .....	5
<b>MATERIALS AND METHODS.....</b>	<b>7</b>
FEATHER COLLECTION.....	7
COLOUR ANALYSIS.....	8
STATISTICAL ANALYSIS.....	9
<b>RESULTS.....</b>	<b>10</b>
YEAR TO YEAR COLOUR VARIATION .....	10
INTERMEDIATE TO DEFINITIVE COLOUR CHANGE .....	11
CHANGE IN DEFINITIVE PLUMAGE COLOUR .....	14
<b>DISCUSSION.....</b>	<b>16</b>
<b>LITERATURE CITED.....</b>	<b>21</b>
<b>APPENDIX A .....</b>	<b>27</b>
<b>APPENDIX B .....</b>	<b>28</b>

## LIST OF FIGURES

- FIGURE 1.** Mean plumage hue varied significantly among years of study for both male (A) and female (B) American redstarts. Represented are the yearly population means with standard deviations .....10
- FIGURE 2.** Changes in brightness (A), chroma (B), carotenoid chroma (C), and hue (D) from SY to ASY1 males. Represented are the results of the mixed-effects model, sample sizes are given in the corners of boxes (\* indicates  $p < 0.05$  significance level, \*\*\* indicates  $p < 0.001$  significance level). Whiskers represent 5<sup>th</sup>/95<sup>th</sup> percentiles, dots represent outliers beyond the 5<sup>th</sup>/95<sup>th</sup> percentile, and the center line indicates the mean .....12
- FIGURE 3.** Changes in brightness (A), chroma (B), carotenoid chroma (C), and hue (D) from SY to ASY1 females. Represented are the results of the mixed-effects model, sample sizes are given in the corners of boxes (\* indicates  $p < 0.05$  significance level, \*\*\* indicates  $p < 0.001$  significance level). Whiskers represent 5<sup>th</sup>/95<sup>th</sup> percentiles, dots represent outliers beyond the 5<sup>th</sup>/95<sup>th</sup> percentile, and the center line indicates the mean .....13
- FIGURE 4.** Change in feather hue of male American redstart from their first to their third occurrences as ASYs. Represented are the results of a paired t-test (\* indicates  $p < 0.05$  significance level, \*\*\* indicates  $p < 0.001$  significance level). Whiskers represent 5<sup>th</sup>/95<sup>th</sup> percentiles, and the center line indicates the mean.....15
- FIGURE 5.** Changes in brightness (A), chroma (B), carotenoid chroma (C), and hue (D) for females in their first to second occurrence as ASYs. Represented are the results of the mixed-effects model, sample sizes are given in the corners of boxes. Whiskers represent 5<sup>th</sup>/95<sup>th</sup> percentiles, dots represent outliers beyond the 5<sup>th</sup>/95<sup>th</sup> percentile, and the center line indicates the mean .....16

## LIST OF TABLES

<b>TABLE 1.</b> Plumage colour characteristics by age and sex of American redstarts (unstandardized values) .....	<b>13</b>
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## INTRODUCTION

Birds are some of the most colourful animals on this planet, often displaying brilliantly pigmented plumage that has a wide range of signalling functions. Colour is used in signalling conspecifics, both to attract mates (reviewed in Anderson and Simmons 2006) and in intrasexual dominance or aggression signalling (Smith 1979; reviewed in Santos et al. 2011). Inconspicuous colour can be used for crypsis (i.e., to hide from predators; Slagsvold et al. 1995), while conspicuous colour can be used for flushing-out prey (Mumme et al. 2006) or to signal to predators that an individual may be difficult to catch (Götmark 1992). The many diverse functions of plumage colour make understanding how it is produced and how it changes especially important.

### *Types of Colour*

The mechanisms producing plumage colours have been studied extensively, and three primary types of colour have been identified. The first mechanism involves the deposition of melanin, a pigment that produces black, grey, brown or other earth tones (Brush 1978). Melanin is manufactured from common amino acids such as tyrosine, phenylalanine, and cysteine (McGraw 2008), and most bird species display at least some melanin-based plumage. A second mechanism of colour production involves feather microstructure: various arrangements of keratin, melanin, air and feather barbules cause light to be scattered and reflected in different ways, most commonly producing blues, greens, purples, and iridescent colours (Doucet 2002; Doucet et al. 2006). Finally, most red, orange, and yellow colours result from the deposition and metabolic conversion of various carotenoid pigments (McGraw et al. 2005).

Unlike melanin- or structurally-based plumage, in which colour is produced developmentally, carotenoids can only be obtained through diet (Hill and Montgomerie 1994). Synthesized in plants (Simpson 1983), carotenoids pass through trophic levels, being acquired and used by most organisms, including invertebrates (reviewed in Bhosale and Bernstein 2007), fish (Page and Davies 2003), reptiles (reviewed in Olsson et al. in press), mammals (Lobo et al. 2012) and birds (Hill and Montgomerie 2002; McGraw et al. 2005). Carotenoids serve multiple purposes. They are precursors for vitamin A (reviewed in Palace



et al. 1999), antioxidants (reviewed in Palace et al. 1999; reviewed in Møller et al. 2000), are involved in immune function (reviewed in Hill 1999; Blount et al. 2003; Poston et al. 2005), as well as being incorporated as a pigment into dead tissues such as hair, scales, and feathers (McGraw et al. 2005).

Thus, in order to display bright orange-red feathers, birds need to have access to sufficient carotenoids in their food at the time of moult and need to be able to deposit these carotenoids into feathers rather than use them for other purposes (Brush 1990; Hill 1992; Hill and Montgomerie 1994; Hill et al. 2002; Fitze et al. 2003). There are a wide range of carotenoids that can be incorporated into feathers, either by themselves or in combination with other pigments to produce colours ranging from red and orange through yellow (McGraw et al. 2005). Some types of carotenoids can be deposited directly into feathers (lutein for example), while others, such as canthaxanthin, must be further metabolically modified before deposition (Brush 1990; McGraw et al. 2001). Because carotenoids must be ingested through the diet, carotenoid-based plumage colouration is generally regarded as an honest signal of physical condition and quality (Hill and Montgomerie 1994; reviewed in Olsen and Owens 1998; Hill et al. 2002). Only those individuals that are efficient at foraging and that can find carotenoid-rich food sources are able to obtain enough carotenoid pigments to incorporate them into their feathers (McGraw et al. 2001). In many species, more intensely coloured feathers provide significant advantages to males both during the breeding season as well as on the wintering grounds.

### *Functions of Colour*

Darwin (1872) first hypothesized that during the breeding season, having brightly pigmented feathers imparts significant reproductive advantages, and since then much research has focused on identifying these advantages. Carotenoid-based plumage often acts as a signal to potential mates advertising the quality or condition of an individual (Hill and Montgomerie 1994; MacDougall and Montgomerie 2003). Females often prefer to mate with brighter or more colourful males, and it is thought that they gain either direct (increased access to resources) or indirect (better quality offspring, more parental care from their mate) benefits from these colourful males (Hill 1991). In species such as the American goldfinch (*Carduelis tristis*) that participate in mutual mate choice, plumage colour also plays an

important role in mate selection: assortative mating takes place, that is, colourful individuals mate with other colourful individuals (MacDougall and Montgomerie 2003). These colourful individuals often pair earlier in the breeding season and have higher reproductive success overall (McGraw et al. 2001).

Carotenoid-based plumage colour is not a simple signal however; different aspects of carotenoid-based plumage are linked to separate reproductive benefits (Badyaev et al. 2001), which may vary from species to species. In the house finch (*Carpodacus mexicanus*), male plumage brightness is correlated with how much a male will provision his offspring (Hill 1991), while plumage redness is linked with a male's fecundity (Badyaev et al. 2001). The size of colour badges acts as yet another signal, signalling male dominance in the red-collared widowbird (*Euplectes ardens*; Pryke et al. 2002) and was linked to viability in the house finch (Badyaev et al. 2001).

### *Changes in Colour*

Because plumage colour serves so many functions, it is important to understand how it is produced and how it changes over the lifetime of a bird. In some species, older birds tend to be brighter or more distinctive than younger individuals (Siitari and Huhta 2002; Probst et al. 2007). Two competing hypotheses have been developed to explain this observation: colour change occurring within individuals as they age (Hill 1996; Delhey and Kempenaers 2006), or colour-biased survival occurs such that less colourful individuals die (reviewed in Jennions et al. 2001; Delhey and Kempenaers 2006). Most of the studies examining these hypotheses have been conducted on species with structurally-based plumage colouration, such as blue tits (*Parus caeruleus*; Delhey and Kempenaers 2006), western bluebirds (*Sialia mexicana*; Budden and Dickinson 2008), and tree swallows (*Tachycineta bicolor*; Bitton and Dawson 2008a, Bitton and Dawson 2008b).

Over time, the hue and ultraviolet reflectance of the crown plumage of both male and female blue tits changed significantly; older birds displayed more chromatic (higher spectral purity) crown plumage with a higher ultraviolet reflectance (Delhey and Kempenaers 2006). Brightness (average light reflected by the feather) also changed, such that older males and females were significantly brighter than younger birds (Delhey and Kempenaers 2006). In

eastern bluebirds, males also became brighter with age and displayed larger areas of blue plumage (Budden and Dickinson 2009). For both blue tits and 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).

Studies of tree swallows, which also display structurally-produced plumage colouration, also examined how colour changes with age. By measuring the colour of males captured in consecutive years, they found that individual male swallows increased in brightness; exhibiting within-individual colour change that supports the age-related colour change hypothesis (Bitton and Dawson 2008a). In the population as a whole, the plumage of older male tree swallows maximally reflected light at shorter wavelengths, resulting in more intensely blue plumage than that of younger males. As individuals themselves did not become bluer in consecutive seasons, this change was related to the probability of returning to the study site, where individuals with less intensely blue plumage had lower survival or lower site fidelity than individuals with bluer plumage (Bitton and Dawson 2008a). Tree swallows thus serve as an example of how both age-related colour change and colour biased survival can be occurring within the same species.

Compared to the research on plumage colour change carried out on structurally-based plumage, few studies have been conducted on colour change in birds with carotenoid-based plumage. Those studies that have investigated age-related colour change in species with carotenoid-based plumage have produced conflicting results. In 2010, Val et al. examined how carotenoid-based plumage colour in great tits (*Parus major*) changes as they age. Great tits displayed yellow feathers that resulted from the deposition of lutein and zeaxanthin, pigments that appeared to be directly deposited without any metabolic conversions (Partali et al. 1987; Val et al. 2010). As great tits age, both males and females increased in plumage colouration, growing feathers that were greener and more saturated in colour than those of younger birds (Val et al. 2010). This colour change was attributed to within-individual changes in foraging ability; as individuals age, they become better foragers and thus are able to grow more colourful feathers. A within-individual colour change was also found in male red bishops (*Euplectes orix*; Edler and Friedl 2012). Males display the brightest, most carotenoid-saturated plumage in the middle of their life (3 to 5 years old), displaying

significantly duller plumage both earlier and later in their lives. The authors speculated that this colour change may be caused by differential allocation of carotenoids during different life stages; in which older males may use carotenoids to maintain their immune function rather than investing them in colour (Edler and Friedl 2012).

Conversely, European serins (*Serinus serinus*) do not undergo any within-individual colour change (Pagani-Núñez and Senar 2012). European serins display bright yellow feathers on their breasts, throats, and some of their heads. This is caused by deposition of primarily xanthophylls A and B, carotenoid pigments that require metabolic modification before they are incorporated into feathers. In this study, brightness, hue, and chroma of feathers from individuals caught in two consecutive years were analyzed. Neither males nor females exhibited a significant change in any of these colour characteristics from one year to the next, indicating that colour is not changing within-individuals as they age (Pagani-Núñez and Senar 2012). However, at the population level, older birds were brighter and more saturated in colour than younger birds (Pagani-Núñez and Senar 2012). Because individuals themselves were not changing, this population level colour change was attributed to differential survival of dull, less colourful individuals, supporting the colour-biased survival hypothesis.

To my knowledge, these studies on age-related change of carotenoid-based plumage colouration are the only ones in publication. Their conflicting results make further studies essential for understanding age-related colour change in species with carotenoid-based pigments. It is often difficult to obtain large samples of birds that are recaptured in multiple years, since individuals may disperse or die. Because of this, long-term study populations of species with high site fidelity (i.e., birds return to the same area each year to breed) are ideally suited for studying age-related colour change. A long-term study population of American redstarts (*Setophaga ruticilla*) in Ontario provides an ideal opportunity to study changes in carotenoid-based plumage colour.

### *Colour and the American Redstart*

The American redstart, a long-distance migratory passerine, has high site fidelity as well as a relatively long lifespan: the same individual birds will return multiple times to the

same breeding area. This makes it an ideal species in which to study age-related colour change. American redstarts are insectivores whose common prey items include *homoptera*, *diptera*, *coleoptera*, and *hymenoptera* species, from which they obtain the carotenoid pigments canthaxanthin and canary xanthophylls A and B that are needed to produce their plumage colour (Robinson and Holmes 1982; McGraw et al. 2005). Redstarts are sexually dichromatic, where the coloured plumage on the wings, flanks, and tails is yellow in females and orange for males. Additionally, redstarts exhibit delayed plumage maturation: males display a yellow and grey “intermediate” plumage that is similar to that of females until after their first breeding season (their second year) when they moult into the black and orange definitive plumage (Rohwer et al. 1980).

Although much work has focused on understanding why this delay in plumage maturation occurs (Rohwer et al. 1983; Flood 1984; Studds and Robertson 1985; reviewed in Hawkins et al. 2012), little is known about how the carotenoid-based colour changes once definitive plumage has been attained. Previous research on a small number of male redstarts (n= 17) suggested that age-related change does occur, with tail feather redness significantly decreasing with age (Reudink et al. 2009a). As seen in other species, possessing colourful plumage imparts significant reproductive advantages for males during the breeding season.

In redstarts, males with brighter tail feathers are more likely to obtain and defend multiple territories, and thus are more likely to be polygynous and ultimately sire more offspring (reviewed in Lozano 1994; Reudink et al. 2009a). This suggests that tail feather colouration plays a role in signalling dominance in male-male interactions. Flank colouration appears to be an important intersexual signal as well; males with intensely red coloured flanks are more likely to retain paternity at their own nest, suggesting that flank colour is an important signal whereby females assess male quality (Reudink et al. 2009a). The end result is that males with either brighter tail or redder flanks will sire more offspring, and thus have higher reproductive success. Carotenoid-based plumage may also signal the level of parental investment a male will make in his offspring. Male American redstarts with brighter flanks make more trips to the nest when provisioning offspring than do males with dull flanks (Germain et al. 2010). This suggests once again that flank colouration is used in intersexual

interactions, as females may be able to judge how much effort a male will put towards provisioning offspring based on his flank brightness.

Carotenoid-based plumage may also serve an important signaling function on the wintering grounds, where male and female redstarts each defend a territory, and where habitat quality varies significantly (Marra 2000). Male redstarts with brighter tail feathers are more likely to occupy high-quality habitat (Reudink et al. 2009c). Individuals living in high-quality winter habitats are in better overall body condition and have lower levels of the stress hormone corticosterone (Marra and Holberton 1998; Studds and Marra 2005). Males occupying high-quality winter territory are thus able to leave the wintering grounds earlier, arrive earlier onto the breeding grounds, and thus have first access to breeding territories (Studds and Marra 2005; Reudink et al. 2009b). These early arriving males then attract females sooner, so that the pair can then begin producing eggs earlier (and thus have more of the breeding season remaining to fledge their young in) than later arriving males (Smith and Moore 2005).

Carotenoid-based plumage colour is clearly an important signal to redstarts; however, we do not yet know how their colour changes with age. In this study we wanted to first compare young and old redstarts of both sexes to determine if plumage colour varies. We then sought to determine if changes in plumage colouration are caused by differential survival based on colour or by changes in colouration within individuals as they age. By examining individuals captured in consecutive years as well as by looking at different age classes in the population as a whole, we aim to be able to differentiate between within-individual colour change and variation occurring at the population level due to differential survival based on colour (see Delhey and Kempenaers 2006; Bitton and Dawson 2008a).

## **MATERIALS AND METHODS**

### *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 fairly high site fidelity (although males return to the same breeding area significantly more often than females; Lemon et al. 1996), resulting in many individuals

being captured in multiple years. The study area was dominated by deciduous trees including sugar maple (*Acer saccharum*) and Eastern hop hornbeam (*Ostrya virginiana*; see Germain et al. 2010). Male redstarts were captured in mist nets, using song playback to simulate territory intrusion; females were captured in mist nets set up near their nests (see Langin et al. 2006; Reudink et al. 2009b; Germain et al. 2010). Females were captured less frequently than males, and in some years were not captured at all (see appendix A), resulting in a skewed sample in favour of males.

Each individual (n= 653) was sexed and then aged as either second year (SY) or after second year (ASY; see appendix A). For males, aging was done based on colour; because of delayed plumage maturation, SY males display a female-like yellow and grey intermediate plumage whereas the definitive plumage of ASY males is black and orange (Rohwer et al. 1980). Females were aged as SY or ASYs according to methods described by Pyle (1997). Each individual was then given a unique band number, and its physical characteristics (i.e., mass, wing length, tail length, tarsus length) were recorded. A single rectrix was removed from each individual to be used in colour analysis.

### *Colour Analysis*

Plumage was quantified by measuring reflectance across the avian visual range (300-700nm) using an Ocean Optics JAZ spectrometer with a PX-2 xenon light source. Light was transmitted through a fiber optic probe which was held in a non-reflective probe holder to consistently measure the feather from a set distance of 5.9 mm and at a 90° angle. To standardize the reflectance measurements, dark (sealed cylinder of Colourline #142 Ebony paper) and white (Ocean Optics white standard) 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 and 10 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 colour analysis.

Reflectance curves were analyzed using RCLR 0.9.29 colour analysis program (Montgomerie 2008). I first performed smoothing on the data points to eliminate local peaks and noise in the curves as they were highly variable, then calculated several colour variables

from the raw spectrometer data. I examined four different colour variables: brightness, chroma, carotenoid chroma, and hue (see Montgomerie 2008). Brightness ( $\sum_{300}^{700} R_i / n_w$ ) was measured as the mean amount of light reflected by the feather (for wavelength from 300-700nm). Chroma ( $\sqrt{(B_r - B_g)^2 + (B_y - B_b)^2}$ ) is a measure of saturation or spectral purity. Carotenoid chroma ( $[R_{\lambda 450} - R_{\lambda 700}] / R_{\lambda 700}$ ) assesses the amount of light reflected that is due to carotenoids, where lower values for carotenoid chroma indicate that more carotenoids are enriched in the feather. Hue ( $\arctan\{[(B_y - B_b) / B_1] / [(B_r - B_g) / B_1]\}$ ) was measured as a ratio of 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). High hue values indicate shorter wavelengths (yellow-shifted) while lower hue values indicate that longer wavelengths (orange-shifted) of light are being reflected.

### *Statistical Analysis*

Statistical analyses were performed using JMP statistical software version 10 (SAS Institute 2012). To control for year effects, I standardized colour variables by sex, year, and age class (SY or ASY) by setting the yearly mean for each colour variable to zero and calculated how different each feather (representing a single individual) was from the mean, using this difference as the standardized colour variable for an individual bird in a given year. However, because SY and ASY males display differently coloured plumage (yellow and orange respectively), the unstandardized colour variables were used to examine the change in colour from SY to ASY plumage. Reflectance spectrometry sometime produces erroneous outliers, so for each colour variable any outliers (individuals falling outside of a 95% confidence interval) were excluded from further analysis.

To quantify how colour changed between SYs and ASYs, I used a mixed-effects model using occurrence (either SY, ASY1, ASY2, or ASY3) as a fixed effect and band number (individual) as a random effect, with each colour variable as a response variable. Occurrence was determined based on the age and the number of years in which an individual has been sighted (e.g., ASY2 would mean the second year in which an individual was sighted as an ASY bird). To detect colour changes in adults of each sex from year to year, this model was used both with all birds (to examine colour change in the general population) as well as



in a longitudinal analysis with only those individuals that were captured in consecutive years (to examine colour change within-individuals themselves longitudinally through time). We then used a paired t-test to test for differences between years within individuals.

## RESULTS

### *Year to Year Colour Variation*

Initial analysis revealed significant yearly variation in all plumage colour characteristics in both SY and ASY males ( $p < 0.0001$  for all, see appendix B1; ASY male hue:  $n = 281$ ,  $F = 80.59$ ,  $p < 0.0001$ ; SY male hue:  $n = 146$ ,  $F = 20.63$ ,  $p < 0.0001$ , figure 1a). Significant yearly variation was also seen in the hue and brightness of ASY females (hue:  $n = 72$ ,  $F = 4.13$ ,  $p = 0.0008$ , figure 1b; brightness  $n = 74$ ,  $F = 2.45$ ,  $p = 0.02$ , see appendix B2) and for the hue and carotenoid chroma of SY females (hue:  $n = 42$ ,  $F = 3.30$ ,  $p = 0.01$ , figure 1b; carotenoid chroma:  $n = 42$ ,  $F = 3.90$ ,  $p = 0.006$ , see appendix B2). Females did not exhibit a significant yearly variation in the other plumage characteristics (appendix B2). Because of these large annual variations in colour, it was necessary to standardize colour variables before any further analyses were conducted.

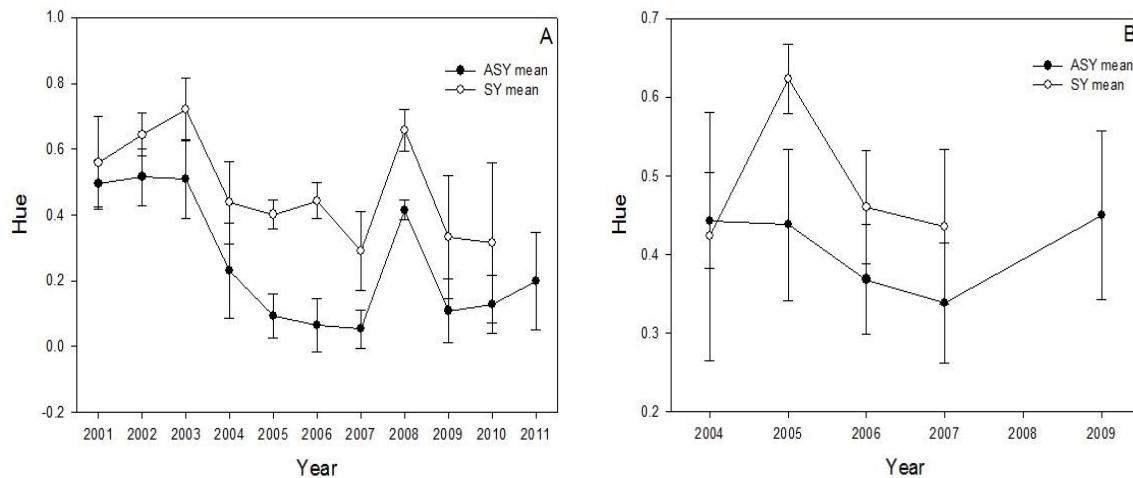


Figure 1. Mean plumage hue varied significantly among years of study for both male (A) and female (B) American redstarts. Represented are the yearly population means with standard deviations.

### *Intermediate to Definitive Colour Change*

At the population level, male American redstarts exhibited high variation in plumage colour characteristics. As predicted, I found significant differences in carotenoid chroma ( $n=342$ ,  $F=17.14$ ,  $p<0.0001$ ; figure 1c) and hue ( $n=345$ ,  $F=264.71$ ,  $p<0.0001$ ; figure 1d) between SY and ASY males (figure 1); ASY males displayed lower carotenoid chroma and hue than SY males (i.e., they displayed plumage that was more orange-shifted and had more enriched carotenoids; table 1). There were no significant difference in brightness ( $n=343$ ,  $F=1.11$ ,  $p=0.29$ ; figure 1a) or chroma ( $n=343$ ,  $F=3.55$ ,  $p=0.06$ ; figure 1b) between SY and ASY males. Paired t-tests with males caught as SYs and again as ASYs also showed that, at the individual level, ASY males significantly decrease in carotenoid chroma ( $n=12$ ,  $t=-2.56$ ,  $p=0.03$ ) and hue ( $n=12$ ,  $t=-2.40$ ,  $p=0.035$ ), while showing no significant changes in chroma ( $n=12$ ,  $t=1.03$ ,  $p=0.32$ ), or brightness ( $n=12$ ,  $t=-0.04$ ,  $p=0.97$ ). This means that individual males became more orange-shifted and enriched more carotenoids as they aged from SYs to ASYs.

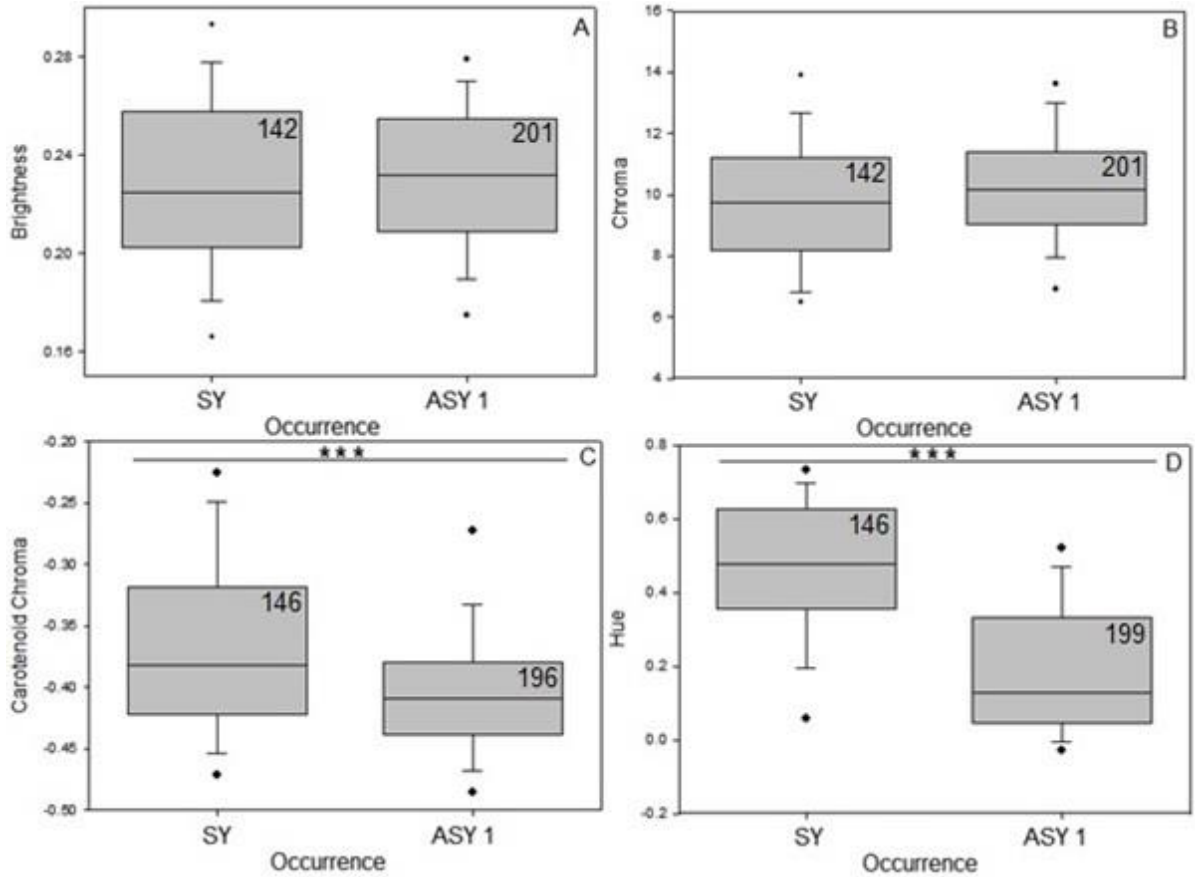


Figure 2. Changes in brightness (A), chroma (B), carotenoid chroma (C), and hue (D) from SY to ASY1 males. Represented are the results of the mixed-effects model, sample sizes are given in the corners of boxes (\* indicates  $p < 0.05$  significance level, \*\*\* indicates  $p < 0.001$  significance level). Whiskers represent 5<sup>th</sup>/95<sup>th</sup> percentiles, dots represent outliers beyond the 5<sup>th</sup>/95<sup>th</sup> percentile, and the center line indicates the mean.

Mixed-effects models revealed that females undergo significant changes in brightness ( $n = 105$ ,  $F = 18.58$ ,  $p = 0.0002$ ; figure 2a) and hue ( $n = 101$ ,  $F = 5.38$ ,  $p = 0.02$ ; figure 2d) between their second (SY) and later years of life (ASY); at the population level, ASY females are brighter and more orange-shifted than SY females (table 1). However, a paired t-test could not be conducted due to the small sample size ( $n=2$ ) of females that were captured as SYs and then again in the subsequent year, so we were unable to determine if this colour change occurs within individuals.

Table 1. Plumage colour characteristics by age and sex of American redstarts (unstandardized values).

	Females (mean $\pm$ SD)		Males (mean $\pm$ SD)	
	SY	ASY	SY	ASY
Brightness	0.18 $\pm$ 0.06	0.22 $\pm$ 0.04	0.23 $\pm$ 0.04	0.23 $\pm$ 0.03
Chroma	8.39 $\pm$ 3.23	9.54 $\pm$ 2.49	9.84 $\pm$ 2.34	10.25 $\pm$ 2.14
Carotenoid Chroma	-0.40 $\pm$ 0.08	-0.38 $\pm$ 0.05	-0.37 $\pm$ 0.08	-0.40 $\pm$ 0.17
Hue	0.46 $\pm$ 0.13	0.41 $\pm$ 0.10	0.44 $\pm$ 0.18	0.17 $\pm$ 0.17

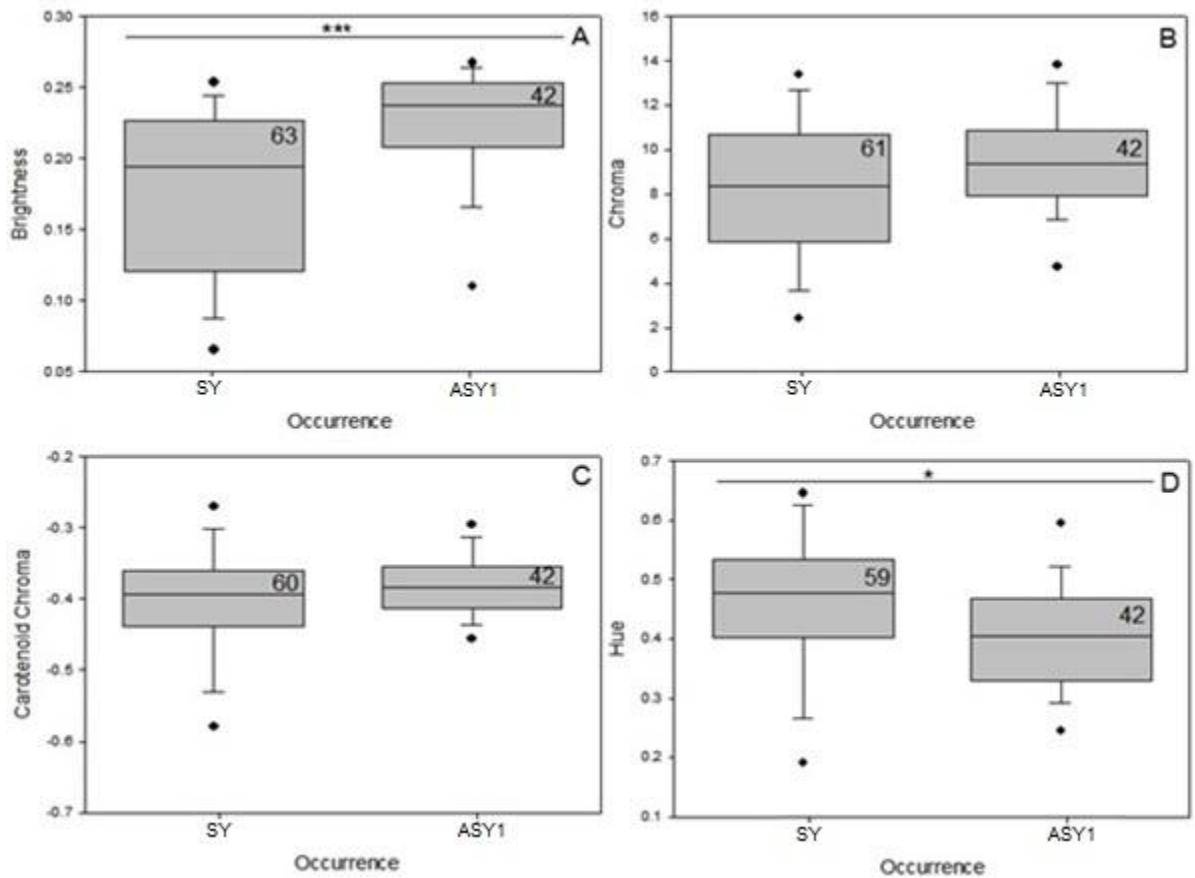


Figure 3. Changes in brightness (A), chroma (B), carotenoid chroma (C), and hue (D) from SY to ASY1 females. Represented are the results of the mixed-effects model, sample sizes are given in the corners of boxes (\* indicates  $p < 0.05$  significance level, \*\*\* indicates  $p < 0.001$  significance level). Whiskers represent 5<sup>th</sup>/95<sup>th</sup> percentiles, dots represent outliers beyond the 5<sup>th</sup>/95<sup>th</sup> percentile, and the center line indicates the mean.

### *Change in Definitive Plumage Colour*

Mixed-effects models using all males captured in one or more occurrences as ASYs revealed that all colour characteristics vary significantly with age (brightness:  $n=281$ ,  $F=5.18$ ,  $p<0.0001$ ; chroma:  $n=282$ ,  $F=3.86$ ,  $p=0.0011$ ; carotenoid chroma:  $n=274$ ,  $F=4.05$ ,  $p=0.0007$ ; hue:  $n=274$ ,  $F=3.83$ ,  $p=0.0012$ ), where older males in the population displayed brighter, more chromatic and more yellow-shifted plumage than younger males. However, when only those male redstarts captured in three consecutive years were tested (i.e., when we examined colour change at the individual level), only hue changed significantly with age ( $n=36$ ,  $F=6.98$ ,  $p=0.004$ ). No significant colour change occurred within individual males for brightness ( $n=37$ ,  $F=0.78$ ,  $p=0.46$ ), chroma ( $n=36$ ,  $F=3.09$ ,  $p=0.06$ ), or carotenoid chroma ( $n=36$ ,  $F=2.42$ ,  $p=0.11$ ). Results of a paired t-test confirmed that males display plumage with the lowest hue in their first occurrence as ASYs and moult into feathers with a significantly higher hue value in the following year ( $n=11$ ,  $t=2.38$ ,  $p=0.04$ ; figure 4). They continued to increase in hue between their second and third year, although not significantly ( $n=10$ ,  $t=0.60$ ,  $p=0.56$ ), and by their third occurrence males still displayed significantly higher hue than when they were in their first occurrence as ASYs ( $n=13$ ,  $t=4.23$ ,  $p=0.001$ ).

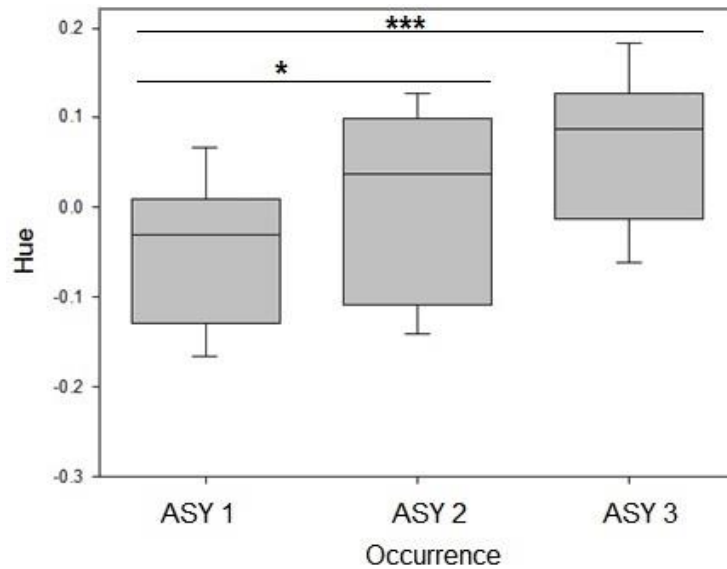


Figure 4. Change in feather hue of male American redstart from their first to their third occurrences as ASYs. Represented are the results of a paired t-test (\* indicates  $p < 0.05$  significance level, \*\*\* indicates  $p < 0.001$  significance level). Whiskers represent 5<sup>th</sup>/95<sup>th</sup> percentiles, and the center line indicates the mean.

Due to a limited sample size of females, I was only able to compare the colour of ASY females captured in two consecutive years (see appendix A). Results of the mixed-effects models revealed that ASY females did not change significantly in brightness ( $n = 72$ ,  $F = 0.47$ ,  $p = 0.49$ ), chroma ( $n = 72$ ,  $F = 2.90$ ,  $p = 0.09$ ), carotenoid chroma ( $n = 70$ ,  $F = 0.01$ ,  $p = 0.91$ ), or hue ( $n = 70$ ,  $F = 1.52$ ,  $p = 0.24$ ) as they age (Figure 5). Paired t-tests could not be performed due to a limited sample size ( $n = 2$ ) of females that were captured in both their first and second occurrences as ASYs.

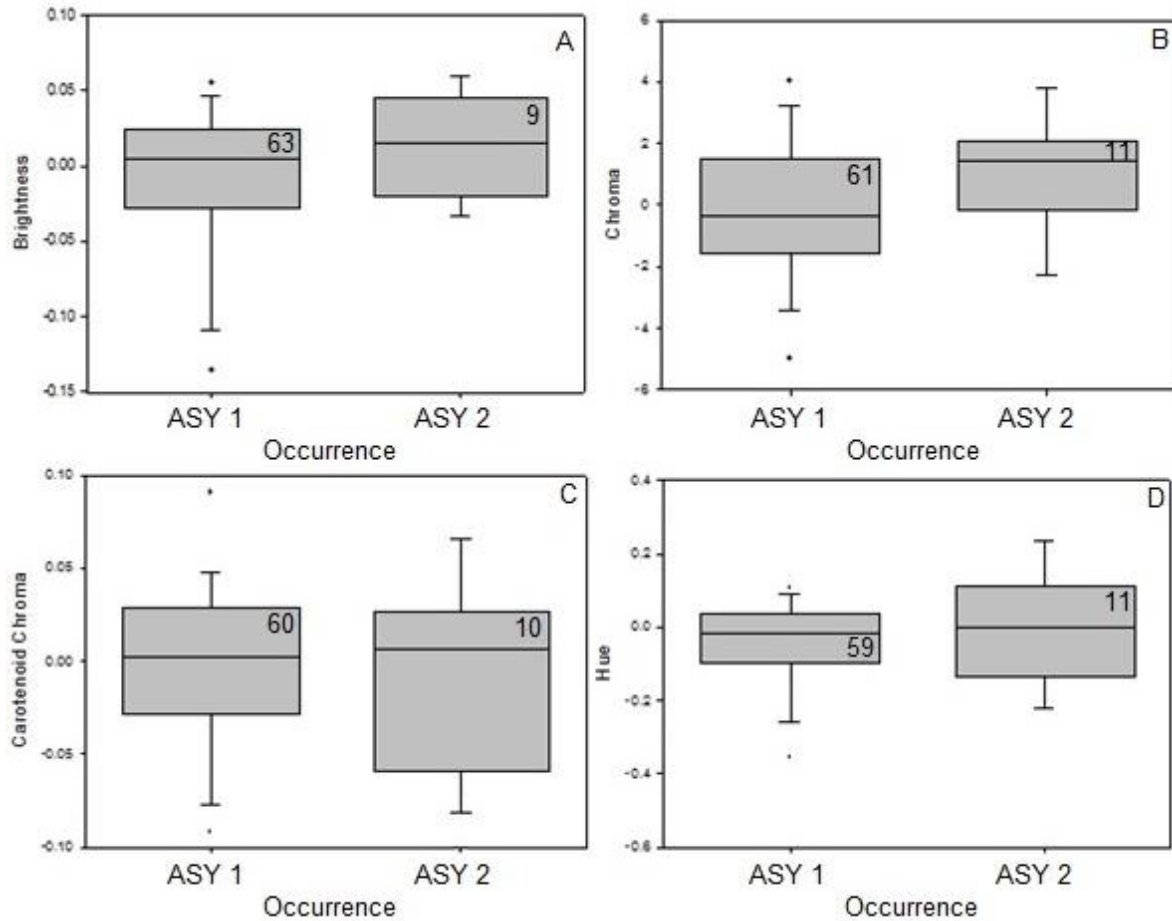


Figure 5. Changes in brightness (A), chroma (B), carotenoid chroma (C), and hue (D) for females in their first to second occurrence as ASYs. Represented are the results of the mixed-effects model, sample sizes are given in the corners of boxes. Whiskers represent 5<sup>th</sup>/95<sup>th</sup> percentiles, dots represent outliers beyond the 5<sup>th</sup>/95<sup>th</sup> percentile, and the center line indicates the mean.

## DISCUSSION

Male American redstarts undergo an age-related colour change after definitive adult plumage has been obtained. At the population level, males in their first occurrence as ASYs displayed the orange-shifted plumage, while older males displayed plumage that is more yellow-shifted in colour. This pattern was also observed in longitudinal analysis where I examined males captured in three consecutive years. Individuals showed a significant yellow

shift to their plumage (measured as an increase in hue value) between their ASY1 and ASY2 occurrence, and between their ASY1 and ASY3 occurrence (though there was no significant change between the second and third occurrence. This finding supports the age-related colour change hypothesis, as the colour change is occurring within individuals themselves over time.

Age-related carotenoid-based colour change has also been documented in great tits; both males and females became greener and more saturated in colour as they age (Val et al. 2010). Because carotenoid-based plumage colouration is often regarded as an honest indicator of body and nutritional condition, it has been suggested that this colour change arises as a result of the fact that older great tits become more efficient foragers (Hill and Montgomerie 1994; Hill 1999; Val et al. 2010). Increased foraging efficiency does not fully explain the colour change seen in redstarts, however, as older females would presumably also increase in foraging efficiency, yet no colour differences were found in female redstarts as they aged.

At the population level, older male redstarts also had brighter plumage with higher carotenoid chroma, but decreased chroma compared to younger males. However, these differences (in brightness, carotenoid chroma, and chroma) were not significant at the individual level. This result is similar to what Pagani-Núñez and Senar (2012) found in European serins, where individuals themselves did not exhibit any 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 favour survival of individuals with brighter plumage (Pagani-Núñez and Senar 2012), and similar selection pressure could explain the observed population level colour change in redstarts. During the winter, redstarts with duller (lower brightness) tail feathers most often occupy lower-quality habitats (Reudink et al. 2009c), and are more likely to be in poor physical condition by the end of winter (Marra and Holberton 1998; Studds and Marra 2005). Individuals in poor physical condition may suffer decreased chances of surviving to subsequent breeding seasons, which could account for why we found the increase in brightness at the population level, but not in individual males themselves.



Analysis at the population level revealed that both male and female American redstarts display significantly different colour characteristics between SY and ASY age classes, and also that it is different colour characteristics that change in each sex. In males, which display delayed plumage maturation, this manifested in ASY birds displaying significantly lower carotenoid chroma and hue, resulting in plumage which was more orange-shifted compared to the yellow-shifted plumage of SY males. In females, ASY birds possess plumage which was significantly brighter and had lower hue, resulting in feathers that were brighter and more orange-shifted (though still appear yellow to the eye) compared to the plumage of SY females. This change in colour between SYs and ASYs is consistent with differences in brightness reported by Osmond et al. (in review).

Because carotenoids can only be obtained through the diet, carotenoid-based colouration is often seen as a measure of male condition or quality: males 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, as individuals age they become more efficient at foraging (Morrison et al. 1978, Gochfeld and Burger 1984); individuals may learn through time which prey species are rich in carotenoids, and thus may be able to produce plumage more enriched with carotenoid pigments as they age. The observed trend for males to display more yellow-shifted plumage with age then is opposite to what would be expected, as it appears that fewer carotenoids are being incorporated into plumage.

A potential explanation for the yellow shift in plumage colouration is that there is a trade-off between the colour that can be produced and reproductive effort; an individual has only a finite amount of energy to allocate among activities or processes such as reproduction, immune function, migration, and moult. There has been considerable research on the trade-offs between clutch size and factors such as parental survival, offspring survival, future reproductive success (reviewed in Godfray et al. 1991), and future ornamentation (Siefferman and Hill 2005). Previous research on eastern bluebirds (*Sialia sialis*) revealed that when clutch size was experimentally decreased, males significantly increased in plumage brightness in the subsequent year (Siefferman and Hill 2005), indicating a trade-off between current reproductive effort and energy left over to invest in future reproduction. This trade-

off has also been shown to influence the degree of ornamental plumage in the house sparrow (*Passer domesticus*), where males with an experimentally increased clutch size (increasing their reproductive effort) grew a smaller badge in the following year (Griffith 2000). Norris et al. (2004) found that male American redstarts that exerted a high reproductive effort, tended to grow feathers that were more yellow-shifted (reflected light at shorter wavelengths) than males that made a smaller reproductive effort. However, not all studies agree that this trade-off exists in redstarts. Reudink et al. (2008) studied the same population of redstarts and found no relationship between reproductive effort and any aspect of plumage colour in the following breeding season.

Due to the conflicting results of previous studies, it is unclear if a trade-off between current reproductive effort and energy left over to invest in future reproduction influences redstart plumage colour. Though sexually mature, SY males are less successful in attracting a mate, pairing success rates in previous studies range from 40-71% (Sturm 1945, reviewed in Procter-Grey and Holmes 1981) compared to 71-100% success in ASY males (reviewed in Procter-Grey and Holmes 1981), resulting in a lower reproductive effort for most SYs. Thus, more of their energy can go towards moulting into very intensely orange coloured feathers. Additionally, because they do not have offspring to care for, unsuccessful males may begin moulting earlier than successfully breeding males, giving them a longer total period of time to complete the moult before the onset of migration.

Time to complete moult also influences plumage colour. In a previous study with house finches, individuals that started the moult earlier, resulting in a longer period of time to complete this process, grew brighter feathers than those individuals that started moulting later (Hill and Montgomerie 1994). Likewise, rock sparrows (*Petronia petronia*) that experienced experimentally shortened moult periods grew feathers with smaller, less pigmented yellow patches (Serra et al. 2007). Redstarts undergo a complete moult during a limited time period between fledging their young and leaving on their migratory journey to the wintering grounds. Previous work suggests that individuals that fledge young late in the season may delay the onset of moult until during migration, a phenomenon referred to as moult migration. Norris et al. (2004) determined that those individuals that do undergo moult migration moult into plumage that is less saturated in orange colouration than those males

that complete moult on the breeding grounds. However, this phenomenon is debated as Reudink et al. (2008) examined the same population and found no evidence of moult migration or that moult timing affected plumage colour in the following season.

A combination of low reproductive effort and increased length of time to complete moult for SY males could cause the pattern of colour change I found, in which ASY males display the most orange-shifted plumage in their first occurrence and then significantly decrease in colour intensity in subsequent years. ASY males in their first occurrence on the breeding ground are much more likely than SY males to successfully pair and raise offspring (i.e., show comparatively high reproductive effort), which could result in these birds having less time and energy to put towards moult; this could explain why in the following year males regrew significantly yellow-shifted plumage in the following year. It could also account for the lack of colour change observed in ASY females. In our study population, females successfully mate as SYs as well as ASYs (unpublished data), meaning that they would likely exert a similar reproductive effort at both ages (and thus would not benefit from the possible trade-off between current and future reproductive effort).

Although these results support the idea that within-individual colour change does occur in American redstarts, the underlying cause of this change is still unclear. Additionally, at the population level, it still may be true that some form of selection is acting against duller individuals. Plumage colour is a complex trait influenced by many factors, and further study is needed to better understand the role that reproductive effort plays in influencing colour. Further research should examine whether an energetic trade-off between current and future reproductive success is indeed occurring, as well as on what specific aspects of reproductive effort influence colour the most (e.g., number of mates, number of fledglings, fledging date). This could potentially be done through a combination of further observations in the field paired with experimental manipulations of clutch size similar to those done with bluebirds. The results of this future research will help further our understanding of how plumage colour and reproductive success are related.

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## APPENDIX A

Table A1. The number of redstarts captured in each year of the study for which feathers were available for colour analysis.

Capture Year	Females		Males	
	SY	ASY	SY	ASY
2001	0	0	11	3
2002	0	0	25	18
2003	1	3	13	18
2004	18	25	29	35
2005	12	16	38	43
2006	17	11	17	40
2007	12	16	13	51
2008	0	1	16	22
2009	9	12	17	25
2010	0	0	13	38
2011	0	1	1	13
<b>Total</b>	<b>69</b>	<b>85</b>	<b>193</b>	<b>306</b>

## APPENDIX B

Table B1. Yearly variation in plumage colour characteristics for male American redstarts (by age). Results of ANOVAs, significant results are displayed in bold.

	SY			ASY		
	n	F	p	n	F	p
Brightness	<b>143</b>	<b>12.90</b>	<b>&lt;0.0001</b>	<b>281</b>	<b>24.49</b>	<b>&lt;0.0001</b>
Chroma	<b>142</b>	<b>4.49</b>	<b>&lt;0.0001</b>	<b>282</b>	<b>6.22</b>	<b>&lt;0.0001</b>
Carotenoid Chroma	<b>146</b>	<b>15.45</b>	<b>&lt;0.0001</b>	<b>274</b>	<b>19.96</b>	<b>&lt;0.0001</b>
Hue	<b>146</b>	<b>20.63</b>	<b>&lt;0.0001</b>	<b>281</b>	<b>80.59</b>	<b>&lt;0.0001</b>

Table B2. Yearly variation in plumage colour characteristics for female American redstarts (by age). Results of ANOVAs, significant results are displayed in bold.

	SY			ASY		
	n	F	p	n	F	p
Brightness	42	2.05	0.09	<b>74</b>	<b>2.45</b>	<b>0.02</b>
Chroma	42	1.12	0.36	74	1.46	0.19
Carotenoid Chroma	42	3.9	0.006	72	1.87	0.08
Hue	<b>42</b>	<b>3.3</b>	<b>0.014</b>	<b>72</b>	<b>4.12</b>	<b>0.0008</b>