CARRY-OVER EFFECTS IN AMERICAN REDSTARTS:
IMPLICATIONS FOR SEXUAL SELECTION AND BEHAVIOUR

by

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Abstract

Migratory birds spend most of the year on the over-wintering grounds or traveling between breeding and wintering areas, but research has focused on the relatively short breeding period. As a consequence, we have only a rudimentary understanding of how life histories of long-distance migrants are shaped by events and selective pressures interacting throughout the annual cycle. In this thesis, I examine the association between plumage traits and performance, both during the over-wintering and breeding phases of the annual cycle and how events during one season carry-over to influence behavioural and evolutionary processes in subsequent seasons in a migratory warbler, the American redstart (*Setophaga ruticilla*).

First, I demonstrate that tail feather brightness is correlated with winter habitat quality in Jamaica, suggesting that plumage may act as a status signal during the non-breeding season. Stable-carbon isotopes analyzed from claws of redstarts arriving on the breeding grounds confirm the association between ornamentation and winter territory quality. Second, I demonstrate that redstarts arriving to breed in southern Ontario from high-quality winter habitats arrive earlier, resulting in a lower probability of paternity loss, a higher probability of achieving polygyny, and higher genetic fledging success. Third, I demonstrate that tail feather brightness, associated with winter territory quality, predicts the likelihood of polygyny during the breeding season, indicating that tail brightness is associated with performance during two phases of the annual cycle. Paternity is predicted by both tail and flank colouration. Finally, I demonstrate that reported trade-offs between reproductive effort and plumage ornamentation as manifested by moult-migration in redstarts is likely an artifact of high variation in local stable-
hydrogen isotope signatures (δD) and occasional feather loss and re-growth during the over-wintering period. Thus, moult-migration does not appear to be an important carry-over effect in redstarts. This work demonstrates that plumage may be under selection during both stationary phases of the annual cycle. Furthermore, it suggests that carry-over effects from the non-breeding season can influence evolutionary processes such as sexual selection and highlights the importance of considering selective pressures and events occurring throughout the annual cycle in studying the behaviour and ecology of migratory animals.


**Co-Authorship**

Chapters 2-5 are co-authored by my advisors, Laurene Ratcliffe and Peter Marra. Both advisors funded data collection and provided input to the design, analysis and interpretation of these studies. Chapters 2, 3, and 5 are co-authored by Kurt Kyser, who provided personnel and equipment at Queen’s Facility for Isotope Research to conduct isotope analyses. Kurt also provided input into study design and interpretation of the isotope data. Chapters 2 and 5 are co-authored by Colin Studds, who collected a number of feather samples in Jamaica and contributed to the statistical analysis. Chapters 3 and 5 are co-authored by Katie Langin, who collected the 2004 breeding season field data and contributed to the study design and interpretation of Chapter 5. Peter Boag is a co-author on chapters 3 and 4 and contributed personnel and equipment at the Queen’s University Molecular Ecology Lab for microsatellite analyses. All co-authors provided editorial comments on manuscripts.

This thesis is in Manuscript format, in accordance with the Department of Biology Guide to Graduate Studies guidelines. The General Introduction (Chapter 1) is a popular press article, as approved by my thesis supervisory committee in December 2007.

Authorship of published papers and anticipated publications:

- **Chapter 1** Reudink, M. (in prep.). Behaviour and ecology of American redstarts throughout the annual cycle.


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Chapter 1

General Introduction
Tracking the year-round behaviour and ecology of migratory birds

From the massive historic herds of bison traversing the plains of western North America to the pole-to-pole journey of the Arctic tern, migration is arguably one of the most staggering of all biological phenomena. Almost none of the earth is left untouched by migratory animals. The yearly movement between breeding and non-breeding areas can span thousands of kilometers, involving billions of individuals moving between continents and across oceans. Aquatic mammals, such as humpback whales migrate thousands of kilometers from the warm, tropical waters off Central America to the krill-rich feeding areas off Antarctica. Tracking the bounty produced by seasonal monsoon rains, African wildebeest, zebra, and elephants embark on vast over-land journeys across the parched lands of the Serengeti from Tanzania to the Masai Mara of Kenya. Migratory systems, however, are not limited by body size. By far the most numerous movements involve billions of flying insects such as butterflies, locusts, and dragonflies traveling thousands of kilometers to breed. Yet, the migratory system that has most captivated the imagination of scientists and casual observers alike belongs to migratory birds.

Of the roughly 10,000 bird species, nearly 40% migrate regularly and that proportion increases dramatically at higher latitudes – an estimated 5 billion landbirds from over 200 species migrate from North America to the wintering grounds in Central and South America (with roughly the same numbers migrating from Europe and Asia to Africa). Both because of the extraordinary magnitude of bird migration as well as the incredible distances traveled, it is no surprise that much work has been directed towards understanding the ecology and behaviour of migratory birds. Perhaps the most impressive
of all avian migrations is the trans-continental journey of the Arctic tern, who travels yearly from its breeding grounds in the Arctic south to the Antarctic seas – a round-trip journey of roughly 30,000 kilometers. Amongst the most energetically demanding of journeys is that of the small, 11 gram blackpoll warbler, who leaves the breeding grounds in southeastern Canada and New England and flies 80-90 non-stop hours over water before touching down in the Caribbean or South America. No less remarkable, however, is the 24-hour trans-Gulf of Mexico flight of the diminutive ruby-throated hummingbird.

Although their capacity for long-distance flight has made migratory birds extremely successful in colonizing nearly every remote corner of the globe, their long-distance movements also make them particularly susceptible to population declines and extinctions. Across the globe, migratory birds are experiencing some of the most drastic population declines of any organisms. Rapidly changing climactic conditions are quickly advancing the spring phenology of the insect prey many migratory birds rely on during the breeding season, resulting in a disconnect between arrival on the breeding grounds and peak food abundance. Moreover, migratory birds must cope with the challenges of deforestation and habitat loss not only on the breeding grounds, but also on the wintering grounds and at stopover sites during migration. From a conservation perspective, one of the major challenges is understanding how to enact policies to protect migratory birds when, despite the vastness of the phenomenon of bird migration and the extraordinary number of individuals involved, we still know little about the factors that influence their life history and behaviour outside of the breeding period. Indeed, most of our knowledge of migratory birds comes from studies conducted on the temperate breeding grounds in
Eurasia and North America, with a paucity of studies addressing the over-wintering and stopover ecology of migratory birds.

It is exceedingly clear from studies of resident animals that events occurring throughout the year can impact survival and reproduction. These events occurring in one season that influence survival or performance in subsequent seasons are termed *carry-over effects*. Carry-over effects may be manifest at the population level (e.g., drought conditions in Africa during the winter influencing population-level reproductive success of barn swallows the following spring) or at the individual level. As an example of individual-level carry-over effects, in Columbian ground squirrels, body condition in late fall carries-over to influence over-winter survival and, in turn, over-winter condition influences reproductive success the following spring. These individual-level carry-over effects have been detected across taxonomic levels. In fishes, such as slippery-dick wrasse, larval growth rates predict juvenile survival in the fall; in wood frogs, juvenile body size predicts over-winter survival. Within birds, dunnocks supplemented with food during the winter breed earlier in the spring. Yet, these seasonal carry-over effects can influence more than just survival and reproductive success. In black-capped chickadees, dominance status during the winter influences both social and genetic female mate choice during the breeding season. Additionally, events occurring early in life can carry-over to influence mate choice and sexual selection later in life. The expression of yellow, carotenoid-based ornamental plumage in first-year blue tits, a signal important for both winter dominance status and for mate choice, is controlled primarily by carotenoid acquisition during the first few days after hatching. In subsequent years as well, the
brilliance of ornamental plumage traits is highly dependent on condition at the time of moult, which, for many species occurs nearly a year prior to breeding.

Yet, clearly carry-over effects are not limited to resident species. At the population level, droughts on North American prairies, which act as migratory staging areas for lesser snow geese, result in reduced clutch sizes during the breeding season in the Arctic. The challenge in tracking individual-level carry-over effects, however, is the inherent difficulty in tracking migratory birds throughout the annual cycle. For large species, such as geese or raptors, radio or satellite transmitters can be attached to individuals to track their movements throughout migration. Yet, this technique is not without its limitations. For most researchers, the costs of satellite tracking are prohibitive and radio tracking is limited by the researchers’ ability to follow the birds using cars or aircraft. For small songbirds, satellite transmitters are too large and radio transmitters that are small enough to be carried lack the battery power and transmitter strength necessary for tracking individuals through migration. Thus, researchers have turned to the use of intrinsic biochemical markers, such as DNA and stable-isotopes.

The challenge with using DNA is that many migratory birds have poor genetic population structuring, meaning that even if we capture a bird on the wintering grounds, we may only, at best, be able to assign it to an eastern or western breeding population. Furthermore, genetic data only allow us to make connections back to the breeding grounds and tell us nothing about over-wintering locations, conditions, or events. Other biochemical markers that have recently been put to extensive use are stable-isotopes. Stable-isotopes are naturally-occurring forms of elements that vary in their atomic mass (e.g., $^{13}$C, $^{15}$N, $^{2}$H). The heavy isotopes of carbon, nitrogen, and hydrogen have one
additional neutron and are ubiquitous in all biological systems, but in much lower abundance compared to their lighter counterparts. Importantly for biologists, the ratio of the heavy to light isotopes (e.g., $\delta^{13}\text{C}$, which written in delta notation and expressed in parts per mil (‰) relative to an international standard) varies predictably within the environment.

Perhaps the most extensively utilized stable-isotope system is that of hydrogen. Because of the high energy available in tropical systems, precipitation originating from equatorial waters is enriched in deuterium. As weather systems move across landmasses, such as continental North America, the heavy isotope deuterium is preferentially lost in precipitation. Thus, as a storm system travels further, less of the heavy isotope remains to be lost in precipitation. The result is that by monitoring precipitation patterns across North America, we can construct an isotopic map to which we can later assign birds (Figure 1.1). Isotopic signatures in the precipitation are then transferred up the food chain, from plants to insects and eventually into the tissues of birds. By sampling a tissue that is inert once grown, such as a feather, we can infer the geographic location of that bird at the time that tissue was grown. This technique has proved invaluable for making connections between wintering and breeding populations, determining migratory stopover sites used for moult, and detecting long-distance dispersal.

However, for many questions, it is necessary to examine tissues grown outside the breeding season. For examining carry-over effects from winter to summer, one technique is to analyze tissues, such as claws, that turnover their isotopic signatures on the order of weeks to months. By sampling claw tissues of birds as they arrive on the breeding grounds, the isotopic signatures in the claws will reflect conditions during winter. This
technique has been used successfully to determine individual winter habitat quality taking advantage of the fact that habitats in the tropics vary isotopically in carbon based on plant photosynthetic systems and water stress. \( \text{C}_4 \) plants and plants under water stress are less able to discriminate against the heavy isotope of carbon resulting in more positive \( \delta^{13}\text{C} \) signatures. Thus, plants in water-stressed habitats, such as dry scrub forests, differ isotopically from plants in \( \text{C}_3 \)-dominated, water-saturated habitats such as mangroves. As with deuterium, these isotopic signatures are transferred up the food chain and eventually incorporated into the tissues of birds.

By sampling tissues such as claws and blood, developed on the wintering grounds from migrating birds and birds arriving on the breeding grounds, we have gained incredible insight into how events during the non-breeding season can carry-over to subsequent seasons. We now know that winter habitat quality can influence individual condition during migration, arrival date on the breeding grounds, condition upon arrival, apparent reproductive success, and even natal dispersal.

For my dissertation work at Queen’s University in Kingston, Ontario, and in conjunction with the Smithsonian Migratory Bird Center in Washington, DC, my goal was to understand how events acting throughout the annual cycle can influence the behaviour of migratory birds and impact evolutionary processes. My primary focus was to ask how plumage is related to performance during both the breeding and non-breeding seasons, and to understand how carry-over effects (both from the breeding and non-breeding seasons) can influence behavioural and evolutionary processes (Figure 1.2). For this work, I studied a striking and charismatic long-distance migratory songbird, the American redstart (\textit{Setophaga ruticilla}). American redstarts are small, insectivorous
songbirds that are common breeders throughout much of North America and over-winter in the Caribbean as well as Central and South America (Figure 1.2). Redstarts are sexually dichromatic and exhibit delayed plumage maturation, meaning that females and first-year males have drab, gray and yellow plumage and males do not moult into their definitive, black and orange adult plumage until after their first breeding season (Figure 1.3). Besides being an excellent candidate for research on plumage colouration, redstarts are one of the few species of migratory birds that have been intensely studied both on the breeding and wintering grounds, making it an ideal species for studying questions related to the year-round ecology and behaviour of migratory birds.

By examining the relationship between plumage colouration and selective pressures during both the breeding and non-breeding seasons, I discovered that the flashy tail-fanning display used by redstarts to scare up their insect prey, is likely under directional selection both during the non-breeding and breeding seasons. On the non-breeding grounds in Jamaica, redstarts aggressively compete for territories in high-quality habitats. I demonstrated that both first-year and adult males over-wintering in high-quality habitats had brighter tails than birds from poor-quality habitats. On the breeding grounds, I analyzed the stable-carbon isotope signatures in the claws of newly arriving males and found that this same relationship between tail brightness and habitat quality held with birds likely arriving on the breeding grounds from a variety of winter locales. These results suggest that plumage may act as a status signal, mediating winter territory acquisition. Interestingly, tail feather brightness was also related to polygyny on the breeding grounds, where polygynous males were brighter than monogamous males. Because polygyny is highly dependent on maintaining multiple territories, it is likely that
tail feather brightness also serves to mediate territory acquisition on the breeding grounds, thus functioning as a status signal during two major phases of the annual cycle. Another interesting possibility is that females may be using information gathered during the non-breeding season (i.e., bright males are the most competitive and best able to secure high-quality territories) to inform mate choice decisions during the breeding season.

Nearly 20 years of intense study on over-wintering American redstarts in Jamaica has demonstrated that the quality of a male’s winter territory can have significant ramifications throughout the year. Redstarts over-wintering in high-quality territories have higher over-winter survival, are in better condition, and depart for the breeding grounds earlier. Critically, stable-carbon isotope analysis of birds arriving to breed in North America has also revealed that winter habitat quality can influence arrival timing, condition upon arrival, and apparent reproductive success. In my work, I found that early arrival, driven by winter habitat quality, can influence the evolutionary process of sexual selection through variation in paternity and polygyny. Early arriving males secure paternity at their own nest, sire extra-pair offspring, achieve polygyny and ultimately fledge one additional offspring per year.

Yet, there may be trade-offs associated with high amounts of reproductive effort. A recent study suggested that, in redstarts, raising many offspring late into the season results in a situation whereby these males are forced to delay some of their moult until migration, a process known as moult-migration. Because of the stress associated with moulting and migrating at the same time, these birds moult in poor-quality feathers, which could then hinder their ability to obtain a high-quality winter territory and affect
their ability to attract mates the following season. However, after recapturing known breeders from the previous season in two years, I failed to detect any birds with hydrogen-isotope signatures indicative of moult outside the known variation in our study population. Furthermore, I found no evidence to support the idea that late season parental effort resulted in poor quality feathers with more positive isotopic values. I suggest instead that the reported phenomenon of moult-migration in redstarts is likely an artifact of high variation in local δD and occasional feather loss and adventitious moult during the over-wintering period. Thus, while carry-over effects of winter habitat quality have significant effects during the breeding period, I was unable to detect a carry-over effect driven by reproductive effort.

So, how does this work inform our understanding of the behaviour and ecology of migratory birds? First, it demonstrates that to understand the evolution of plumage ornamentation in migratory birds, we must examine selective pressures acting throughout the annual cycle. While many bird species moult into drab, non-breeding plumage, a large number maintain their showy nuptial plumage throughout the year, yet scant attention has been paid to the selective forces acting on plumage outside the breeding season. Furthermore, we may soon learn that, much like resident species, interactions between individuals during the non-breeding season help inform mating decisions.

Second, in much of the tropics, areas that provide high-quality habitat for many migratory birds, such as mangroves and wet lowland forests, are also those most at risk from human development. While the loss of high-quality habitats in the tropics will undoubtedly result in large-scale population declines, this loss of habitat may also alter evolutionary processes such as sexual selection in ways we had never imagined. Yet this
research is just the beginning for gaining a holistic view of the events and pressures that influence the life history migratory birds throughout the annual cycle. Future research will need to be dedicated to discovering and understanding carry-over effects, not just from the stationary breeding and over-wintering periods, but also those events occurring during even more poorly understood migratory period.

**Suggested Reading**


Hobson, K. A. and Wassenaar, L. I. 1997 Linking breeding and wintering grounds of
neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers.


Figure 1.1. Hydrogen isotopic base map of North America created by Hobson and Wassenaar (1997) based on growing-season precipitation. Dots represent International Atomic Energy Association (IAEA) precipitation sampling stations and contour lines indicate general patterns isotopic variation.
Figure 1.2. (A) Map of the breeding and wintering range of American redstarts from Sherry and Holmes (1997). Overlaid on the map are the titles of the data chapters of the thesis. The placement corresponds to the periods of the annual cycle being investigated. (B) Schematic representation of the questions asked in this thesis. Numbers in the orange dots represent the questions being asked in the different data chapters.
Figure 1.3. A pair of American redstarts feeding young at the nest (photos: M. Reudink). Top photo is a female in the gray and yellow plumage that is also exhibited by first year males. Only adult males display the black and orange plumage shown in the lower photo.
Chapter 2

Plumage brightness predicts non-breeding season territory quality in a long-distance migratory songbird
Abstract  Many species of birds exhibit brilliant ornamental plumage, yet most research on the function and evolution of plumage has been confined to the breeding season. In the American redstart (*Setophaga ruticilla*), a long-distance Neotropical-Nearctic migratory bird, the acquisition of a winter territory in high-quality habitat advances spring departure and subsequent arrival on breeding areas, and increases reproductive success and annual survival. Here, we show that males holding winter territories in high-quality, black mangrove habitats in Jamaica have brighter yellow-orange tail feathers than males occupying territories in poor-quality second-growth scrub habitats. Moreover, males arriving on the breeding grounds from higher-quality winter habitats (inferred by stable-carbon isotopes) also had brighter tail feathers. Because behavioural dominance plays an important role in the acquisition of winter territories, plumage brightness may also be related to fighting ability and the acquisition and maintenance of territories in high-quality habitat. These results highlight the need for further research on the relationships between plumage colouration, behaviour, and the ecology of over-wintering migratory birds.
Introduction

Study of the function of plumage colouration during the breeding season has informed much of what we know about the elaboration of ornamental traits through sexual selection and female choice (Hill 2006). In many species, plumage is highly variable and phenotypically plastic and can be influenced by both the environment (Linville and Breitwisch 1997, McGraw and Hill 2001) and individual condition at the time of moult (Figuerola et al 2003, Saks et al. 2003). This variation in plumage colouration can be used by conspecifics to gather information about an individual’s quality as a potential mate or competitor (Andersson 1994, Zahavi 1977). Many breeding season studies have now confirmed that females can use this information to inform social (e.g., Hill 1990, Johnsen et al. 1998, MacDougall and Montgomerie 2003, Senar et al. 2005, for review, see Hill 2006) and extra-pair mating decisions (Sundberg and Dixon 1996, Yerzinac and Weatherhead 1997, Thusius et al. 2001) – the result of which can lead to increased variance in male mating success and the elaboration of ornamental traits (Webster 2007, Albrecht et al. 2008, Dolan et al. 2008).

However, plumage can also function as a signal of individual quality during the non-breeding season. Variation in plumage colouration can indicate social dominance (Maynard Smith and Harper 1988, Mennill et al. 2003), and experimental studies have shown that manipulation of signals can alter dominance status (Peek 1972, Rohwer 1985, Holberton 1989, Pryke et al. 2002, Pryke et al. 2003), suggesting that social selection can be an important force in the evolution of plumage traits (for review, see Senar 2006). Yet, despite the widespread evidence for plumage-based status signaling systems in temperate
(and some tropical) species, little is known about whether plumage functions as a signal in migratory birds during the non-breeding season (but see Stutchbury 1994). This is not surprising given the relative paucity of information on the over-wintering ecology and behaviour of migratory birds (Greenberg and Marra 2005). By examining only one phase of the annual cycle, we ignore potential factors that may influence the evolution of ornamental plumage. This work is of particular relevance, as new studies are beginning to reveal how non-breeding season events may influence an individual’s life-history traits, ecology, and behaviour (e.g. Studds et al. 2008).

For plumage to act as a status signal, it must advertise information about an individual’s dominance status or competitive ability (Rohwer 1975). These plumage-based signals may include, but are not limited to, badge size (e.g., epaulet size: Eckert and Weatherhead 1987, bib size: Møller 1987, McGraw et al. 2003) or colouration based on melanin (Mennill et al. 2003), carotenoids (Wolfenbarger 1999, McGraw and Hill 2000, Pryke et al. 2002), or feather microstructure (Alonso-Alvarez et al. 2004). Melanin is synthesized internally from amino acids and is not acquired directly from the diet (Hill 2002), although the honesty of the signal may be maintained by nutrient limitation (McGraw 2007) and social reinforcement mechanisms (Senar 2006), or mediated by testosterone levels during moult (Hill 2002). Carotenoids cannot be synthesized naturally and therefore must be ingested through diet, modified (depending on the pigment), and deposited (Hill 2002, 2006). Thus, only males in good condition with access to high-quality food should be able to signal with carotenoid pigments, making this a potential honest indicator of male quality (Hill 1999; Pryke et al. 2001).
For a plumage-based status signaling system to exist, there must be variation in plumage that can be used to assess individual quality and a system in which assessing the fighting ability or dominance status of conspecifics confers an advantage (Rohwer 1975). American redstarts (*Setophaga ruticilla*), a small long-distance migratory songbird, are ideally suited to this study because they are highly variable in both melanin- and carotenoid-based plumage (Sherry and Holmes 1997; see Methods: Study Species), and dominance relationships play a critical role in obtaining and maintaining high-quality winter territories (Marra 2000). Furthermore, redstarts use a distinctive tail fanning display during aggressive interactions (Sherry and Holmes 1997), suggesting that aspects of tail colouration could be important signals. Dominance behaviour and competitive interactions lead to age- and sex-biased habitat segregation, with adult males occupying the majority of territories in high-quality habitats in our study sites in Jamaica (Marra et al. 1993, Marra 2000). Individuals over-wintering in high-quality, mangrove territories experience higher food availability than those in second-growth scrub and this influences arrival date and reproductive success on breeding areas (Marra et al. 1998; Norris et al. 2004a; Studds and Marra 2007), as well as annual return rates (Marra and Holmes 2001). Interestingly, female redstarts able to acquire and maintain territories in high-quality habitats are larger and more aggressive than females in poor-quality habitat (Marra 2000). However, male redstarts do not differ in body size between habitat types (Marra 2000). One hypothesis for this sex-specific difference is that for males, territory acquisition is mediated through plumage-based status signaling.

Here, we test if plumage colouration is associated with territory occupancy across a habitat quality gradient in Jamaica. Specifically, we test for differences in brightness,
chroma, and hue in tail feathers of male American redstarts holding territories in black mangrove forest (high-quality) and second-growth scrub (low-quality). Additionally, we test if the relationship between plumage colour and winter habitat quality carries over onto the breeding grounds in Ontario, Canada.

Methods

Study species

Our study species was the American redstart, a small (7-8g) and widespread Nearctic-Neotropical migratory songbird. American redstarts breed throughout much of North America and winter throughout the Caribbean, Mexico, and parts of Central and northern South America (Sherry and Holmes 1997). Recent work suggests that American redstarts over-wintering in the Caribbean breed in the northeastern United States and southeastern Canada, including Ontario (Norris et al. 2006). American redstarts are sexually dimorphic; males exhibit delayed plumage maturation, with highly variable plumage colouration both within and between age classes. Individuals undergo a single pre-basic moult at the end of the breeding season and retain those feathers through the following breeding season (Sherry and Holmes 1997). In their first winter (and subsequent breeding season), males are greenish-gray with yellow carotenoid-based patches on their wings, tail, and flanks. These males lack bibs entirely, but some individuals adventitiously moult small patches of black feathers on the breast, back and head during winter. After their first breeding season, males moult into their definitive plumage: black upperparts and head with a white belly, salmon-orange carotenoid-based patches on the wings, tail and
flanks and a black bib (Sherry and Holmes 1997). Studies from the breeding grounds suggest that bib size is related to breeding-season performance (Perrault et al. 1996), though the function of the carotenoid-based patches has not yet been investigated.

**Study sites**

Over-wintering American redstarts were captured in high-quality (black mangrove) and low-quality (second-growth scrub) habitats from Dec-Mar 2002-2006 at Font Hill Nature Preserve, Westmoreland Parish, Jamaica, West Indies (18°02’ N, 77°57’W; see Marra 2000). Sample numbers varied yearly based on accessibility to different habitats and the duration of our stay. Work on the breeding grounds was conducted May-July 2006 at the Queen’s University Biological Station, Chaffey’s Lock, Ontario, Canada (44°34’ N, 76°19’W).

**Tissue sampling**

All birds were captured in mist nets either through passive blanket netting or using song playbacks accompanied by a decoy, and banded with a single US Fish and Wildlife Service or Canadian Wildlife Service band and 2-3 colour bands for individual identification. On the breeding grounds in May 2006, birds were captured within 5 days of arrival and 2-3 mm of the central claw from each foot was collected; claw samples in Jamaica were obtained in March 2006. For all birds, we recorded wing chord (mm) and plucked a single tail feather (R3) for colour and stable-hydrogen isotope analysis.
Colour analysis and bib size scoring

Reflectance spectra from tail feathers was obtained by measuring percent reflectance across the bird visual spectrum (320-700nm) using an Ocean Optics USB2000 spectrometer attached to a PX-2 xenon pulsed light source. The probe was held at a 90° angle to the feather surface and housed in a rubber sheath to keep the probe at a constant distance from the feather surface and ensure we captured light only from the PX-2 light source. The probe captured a feather area of approximately 2 mm². All feathers were mounted on minimally reflective (<5% reflectance) black paper (Colorline #142 Ebony). To standardize our measurements, we took readings from a dark (sealed black velvet lined box) and white (spectralon) standard between each measurement. Three to five measurements were taken haphazardly within the yellow/orange region of each tail feather, avoiding the rachis (see Fig. 2.1 for reflectance spectra). Tail feathers were chosen for our analysis because they are frequently fanned in aggressive displays (Sherry and Holmes 1997). Plumage colouration was quantified by calculating standard measures of brightness, hue, and chroma (Montgomerie 2006; Table 1). Brightness represents the mean reflectance across the bird visual spectrum (320-700nm). Hue was calculated via segment classification (adapted from Endler 1990; Montgomerie 2006; Table 2.1). Because American redstart feathers exhibit two distinct spectral peaks (UV and yellow/red), we measured both UV and red chroma, which is the light reflected in the UV or red portion of the spectrum (320-415nm and 575-700nm respectively) divided by the
total reflectance. Bib size was ranked in the hand on a scale of 1-5 (1 = small, 5 = large; Lemon et al. 1992).

**Stable-carbon isotope analysis**

Because of differences in plant water stress and photosynthetic system, $\delta^{13}C$ signatures in plant tissues vary among different habitat types in the tropics (Lajtha and Marshall 1994). $C_3$ plants and plants experiencing little water stress generally have more negative $\delta^{13}C$ signatures compared to $C_4$ plants and water-stressed plants (Lajtha and Marshall 1994). These signatures are transferred up the food chain, and eventually incorporated into birds’ tissues. Thus, by sampling bird tissues, such as claws, on arrival in Canada, we can infer information about the habitat that bird occupied during the non-breeding period (Marra et al. 1998). In Jamaica, black mangrove forests are inundated with water for much of the year and plants experience less water-stress than the highly seasonal second-growth scrub, resulting in more negative $\delta^{13}C$ signatures in plants and animals in that environment (Marra et al. 1998). Thus, by capturing birds upon arrival on the breeding grounds, it is possible to infer the quality of winter habitat. In separate populations of breeding American redstarts, Marra et al. (1998) and Norris et al. (2004a) showed that birds arriving earlier on the breeding grounds had more negative $\delta^{13}C$ signatures. Furthermore, Norris et al. (2004a) found birds with more negative $\delta^{13}C$ signatures ultimately had higher reproductive success. Compared to blood, $\delta^{13}C$ in claw tissues has a relatively slow turnover rate (weeks to months) and, for studies of migratory birds, this signature should be retained post-arrival on the breeding grounds, though some
integration of local isotopic signatures may occur (Bearhop et al. 2003, 2004). For this study we included only birds that arrived within 25 days of the first bird to arrive on the breeding grounds. Claws samples were weighed, then converted to CO$_2$ in an oxidation/reduction furnace, separated by gas chromatography, then measured for $\delta^{13}$C with an isotope-ratio mass spectrometer (Lajtha and Marshall 1994; Norris et al. 2004a).

**Stable-hydrogen isotope analysis**

Previous work on American redstarts suggested that plumage colouration varies geographically, with birds moulting feathers at more northerly latitudes having more red chroma than those birds moulting at more southerly latitudes (Norris et al. 2007), and that some birds that invest heavily in late-season parental effort may moult south of the breeding grounds, resulting in duller plumage (Norris et al. 2004b). Because our wintering population breeds across a relatively large geographic range (Studds et al. 2008), we used stable-hydrogen isotope ($\delta^D$) analysis to determine if there was a relationship between moult location and brightness or red chroma and if moult location helped explain winter habitat occupancy. Details of our stable-hydrogen isotope analysis are reported in Langin et al. (2007). Briefly, all feathers were washed in a 2:1 chloroform:methanol solution, 0.1-0.15mg of feather was weighed and combusted in a Finnigan TC/EA reduction furnace at 1,450°C and introduced into a Finnigan MAT Delta Plus XL isotope ratio mass spectrometer. All $\delta^D$ values are reported in parts per mil notation ($‰$) relative to Vienna Standard Mean Ocean Water. Only feathers from adult males were used in this analysis.
Statistical analyses

Each component of redstart feather colour (brightness, UV chroma, red chroma, and hue) was analyzed separately. Data on feather colour of birds captured in Jamaica were examined by using analysis of variance (ANOVA) with habitat and bird age as main effects. Unflattened wing chord was included in the model as a linear covariate to adjust for variation in feather colour due to body size differences. Because we conducted separate tests for each colour variable, we applied Bonferroni correction. Due to small sample sizes, we pooled data from multiple years (2002: n = 5 adult, 0 first-year; 2003: n = 3 adult, 0 first-year; 2004: n = 11 adult, 1 first-year; 2005: n = 17 adult, 16 first-year; 2006: n = 2 adult, 7 first-year). Because of uneven sampling of adult and first-year males in different years, we cannot clearly differentiate between age effects and year effects; however, we have no a priori reason to expect differences between years in which a higher proportion of adults or first-year males were captured. Differences in bib size between adult males over-wintering in mangrove and scrub habitats were analyzed with an independent samples t-test. The relationship between feather colour and stable-carbon isotope ratios in the claws of redstarts arriving in Ontario was evaluated by using linear regression. To validate that stable-carbon isotope ratios in claws reflect moisture gradients in nonbreeding habitat, we also evaluated differences between claws of Jamaican birds occupying mangrove and scrub habitats. The residuals of this analysis were markedly non-normal and were therefore analyzed with a Wilcoxon rank-sum test.
The effect of moulting latitude on feather colour was analyzed using linear regression. All tests were performed in JMP 6.0.2 (SAS Institute 2006).

Results

Relationship between habitat, bird age and plumage colour

Tail feathers of redstarts occupying territories in black mangrove habitat were brighter than those of birds holding territories in second-growth scrub, but did not differ in UV chroma, red chroma, or hue (Table 2.2). This relationship remained significant after applying Bonferroni correction, which lowered our $\alpha$ value to 0.0125. Feathers of adult males had significantly higher UV chroma, red chroma, and redder hue compared to first-year males and marginally, but not significantly, higher brightness. After applying Bonferroni correction, only differences in hue remained significant (Table 2.2). Birds with longer wings also tended to have brighter feathers, but did not have higher UV chroma, red chroma, or redder hue (Table 2.2). Bib size of adult males did not differ between habitats (independent samples t-test: $t_{22} = 1.21, P = 0.24$).

Relationship between habitat (stable-carbon isotopes) and plumage colour

Stable-carbon isotope signatures ($\delta^{13}$C) in claws of adult males sampled in mangrove habitats in Jamaica were significantly more negative than those sampled in scrub habitats (Wilcoxon rank-sum test, $z = 2.66, P = 0.008, n = 26$; Fig. 2.2A). Adult males arriving on
the breeding grounds with signatures consistent with high-quality, wet non-breeding season habitats (more negative $\delta^{13}$C signatures) had brighter plumage ($r^2 = 0.31, P = 0.03, n = 15$; Fig. 2.2B), but not UV chroma ($r^2 = 0.15, P = 0.14, n = 15$), red chroma ($r^2 = 0.0002, P = 0.96, n = 15$), hue ($r^2 = 0.009, P = 0.74, n = 15$), or bib size ($r^2 = 0.06, P = 0.35, n = 16$).

**Relationship between moulting latitude ($\delta$D) and plumage colour/habitat**

Stable-hydrogen isotope analysis revealed that our Jamaica population of over-wintering redstarts moulted over a broad geographic range ($\delta$D range: -59 to -100). We detected no relationship between $\delta$D and brightness ($r^2 = 0.08, p = 0.15, n = 26$), red chroma ($r^2 = 0.0004, p = 0.92, n = 26$), UV chroma ($r^2 = 0.0003, p = 0.93, n = 26$), or hue ($r^2 = 0.06, p = 0.39, n = 26$). Furthermore, we found no difference in $\delta$D between habitat types (two-tailed $t$-test, $t_{26} = 1.37, p = 0.18$).

**Discussion**

In this study we used two different approaches to demonstrate that the brightness of a carotenoid-based plumage patch, moulted at the end of the breeding season, is associated with occupancy of high-quality winter habitats. In our first approach we showed that birds in Jamaica holding territories in high-quality black mangrove forest have brighter tail feathers than birds in low-quality second-growth scrub. We controlled for both age and wing length in our analyses and demonstrated that plumage brightness predicts
habitat occupancy in both age-classes, and does so beyond any effect of body size.

Second, we tested if this pattern carried over to the breeding period and was detectable over broad spatial scales (i.e., for birds likely over-wintering in a range of habitat types) and found that birds arriving on the breeding grounds in Ontario, Canada from higher-quality winter habitats (inferred by stable-carbon isotope analysis) were brighter than birds arriving from poor quality habitats. Our ability to detect a relationship between habitat quality (δ^{13}C) and plumage brightness is surprising because redstarts breeding at our Ontario study site are likely arriving from a variety of winter localities (see methods: Study Species) and suggests that the relationship between plumage brightness and winter habitat occupancy occurs at broad spatial scales and across various habitat types. Because dominance and aggression play a critical role in territory acquisition in American redstarts (Marra 2000), we suggest that the most plausible explanation of our results is that plumage brightness serves as a status signal, indicating superior fighting ability.

Under this scenario, habitat-specific plumage variation may arise from differences in dominance and competitive ability, where brighter birds out-compete less bright birds for high-quality territories. Previous work on American redstarts in Jamaica has shown that all age- and sex-classes initially settle disproportionately in high-quality, mangrove habitats, but that most females and first-year males are subsequently displaced, generally by adult males (Marra 2000). These displacements are driven both by prior residents (i.e., colour-banded birds that held territories in the previous year) and by newly arriving birds, suggesting that both prior residency and intrinsic dominance are important in determining the outcomes of dominance interactions. Also, playback experiments demonstrate that birds in mangrove territories show higher aggressive territorial responses compared to
those in scrub, suggesting that differences in aggression likely determine the outcome of dominance-based territory occupancy (Marra 2000). However, additional experiments are necessary to determine if plumage brightness reflects competitive ability and aggression. For example, it would be informative to measure the intensity of aggressive encounters (e.g., by presenting models and vocalization playbacks to territory holders, sensu Marra 2000) in relation to male plumage or examine the effects of plumage manipulations on males’ ability to obtain and retain a territory in high-quality habitat. Furthermore, if feather brightness is indeed acting as a status signal, it will be necessary to understand the mechanisms that maintain signal honesty.

Our finding that feather brightness, not red chroma, is positively related to territory quality is counter-intuitive. Generally, yellow-orange plumage colouration acts as an honest signal of quality by revealing carotenoid deposition, whereby increasing carotenoid deposition increases red chroma and decreases feather brightness (Andersson and Prager 2006). However, highly reflective (bright) yellow-orange colouration is the product of both carotenoid deposition and structural colouration (Shawkey and Hill 2005), and brightness has been shown to be an indicator of condition and quality (Saks et al. 2003; Stein and Uy 2006). Regardless of the mechanisms that lead to increased brightness, our results indicate that plumage brightness is a good predictor of habitat quality and suggest that further investigation is warranted.

Factors other than dominance-based status signaling might also be responsible for our observed patterns of habitat-specific plumage variation. One possible explanation is that birds in the more open, scrub habitat may be exposed to harsher environmental conditions, such as UV irradiation and feather abrasion, leading to decreased feather
Plumage of male linnets (*Carduelis cannabina*) exposed to higher sunlight UV irradiation increased in hue, saturation, and brightness (Blanco et al. 2005). If UV irradiation similarly affects plumage colour in American redstarts, birds in the more open and harsh scrub habitats should be brighter than those in mangrove, yet we observe the opposite pattern, with birds in the shady, mangrove habitat having brighter plumage.

Another hypothesis is that because the light environment differs between scrub and mangrove forests, brighter individuals may be better suited to the dark environment of the mangrove forests (Endler 1992, Marchetti 1993). For example, the amount of white on the tail feathers of *Myioborus* redstarts increases the effectiveness of flush-pursuit foraging (Mumme et al. 2002) and darker conditions favor larger white tail patches (Jablonski et al. 2006). American redstarts also use a moderate amount of tail-fanning to elicit a flush response from prey (Sherry and Holmes 1997). However, all age- and sex-classes of redstarts settle disproportionately in mangrove habitats upon arrival to Jamaica, but as density increases, dominant individuals then displace subordinates to scrub habitat (Marra 2000). Furthermore, removal experiments in Jamaica demonstrate that redstarts in poor-quality scrub habitat readily move in and occupy mangrove habitat (Marra et al. 1993, Studds and Marra 2005). Thus, it is unlikely that birds settle differentially in mangrove and scrub habitats based on the suitability of their feather brightness for eliciting a flush response from prey items.

A final hypothesis is that patterns of habitat occupancy in Jamaica may be related to geographic variation in plumage colouration across the breeding range, whereby birds from different breeding/moulting locations settle differentially in high- and low-quality habitats. A recent study by Norris et al. (2007) suggested that red chroma in American
redstart feathers varies geographically across the breeding range, with birds from more
northerly locations (inferred by stable-hydrogen isotope analysis) having higher red
chroma values, indicative of higher carotenoid content. Although, our stable-hydrogen
isotope (δD) analyses indicate that our over-wintering population moults over a broad
latitudinal range (δD range: -59 to -100 ‰), we failed to detect a relationship between δD
and plumage colour. However, because the Norris et al. (2007) study was based on
samples collected across the wintering range, it is possible that geographic variation in
feather colour is detectable at broad spatial scales (i.e., across the entire range of the
species), but not within a single over-wintering population. In addition to the lack of
relationship between brightness or red chroma and moulting latitude, we found no
difference in δD between habitat types, suggesting that plumage brightness, not moult
location, is a good predictor of non-breeding habitat occupancy.

Studies that have examined the role of plumage traits under varying scenarios
(e.g., male-male competition versus female mate choice) and throughout the annual cycle
have provided insight into the dual utility of ornamental traits (McGraw and Hill 2000,
Alonso-Alvarez et al. 2004, Griggio et al. 2007) and the evolution of multiple ornaments
(Andersson et al. 2002). Although our study suggests that tail feather brightness may be a
potential signal on the wintering grounds, the function of this and other plumage traits
must be examined within the context of the entire annual cycle and under different
conditions (e.g., female mate choice). In breeding populations of American redstarts,
previous work suggests that birds with smaller bibs have higher pairing success (Lemon
et al. 1996) and our preliminary analyses suggest a potential role for tail feather
brightness in sexual signaling (Reudink MW, unpublished data). Longitudinal studies on
the same individuals across years, combined with experimental studies, will provide important insights into the dynamics of these signaling systems. Such approaches are important for understanding if high-quality individuals are able to produce bright feathers each year regardless of environmental conditions, or if plumage colouration is highly dependent on environment and is thus a phenotypically plastic trait that reveals more about condition/environment at moult rather than intrinsic quality. Finally, studies conducted during different phases of the annual cycle can provide insight into how selection acts on both single and multiple ornaments throughout the year. We suggest that study of plumage during the non-breeding season in long-distance migratory birds is an overlooked avenue that may provide insight into the function and evolution of ornamental traits.

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methods conducted in this study complied with the laws of the nations of Jamaica and Canada.

References


Table 2.1

Formulas used to calculate color variables. Percent reflectance was measured at each 1nm interval across the spectrum. \( B_{\lambda_{i} \rightarrow \lambda_{n}} = \) total light reflected from the \( i \)th wavelength to the \( n \)th wavelength \((\lambda_{i} – \lambda_{n})\) after summing across each 1nm interval:

<table>
<thead>
<tr>
<th>colour variable</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>brightness ( B_{320-700} ) = ( \sum_{\lambda=320}^{700} )</td>
<td>( \lambda_{700} )</td>
</tr>
<tr>
<td>UV chroma ( C_{UV} = B_{320-415} / B_{320-700} )</td>
<td></td>
</tr>
<tr>
<td>red chroma ( C_{red} = B_{575-700} / B_{320-700} )</td>
<td></td>
</tr>
<tr>
<td>hue ( H = \arctan \left( \frac{(B_{512-575} - B_{320-400})/B_{320-700}}{(B_{575-700} - B_{400-512})/B_{320-700}} \right) )</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2

Colour variables (mean±SE) calculated from reflectance spectra of adult and first-year males in over-wintering in high-quality black mangrove forest and low-quality second-growth scrub. Bottom of table shows the results of a two-way ANOVA with age, habitat and wing length as main effects and a habitat×age interaction term (first-year males: n = 12 mangrove, 12 scrub; adult males: n = 24 mangrove, 14 scrub). Bolded values are significant at α = 0.05.

<table>
<thead>
<tr>
<th>age</th>
<th>habitat</th>
<th>brightness</th>
<th>UV chroma</th>
<th>red chroma</th>
<th>hue</th>
</tr>
</thead>
<tbody>
<tr>
<td>adult</td>
<td>combined</td>
<td>23.46±0.52</td>
<td>0.22±0.002</td>
<td>0.41±0.005</td>
<td>-0.02±0.01</td>
</tr>
<tr>
<td></td>
<td>scrub</td>
<td>22.56±0.93</td>
<td>0.22±0.004</td>
<td>0.41±0.008</td>
<td>-0.06±0.02</td>
</tr>
<tr>
<td></td>
<td>mangrove</td>
<td>24.00±0.63</td>
<td>0.22±0.003</td>
<td>0.41±0.006</td>
<td>-0.02±0.01</td>
</tr>
<tr>
<td>first-year</td>
<td>combined</td>
<td>21.72±0.63</td>
<td>0.21±0.003</td>
<td>0.39±0.004</td>
<td>0.06±0.03</td>
</tr>
<tr>
<td></td>
<td>scrub</td>
<td>20.17±0.89</td>
<td>0.21±0.005</td>
<td>0.39±0.006</td>
<td>0.04±0.04</td>
</tr>
<tr>
<td></td>
<td>mangrove</td>
<td>23.26±0.68</td>
<td>0.20±0.004</td>
<td>0.39±0.006</td>
<td>0.08±0.03</td>
</tr>
</tbody>
</table>

**Effect of age**

\( F = 0.82 \)

\( p = 0.37 \)

**Effect of habitat**

\( F = 9.90 \)

\( p = 0.003 \)

**Effect of habitat×age**

\( F = 1.80 \)

\( p = 0.08 \)

**Effect of wing length**

\( F = 2.62 \)

\( p = 0.01 \)
Fig. 2.1. Average reflectance spectra from the yellow-orange region of the tail feather (R3) in A) adult males and B) first-year males holding territories in high-quality black mangrove forest (black line: adult, n = 24; first-year, n = 12) and low-quality second-growth scrub (gray line: adult, n = 14; first-year, n = 12). Standard error bars are placed periodically along the reflectance spectra.
Fig. 2.2. (A) $\delta^{13}$C signatures in claws of birds collected in mangrove and scrub habitats. Sample size is indicated on top of the boxes; horizontal lines represent 90th, 75th, 50th, 25th, and 10th percentiles. (B) Relationship between brightness and $\delta^{13}$C in claws ($n = 15$) collected upon arrival on the breeding grounds in Canada. Birds from wetter winter habitats (more negative $\delta^{13}$C signatures) have higher brightness; regression line shown.
Chapter 3

Non-breeding season events influence sexual selection in a long-distance migratory bird
SUMMARY

The study of sexual selection has traditionally focused on events and behaviours immediately surrounding copulation. In this study, we examine whether carry-over effects from the non-breeding season can influence the process of sexual selection in a long-distance migratory bird, the American redstart (*Setophaga ruticilla*). Previous work on redstarts demonstrated that over-wintering in a high-quality habitat influences spring departure dates from the wintering grounds, advances arrival dates on the breeding grounds, and increases apparent reproductive success. We show that the mixed-mating strategy of redstarts compounds the benefits of over-wintering in high-quality winter habitats. Birds arriving to breed in Canada from high-quality habitats arrive earlier than birds from poor-quality habitats, resulting in a lower probability of paternity loss, a higher probability of achieving polygyny, and ultimately higher realized reproductive success. Such results suggest that the process of sexual selection may be influenced by events interacting throughout the annual cycle.
1. INTRODUCTION

For long-distance migratory birds, individual life-history, ecology, and behaviour is shaped by events and selective pressures acting throughout the annual cycle (Greenberg & Marra 2006). The challenge in understanding seasonal interactions lies in the inherent difficulty of tracking individuals and tracing the impacts of carry-over effects (i.e., events occurring during one season that carry-over to influence an individual or population’s performance in subsequent seasons; Marra et al. 1998) between phases of the annual cycle, often between continents. Until recently, making connections between breeding and wintering populations and detecting potential carry-over effects has remained elusive. However, advances in the utilization of naturally occurring biochemical markers, such as stable isotopes, in animal tissues have allowed us to begin making connections throughout the annual cycle (Webster et al. 2002; Rubenstein & Hobson 2004). Studies using stable-hydrogen isotopes have revealed connections between wintering and breeding populations (Webster et al. 2002), use of migratory stopover sites (e.g., Yohannes et al. 2007), and patterns of migration (e.g., Kelly et al. 2002). This technique has also revealed previously unknown carry-over effects. Studds et al. (2008) recently demonstrated that natal dispersal is influenced by conditions during the non-breeding season, whereby individuals over-wintering in high-quality habitat disperse south of their natal origin, while individuals in poor-quality winter habitats migrate much further and ultimately breed north of their natal origins. Indeed, carry-over effects from the non-breeding period have now been shown to impact many aspects of individual life history in a variety of species.
In European barn swallows (Hirundo rustica), favorable winter conditions in Africa (inferred by the normalized difference vegetation index [NDVI]) advanced population-level arrival onto the breeding grounds in Italy (Saino et al. 2004a). Furthermore, NDVI in winter was positively correlated with population-level breeding success and the length of tail streamers, a sexually-selected trait moulted on the wintering grounds (Saino et al. 2004b). At the individual level, territory acquisition in high-quality winter habitats can have significant fitness consequences. American redstarts (Setophaga ruticilla) holding winter territories in high-quality habitat have higher annual return rates (Marra & Holmes 2001), are in better condition (Marra & Holberton 1998; Studds & Marra 2005), and ultimately depart the wintering grounds earlier than individuals from low-quality habitat (Marra et al. 1998; Studds & Marra 2005). Moreover, these consequences can carry-over to subsequent seasons. Stable-carbon isotope analysis has revealed that the quality of an individuals’ winter territory can influence condition during migration (black-throated blue warblers (Dendroica caerulescens): Bearhop et al. 2004) and the timing of arrival on breeding areas (American redstarts: Marra et al. 1998; Norris et al. 2004). Norris et al. (2004) demonstrated that the carry-over effect of winter habitat on arrival date ultimately influenced apparent reproductive success, with early arriving birds fledging more offspring. However, carry-over effects may also influence whether males achieve polygny as well as within-pair and extrapair paternity – processes that are important drivers of sexual selection in many songbirds (Webster et al. 2007). Thus, examining carry-over using only apparent reproductive success is likely to considerably underestimate the variance in true (genetic) reproductive success (Albrecht et al. 2007;
Webster *et al.* 2007), and miss the critical factors influencing individual fitness and the opportunity for sexual selection.

The study of sexual selection has traditionally focused on the events and behaviours immediately surrounding copulation (i.e., courtship through insemination) and relegated events spatially or temporally separated from copulation to the realm of natural selection. We investigate if the process of sexual selection may be influenced by non-breeding season carry-over effects through variation in arrival dates. Arrival scheduling may be particularly important for species that employ a mixed mating strategy, whereby polygyny and extrapair paternity depend heavily on individuals arriving early enough to pair and secure paternity at the primary nest and advertise for a secondary female and/or extrapair partners (Spottiswoode *et al.* 2006).

In this study, we investigate whether carry-over effects from the non-breeding season influence polygyny and extrapair paternity through variation in arrival dates on the breeding grounds in a long-distance Neotropical migratory bird, the American redstart (figure 3.1). Previous studies have shown that redstarts employ a mixed-mating strategy with high-levels of extrapair paternity (59% of broods, 40% of offspring; Perreault *et al.* 1996), and a moderate level of polygyny (5-16% of males; Secunda and Sherry 1991). We predict that by over-wintering in a high-quality habitat, birds will arrive on the breeding grounds earlier, will be more likely to achieve polygyny (i.e., a second female), and will secure more paternity at their own nest. Additionally, early arriving males should sire more extrapair offspring in the nests of late arriving males. Ultimately, we predict that early arrival on the breeding grounds will result in increased realized fertilization success and fledging success (figure 3.1).
2. MATERIALS AND METHODS

(a) Field data collection

Field work was conducted May-July 2004-2007 at the Queen’s University Biological Station, Chaffey’s Lock, Ontario, Canada (44°34’ N, 76°19’W). Our study area is composed of mixed-deciduous forest, dominated by sugar maple (Acer saccharum) and Eastern hop hornbeam (Ostrya virginiana). When males arrive on the breeding grounds, they immediately begin singing for territory advertisement and to attract females. Each year, from May 1 – May 31, we surveyed our 60ha study area daily from 0600-1200, detecting males by the presence of singing and subsequent visual identification. Arrival date was standardized as the number of days after the first male arrived (first-male arrival date = 0). All adults were captured in mist-nets within 7 days of arrival by simulating territorial intrusions using song playbacks accompanied with a decoy. Once captured, redstarts were individually marked with a single Canadian Wildlife Service aluminum band and 2-3 colour bands. We then extracted 50ul of blood for paternity analysis by piercing the brachial vein and clipped 2-3 mm of the central claw for stable-isotope analysis (2006 and 2007 only).

Upon arrival, all males were observed and mapped for at least 20-30min/day throughout the breeding season to determine territory boundaries and pairing date. Females typically begin nest-building within a few days of pairing. Once nest-building began, we monitored nest status every other day, noting the onset of egg-laying, number of eggs laid, hatching, and fledging success. Males were monitored daily to detect individuals that paired with secondary females (i.e., polygynous mating). At day 5 after
hatching, we banded nestlings with a single aluminum band and collected 15-20ul of blood for paternity analysis. Offspring from nests that were too high to access on day 5 were captured on the day of fledging.

American redstart males exhibit delayed plumage maturation, wherein males resemble females during their first breeding season and do not mature into the full adult breeding plumage until their second prebasic moult, which follows their first breeding season. Because of differences in plumage, and the fact that first-year redstarts have greatly reduced reproductive performance (Sherry & Holmes 1997; Reudink unpublished data), we limited our analyses to only adult (after second-year or ASY) males.

(b) Stable-carbon isotope analysis

Stable-carbon isotope signatures of plants in the tropics vary by habitat type due to differences in plant water stress and photosynthetic system (Lajtha & Marshall 1994). These signatures are transferred up the food chain, and eventually incorporated into birds’ tissues (Marra et al. 1998). By capturing birds upon arrival on the breeding grounds we can infer the quality of winter habitat; more negative δ13C signatures are indicative of higher-quality territories (Marra et al. 1998; Norris et al. 2004; Reudink et al. accepted). Stable-carbon isotopes in claw tissue turnover on the rate of weeks to months, making claws an ideal tissue to sample across the migratory period (Bearhop et al. 2003, 2004). We analyzed only birds that were captured within 7 days of arrival (May 1 – May 31) to ensure carbon isotope signatures reflected winter habitat type. Claws samples were weighed, converted to CO2 in an oxidation/reduction furnace, separated by
gas chromatography, then measured for $\delta^{13}$C with an isotope-ratio mass spectrometer (Lajtha & Marshall 1994; Reudink et al. accepted).

(c) **Paternity analysis**

We collected blood samples from putative parents and offspring and stored the samples in Queen’s lysis buffer (Seutin et al. 1991) (2005-7) or on blotting paper (2004). DNA was extracted using an Invitrogen Blood and Tissue Kit. gDNA was then quantified via agarose gel electrophoresis and diluted or concentrated to ~10ng/µl. All loci were amplified using a Biometra Thermogradient or Biometra UNOII PCR machine under the following conditions: 94ºC for 3 min followed by 35 cycles of 94ºC for 15sec, 58ºC for 15sec, 72ºC for 30 sec, and a final extension of 72ºC for 10 min. Each sample included 1µl DNA (10ng/µL), 1µl 10X Qiagen PCR buffer, 0.03µL (100mM) dNTPs, 0.03µl (100µM) forward primer, 0.03µL (100µM) reverse primer, 0.025µL M13 F 700IRD licor primer, 0.005µL (5U/µL) Taq polymerase, brought up to 10µL total volume with sterile ddH$_2$O. Amplified samples were run on a Licor IR2 Global Sequencer and allele scoring was conducted by a trained observer blind to the identity of individuals.

Paternity analysis was conducted using five microsatellite loci ($Dp\mu 01$, $Dp\mu 03$, $Dp\mu 05$, $Dp\mu 15$, $Dp\mu 16$) originally isolated from yellow warblers ($Dendroica petechia$; Dawson et al. 1997, table 3.1). Over the four years of this study, we analyzed DNA from 265 offspring from 75 nests and all putative parents. The use of five highly variable microsatellite loci ensured a high probability of paternity exclusion (>0.999, Table 3.1). Because of limitations in detecting 2bp differences and the relatively high frequency of null alleles, we followed the conservative approach of Reudink et al. (2006): offspring
were excluded only if they mismatched the putative sire at >2bp and at ≥2 loci. All extrapair offspring were then compared to all sampled potential sires in the population to assign paternity using CERVUS 2.0 (Marshall et al. 1998). All assignments were then double-checked by hand by two trained, independent observers. Extrapair paternity was assigned when the putative sire matched at least 4 of 5 loci within 2bp. Mis-matches at single loci were only allowed when the mis-match was due to a likely null allele. Total fertilization success was calculated as the number of within-pair (WP) offspring at the primary and secondary (if polygynous) nests and the number of extrapair (EP) offspring sired. We calculated genetic fledging success by multiplying the number of offspring fledged by the proportion of within-pair offspring for the primary and secondary nests and added the number of extrapair offspring * fledging success at the extrapair nest.

(d) Realized success and the opportunity for sexual selection

We calculated the opportunity for selection, $I_s$, given only apparent fertilization success at the primary nest, apparent fertilization success at both the primary and secondary nest, and realized genetic success (within-pair [genetic] offspring at primary and secondary nests plus number of extrapair offspring). $I_s$ was calculated as variance in reproductive success divided by the square of the mean reproductive success (Arnold & Wade 1984).

(e) Predicting paternity and polygyny

To illustrate the potential carry-over effects resulting from holding a territory in range of tropical winter habitats of varying quality, we created a simple model based on $\delta^{13}C$ signatures of tissues from individuals collected in four different winter habitats (sensu
Norris et al. 2004). Because winter habitat quality is unlikely to influence paternity directly, but rather through factors associated with arrival timing, we first predicted arrival dates for four winter habitat types of varying quality (from wet to dry: wet forest, mangrove, citrus, scrub; Marra et al. 1998; Marra unpublished data), based on the regression of $\delta^{13}$C on arrival date for birds arriving on the breeding grounds (figure 3.2a). Next, we used those four arrival dates (days 5.8, 8.8, 10.4, 11.8) to predict the proportion of offspring an individual was likely to sire based on the regression of the proportion of within-pair offspring on arrival date (figure 3.2b). We then predicted the probability of being polygynous based on a regression of the probability of polygyny on arrival date. Finally, we calculated predicted realized fledging success based on a regression of realized fledging success on arrival date (figure 3.2c).

(f) **Statistical analysis**

All statistical analyses were performed in JMP 5.1 (SAS Institute 2006) and SAS version 8.2 (SAS Institute 1999). We used a mixed-model with year as a random effect to test the relationship between $\delta^{13}$C and arrival. Because some individuals were present in multiple years, we tested if arrival date predicted binary reproductive variables (extrapair paternity (y/n) and polygyny/monogamy) using logistic regression with individual as a repeated measure and year as a random effect. To test if arrival date predicted linear response variables (fertilization success, fledging success), we used a mixed-model with individual as a random effect and standardized arrival date as a linear co-variate. To test for a relationship between arrival date and the proportion of within-pair offspring, we used a non-parametric mixed-model with proportion paternity as a response variable, year as a
random effect, individual as a repeated measure, and standardized arrival date as a linear co-variate. Comparisons between within-pair and extrapair offspring were analyzed using matched-pairs t-tests.

3. RESULTS

(a) Winter habitat quality and arrival date

Adult males that had over-wintered in high-quality habitats (more negative claw $\delta^{13}$C signatures) in 2006-7 arrived on the breeding grounds earlier than males from low-quality habitats (Pearson correlation: $n = 43$, $r^2 = 0.20$, $P = 0.003$; figure 3.2a). This relationship remained significant when controlling for year effects ($n = 43$, $t = 7.87$, $P = 0.008$), with no confounding effect of year ($n = 43$, $t = 1.65$, $P = 0.14$).

(b) Paternity

Of the 75 nests analyzed, 32 (43%) contained one or more extrapair offspring and 56 of the 239 (23%) offspring analyzed were extrapair. A subset of males (9/75) were polygynous, but there was no significant difference in paternity at the nest of the primary female or the secondary female ($1^{st}$ females: 28/66 (42%) of nests contained extrapair offspring (EPO), 52/209 (25%) of offspring were EPO; $2^{nd}$ females: 4/9 (45%) of nests contained EPO, 4/30 (13%) of offspring were EPO; Fisher’s exact test for presence of EPO: $n = 9$, $r^2 = 0.42$, $P = 0.17$; paired t-test for proportion EPO: $n = 9$, $t = 0.58$, $P = 0.58$). Realized success (within-pair offspring at each nest + extrapair offspring sired) for 62 adult males with complete reproductive data ranged from 0 to 7 offspring (mean = $3.13 \pm 0.24$SE).
(c) Arrivial date and paternity

Adult males that sired all the offspring at their own nest arrived earlier on the breeding grounds than males that lost paternity (logistic regression with correlated data: arrival: \( n = 64, z = 2.00, P = 0.045 \); year: \( n = 64, z = -0.65, P = 0.52 \)). Arrival date of adult males was also significantly correlated with the proportion of within-pair offspring a male sired (non-parametric random effects mixed-model: \( n = 64, \chi^2 = 5.95, P = 0.01 \); figure 3.2b). At nests that lost paternity, extrapair sires arrived on average \( 4.19 \pm 1.83 \)SD days earlier than the males they cuckolded (matched-pairs t-test: \( n = 26, t = -2.28, P = 0.03 \); figure 3.4).

(d) Arrivial date and polygyny

Males that achieved polygyny arrived significantly earlier than males who remained socially monogamous (logistic regression with correlated data: \( n = 115 \) (86 monogamous, 29 polygnyous), arrival: \( z = -2.04, P = 0.04 \); year: \( z = -1.78, P = 0.08 \), figure 3.3). These results did not change qualitatively when we removed the non-significant year effect (\( z = -2.59, P = 0.01 \)).

(e) Realized success and the opportunity for sexual selection

Males that arrived early had higher realized fertilization success (mixed-model; \( n = 65, F = 4.03, P = 0.05 \)) and higher realized fledging success (mixed-model; \( n = 65, F = 7.52, P = 0.008 \)). The index of opportunity for selection, \( I_s \), ranged from 0.10 (considering only apparent fertilization success at a male’s primary nest), to 0.15 (examining apparent
fertilization success at both the primary and, if polygynous, secondary nest), to a maximum of 0.39 (examining realized fertilization success, the number of within-pair offspring sired at primary and secondary nest plus number of extrapair offspring sired).

(f) Predicting paternity, polygyny, and total fledging success

Our model suggests that males arriving on the breeding grounds from high-quality winter habitats sire 13% more offspring at their primary nest and are 16% more likely to be polygynous than males arriving later from poor quality habitats (figure 3.5a,b). Ultimately, males from high-quality winter habitats fledge nearly one additional offspring than birds over-wintering in poor-quality habitats (figure 3.5c).

4. DISCUSSION

Our results indicate that arrival timing on the breeding grounds, driven by conditions experienced thousands of kilometers away on tropical wintering grounds, influences rates of polygny and extrapair paternity in American redstarts, suggesting that non-breeding season carry-over effects may influence the process of sexual selection. Specifically, we demonstrate that not only does winter territory quality influence apparent success through arrival timing (wherein variation in apparent success is driven largely by predation; Norris et al. 2004), but carry-over effects from winter can influence behavioural processes such as female mate choice and male-male competition. Males over-wintering in high-quality habitats arrive earlier on the breeding grounds than birds over-wintering in poor quality habitats (inferred by stable-carbon isotope analysis). In turn, early arriving males sire a higher proportion of their own offspring, sire extrapair offspring in the nests
of late-arriving males, are more likely to achieve polygyny, and ultimately fledge a greater number of genetic offspring. These results suggest that early male arrival, driven by winter habitat quality, may directly influence evolutionary processes (Spottiswoode et al. 2006).

Several species of migratory birds are returning earlier to the breeding grounds and recent work suggested that long-distance migrants breeding in Scandinavia have advanced arrival dates as a result of rapid, climate-driven evolutionary change (Jonzen et al. 2006; but see Both 2007). Our data indicate that early arriving males may increase fledging success by roughly 25% through variation in polygyny and extrapair paternity, suggesting that early arrival is strongly favored by selection and may be a mechanism by which rapid evolutionary change may occur.

Previous studies of American redstarts on the wintering grounds have shown that individuals holding territories in poor-quality habitats delay their spring departure northward from the wintering grounds (Marra et al. 1998; Studds & Marra 2005), arrive later (Marra et al. 1998; Norris 2004; this study), and arrive in poorer condition (Marra et al. 1998) to the breeding grounds. Furthermore, by experimentally upgrading birds from low- to high-quality winter territories, birds advance spring departure dates (Studds & Marra 2005), confirming the idea that spring departure and subsequent arrival on the breeding grounds, is influenced by ecological conditions during winter, not just individual condition/quality. While previous work on our breeding population of redstarts has shown that late arrival results in reduced apparent reproductive success (Norris et al. 2004), our study demonstrates clear effects of winter territory quality influencing rates of extrapair paternity and polygny through variation in arrival scheduling and suggests that
non-breeding season events may influence sexual selection. We show that the costs of wintering in a low-quality territory are compounded by the mixed-mating strategy employed by redstarts. Late arrival on the breeding grounds leads not only to a shortened breeding season and higher probability of nest predation (Lozano et al. 1996; Norris et al. 2004), it also results in an increased probability of cuckoldry from early arriving males. Furthermore, late-arriving males are less likely to mitigate the costs of lost paternity through either extrapair copulations or polygny. Our model suggests that the acquisition of a poor-quality territory during winter may result in a paternity loss of roughly 13% and a 16% lower probability of polygynous mating, ultimately resulting in an over 25% reduction in total fledging success, or one less successful offspring fledged per season (Fig. 3.5c). To our knowledge, this is the first study to demonstrate that genetic success may be influenced by non-breeding season events in a migratory songbird.

Previous studies have suggested that protandry and early male arrival is favored by sexual selection (Thusius et al. 2001; Coppack et al. 2006; Kokko 2006; Spottiswoode et al. 2006), where early arriving individuals increase their probability of obtaining mates (Lozano et al. 1996), achieving polygyny (Hasselquist 1998), and increasing reproductive performance (Norris et al. 2004; Smith & Moore 2005). Early arrival may also influence extrapair paternity through density-dependent effects and breeding synchrony/asynchrony (Westneat et al. 1990; Birkhead & Møller 1992; Chuang et al. 1999; Lindstedt et al. 2007). However, these studies have assumed individual variation in arrival date is dependent on individual quality and condition during migration, largely ignoring factors affecting variation in arrival scheduling, such as non-breeding season carry-over effects. Ample evidence has now accumulated that demonstrates spring migration scheduling and
condition during migration is influenced by conditions experienced during the non-breed season (Marra et al. 1998; Bearhop et al. 2004; Norris et al. 2004; Saino et al. 2004a; Studds & Marra 2005). We suggest that processes which can influence the opportunity for sexual selection, such as variation in extrapair paternity and polygny (Andersson 1994), should be viewed not only in terms of events and processes occurring during the breeding season, but rather as a continual process that may be influenced by events occurring throughout the annual cycle.

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Table 3.1. American redstart microsatellite data characterization from CERVUS 2.0 (Marshall et al. 1998) over the four years of this study (2004-2007).

*Significantly different from expected (goodness-of-fit test: $X^2 = 31.90, p < 0.001$)

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<th>locus</th>
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<th>observed heterozygosity ($ho$)</th>
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<td>0.996</td>
<td>&gt;0.999</td>
<td>+0.040</td>
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Figure 3.1. Diagram illustrating the predicted pathway by which winter habitat quality may carry-over to the breeding season to influence total genetic success.
Figure 3.2. Significant relationships between standardized arrival date (number of days after first male to arrive) on the breeding grounds and a) claw δ\(^{13}\)C, b) proportion of offspring sired by the social male at a nest, and c) total genetic fledging success (within-pair + extrapair offspring).
Figure 3.3. Proportion of adult males arriving during weeks one through four of arrival that were polygynous (black bars) and monogamous (gray bars) during the season.
Figure 3.4. Difference in arrival date between within-pair and extrapair sires at nests that we were able to assign extrapair sires. Bars above zero indicate that the extrapair male arrived earlier than the cuckolded within-pair male, while bars below the line indicate that the extrapair male arrived later. No bars indicate that both the within-pair male and extrapair sire arrived on the same day.
Figure 3.5. Predicted values for a) proportion of offspring sired by within-pair males, b) probability of achieving polygyny, and c) total number of genetic offspring fledged, if males arrived from four different tropical habitat types. Hypothetical arrival dates for individuals wintering in the four tropical habitat types were obtained by predicting arrival dates for the four given $\delta^{13}C$ values based on the regression of $\delta^{13}C$ and arrival date. Those four arrival dates (days 5.8, 8.8, 10.4, and 11.8 respectively) were then used to predict a) the proportion of offspring sired, b) the probability of being polygynous, and c) total number of genetic offspring fledged.
Chapter 4

Plumage colouration predicts paternity and polygyny in the

American redstart
ABSTRACT

Many animals display multiple signals that can be used by conspecifics to gather information about the condition or quality of potential mates or competitors. Different signals can indicate different aspects of individual quality or function in spatially or temporally separated periods. However, for long-distance migratory birds, it is unclear if signals, such as plumage, function in different phases of the annual cycle. In this study, we investigate the potential role of carotenoid-based tail and flank plumage, and bib size, in relation to extra-pair paternity and polygyny in the American redstart (*Setophaga ruticilla*). This work complements our previous research suggesting tail feather brightness acts as a status signal, mediating territory acquisition during the non-breeding season in Jamaica. Here, we show that tail feather brightness also serves as an important signal during the breeding season. Specifically, our results indicate that polygyny, a behaviour highly dependent on obtaining and defending multiple territories, is significantly predicted by tail brightness. Interestingly, flank redness best predicted whether individuals secured paternity at their nest, while the proportion of sired offspring was predicted by arrival date, flank redness, and tail brightness and redness. Extra-pair sires had lower tail and flank redness than cuckolded males. We suggest that by expanding the study of plumage function in long-distance migrants to events occurring throughout the annual cycle, we gain a critical perspective on the function and evolution of ornamental traits.
INTRODUCTION

Sexual dimorphism and the widespread occurrence of exaggerated, ornamental traits in animals are generally attributed to evolution through sexual selection, where more ornamented males obtain a disproportionate share of matings (Andersson 1994). If these traits are costly to produce, they can thus act as honest signals of individual quality (Zahavi 1975; 1987; Kodric-Brown & Brown 1984). Many species have more than one trait that may convey information, such as vocalizations, behavioural displays, and plumage or multiple plumage characters (Andersson 1994). The presence of multiple ornaments is perplexing, and a number of theories have been put forth to explain their evolution. Multiple ornaments could evolve under arbitrary Fisherian selection (Fisher 1958; Kirkpatrick 1982; Möller & Pomiankowski 1993), selection for a single most-revealing trait (Schluter & Price 1993; Iwasa & Pomiankowski 1994), or selection for multiple quality-revealing traits (Johnstone 1995; Doucet & Montgomerie 2003; Papke et al. 2007; Taylor et al. 2007). Preference for male traits can also vary based on plasticity in female choice under differing demographic conditions or female reproductive states (Uetz & Norton 2007) or preference for different traits in different years (Chaine & Lyon 2008).

Furthermore, multiple signals may be intended for different receivers (Pryke et al. 2001; Andersson et al. 2002; Pryke et al. 2002; Braune et al. 2005), indicate different aspects of individual quality (Doucet & Montgomerie 2003; Jawor & Breitwisch 2004), or function in spatially or temporally separated periods (Marchetti 1998; Braune et al. 2005). For example, red-collared widowbirds (Euplectes ardens) exhibit an elongated tail
and a red, carotenoid-based collar. Both tail length and carotenoid colouration are classic examples of sexually selected traits; however, only tail length is preferred by females and associated with mating success (Pryke et al. 2001). The red collar, on the other hand, appears to function in agonistic, intrasexual interactions (Pryke et al. 2002). Such studies highlight the need to investigate the function of multiple ornaments throughout the annual cycle, both in the context of sexual signaling during the breeding season as well as agonistic signaling during the non-breeding period. For migratory birds, elucidating the function of plumage-based signals throughout the annual cycle can be particularly onerous, as some signals may function during migration or on the wintering grounds, thousands of kilometers removed from the breeding grounds. In particular, plumage may act to mediate access to food resources at migratory stopover sites (Moore & Yong 1991), or territory acquisition (Reudink et al. accepted) and agonistic interactions during the non-breeding season (Rohwer 1975).

American redstarts (Setophaga ruticilla) provide a unique opportunity to study the function of multiple plumage traits on both the breeding and wintering grounds. Plumage in ASY male redstarts is highly variable in both carotenoid-based (orange) regions and in the size of the melanin-based (black) bib, making this an ideal species for the study of the relative importance of multiple potential signals. Both carotenoid- and melanin-based plumage traits may reveal individual quality, though the mechanisms that maintain signal honesty can vary between pigment types. Carotenoid-based pigments cannot be synthesized naturally by birds and must be ingested as part of the diet. Thus, only males that are in good condition and can secure access to high quality food sources should be able to acquire high-quality plumage (Hill 1992; Hill & Montgomerie 1994; Hill 1999;
Brawner et al. 2000). Carotenoid-based plumage has been shown to be a reliable sexual signal in number of passerines (for review, see: Hill 2006; Griffith and Pryke 2006). Unlike carotenoids, melanin is synthesized from naturally occurring amino acids, but signal honesty can be maintained through social reinforcement mechanisms (Senar 2006) or nutrient limitation (McGraw 2007). Melanin-based signals, especially badge size, have been shown to signal status in temperate resident species during the non-breeding season (Rohwer 1975, Fugle et al. 1984, Grasso et al. 1996, Woodcock et al. 2005), but can also act as a sexual signal during the breeding season (Møller 1988; Møller, 1990; Thusius et al. 2001; Jawor et al. 2003; Tarof et al. 2005).

Redstarts undergo a single pre-basic moult at the end of the breeding season and retain those feathers throughout the winter and subsequent breeding season (Sherry & Holmes 1997). They are sexually dimorphic and males have delayed plumage maturation; females and males in their first breeding season (second year, or SY males) are gray with yellow patches on their tails, wings, and flanks, while males in their second breeding season or later (after second year, or ASY males) are jet black with orange patches (Sherry & Holmes 1997). SY males have severely reduced reproductive success (see below), suggesting that, at least on a gross level, plumage colouration is associated with gaining reproductive opportunities. American redstarts have a mixed mating strategy, with moderate levels of polygyny (25% of ASY males; Reudink et al. in review) and high levels of extrapair paternity (59% of nests, 40% of offspring: Perreault et al. 1997; 43% of nests, 25% of offspring: Reudink et al. in review).

Polygyny and extrapair paternity require different strategies to maximize success. Because restart extrapair sires provide no parental investment, female mate choice for
extrapair sires should be based on signals that convey genetic quality (Griffith et al. 2002). Polygynous males generally retain two territories, which can be adjacent or separated up to 300m, but sometimes maintain a single, large territory on which the primary and secondary females generally nest at opposite sides. Polygyny requires defending multiple (or much larger) territories throughout the breeding season (Secunda & Sherry 1991), suggesting that male-male interactions may be critical in achieving polygyny. After hatching, redstart males provide parental care at both nests, although the amount of care provided at the secondary nest is reduced relative to the primary nest (Secunda & Sherry 1991; R. Germain unpublished data). While tertiary females have been reported (Secunda & Sherry 1991), we have not recorded any incidences of males pairing with more than two females in our study population.

To the best of our knowledge, no work has yet addressed the function of plumage traits during both the breeding and non-breeding period in a long-distance migratory songbird. However, our previous work conducted in Jamaica showed that male redstarts occupying territories in high-quality habitats during the non-breeding season had brighter tail feathers, suggesting that tail brightness functions as a signal of competitive ability during the non-breeding season (Reudink et al. accepted). By investigating how tail feather brightness and other plumage traits are related to breeding events, we expand upon our previous work to examine if these plumage traits function during the breeding and non-breeding periods and/or if different traits are signals intended for different receivers.

To investigate the potential role of tail feather colouration as a multiple-use signal, and to investigate a role for flank feather colouration and bib size, we examine the
relationship between these plumage characters and extrapair paternity, polygyny, and total genetic fledging success. Because the tail feathers of male redstarts are associated with winter territory acquisition, we predict that males with brighter tail feathers should be more successful at polygyny and polyterritoriality (Secunda & Sherry 1991). We then compare the plumage characteristics of males that sire all their own offspring versus those that lose paternity at their nest. Next, we compare the characteristics of the male that lost paternity to those of the extrapair sire. Finally, we examine the plumage correlates of male genetic fledging success.

METHODS

Field Data Collection

Field work was conducted May-July 2005-2007 at the Queen’s University Biological Station, Chaffey’s Lock, Ontario, Canada (44°34’ N, 76°19’W). Our study area is composed of mixed-deciduous forest, primarily dominated by sugar maple (Acer saccharum) and Eastern hop hornbeam (Ostrya virginiana). When males arrive on the breeding grounds, they immediately begin singing for territory advertisement and to attract females. From May 1 – May 31, we surveyed our 60ha study area from 0600-1200, detecting males by the presence of singing and subsequent visual identification. ASY males arrive first on the breeding ground, followed a few days later by females, then SY males. Most ASY males were captured in mist-nets within 7 days of arrival by simulating territorial intrusions using song playbacks (recorded at Hubbard Brook, New Hampshire) accompanied with either a stuffed ASY male decoy or a caged live male (SY or ASY). Females were captured either by simulating territorial intrusions or by using
song playback of fledgling alarm calls accompanied by a female decoy. Redstarts were individually marked with a single Canadian Wildlife Service aluminum band and 2-3 colour bands. We then extracted 50 µl of blood for paternity analysis by piercing the brachial vein. Consistent with our previous work, we used wing length (mm) as our measure for relative body size (Reudink et al. accepted). From each individual, we plucked a single rectrix (R3) and 12-15 feathers from the center of the orange portion of the flanks.

Upon arrival, all males were observed and mapped for at least 20-30 min/day to determine territory boundaries and pairing date. Females typically begin nest-building within a few days of pairing. Once nest-building began, we monitored nest status every other day, noting the onset of egg-laying, number of eggs laid, hatching, and fledging success. Males continued to be monitored daily to ensure we detected polygynous mating. At day 5 after hatching, we banded nestlings with a single aluminum band and took 15-20 µL of blood for paternity analysis. When nests were inaccessible, the offspring were captured on the day of fledging.

Because redstarts exhibit delayed plumage maturation and few SY males pair and fledge offspring (one of 22 males that stayed in our study area throughout the entire season fledged a single offspring), we restricted our analyses to ASY males.

**Paternity Analysis**

Blood samples taken from putative parents and offspring were stored in Queen’s lysis buffer (2005-7). DNA was extracted using an Invitrogen Blood and Tissue Kit. gDNA was then quantified via agarose gel electrophoresis and diluted or concentrated to
~10ng/µL. All loci were amplified using a Biometra Thermogradient cycler or Biometra UNOII cycler PCR machine under the following conditions: 94°C for 3 min followed by 35 cycles of 94°C for 15 sec, 58°C for 15 sec, 72°C for 30 sec, and a final extension of 72°C for 10 min. Each sample included 1µL DNA (10ng/µL), 1µL 10X Qiagen PCR buffer, 0.03µL (100mM) dNTPs, 0.03µL (100µM) forward primer, 0.03µL (100µM) reverse primer, 0.025µL M13 F 700IRD Licor primer, 0.005µL (5U/µL) Taq polymerase, and up to 10µL total volume sterile ddH2O. Amplified samples were run on a Licor IR2 Global Sequencing Unity and allele scoring was conducted by a trained observer blind to the identity of individuals.

Paternity analysis was conducted using five microsatellite loci (Dpµ01, Dpµ03, Dpµ05, Dpµ15, Dpµ16) originally isolated from yellow warblers (Dendroica petechia; Dawson et al. 1997). Over the three years of this study, we analyzed the DNA of 196 offspring from 62 nests and all putative parents; however, our total sample size decreased to 44 nests due to exclusions because of inadequate colour data (see below) and exclusion of SY males. The use of five highly variable microsatellite loci, ensured a high probability of exclusion (>0.999). Paternity exclusion and assignment was conducted using CERVUS 2.0 (Marshall et al. 1998) and double-checked by hand. Because of limitations in detecting 2bp differences, and relatively high frequency of null alleles, we followed the relatively conservative approach of Reudink et al. (2006); offspring were only excluded if they mismatched the putative sire at >2bp and at ≥2 loci.

Colour Variables
All feather samples were mounted on low-reflectivity black paper (< 5% reflectance; Colorline #142 ebony). The single R3 feather was mounted alone, while 12-15 flank feathers were stacked as they would lie on the bird (Quesada and Senar 2006). In cases where we had an insufficient number of flank feathers or too little orange on the R3, that colour patch was excluded from further analyses. Percent reflectance across the bird visual spectrum (320-700nm) was recorded using an Ocean Optics USB2000 spectrometer attached to a PX-2 xenon pulsed light source (Figure 4.1). The probe was held at a 90° angle from the feather surface and housed in a sheath to keep the probe a constant distance from the feather surface. For both feather regions, we took 25 readings haphazardly throughout the region of interest. Using CLR1.0.3 (Montgomerie 2008), we gathered the raw reflectance data into 10nm bins from 320-700nm and averaged across the 25 measurements. To quantify tail and flank colouration, we first calculated brightness ($B_{320-700}$) by averaging percent reflectance from 320-700nm (Figure 4.1). Because redbreast tail and flank feathers consist of multiple peaks (UV and red/orange), we used PCA to collapse the spectrum into a small number of independent variables that described the shape of the curve (and thus measures of hue and chroma) independent of brightness (Cuthill et al. 1999; Grill & Rush 2000; Montgomerie 2006). To verify this method, we also calculated hue and chroma using the following calculations, where $B_{\lambda_{i-n}}$ = total light reflected from the $i$th wavelength to the $n$th wavelength ($\lambda_i - \lambda_n$) after summing across each 1nm interval:

$$\text{Hue} = \arctan\left(\frac{(B_{\lambda_{415-510}} - B_{\lambda_{320-415}})/B_{\lambda_{320-700}}}{(B_{\lambda_{575-700}} - B_{\lambda_{415-575}})/B_{\lambda_{320-700}}}\right)$$

$$\text{UV chroma} = \frac{B_{\lambda_{320-415}}}{B_{\lambda_{320-700}}}$$

$$\text{Red chroma} = \frac{B_{\lambda_{575-700}}}{B_{\lambda_{320-700}}}$$
Tail PC1 explained 67.7% of the variation in the shape of the curve and loaded positively on the shorter wavelengths and negatively on the red/orange region of the spectrum. Correspondingly, tail PC1 was negatively correlated with hue ($R^2 = 0.33, N = 118, P < 0.001$) and red chroma ($R^2 = 0.87, N = 118, P < 0.001$) and positively correlated with UV chroma ($R^2 = 0.09, N = 118, P = 0.007$). Flank PC1 described 88.9% of the variation in curve shape and, like the tail feathers, loaded positively on the shorter wavelengths and negatively on the red/orange region of the spectrum. Again, flank PC1 was negatively correlated with hue ($R^2 = 0.81, N = 109, P < 0.001$) and red chroma ($R^2 = 0.99, N = 109, P < 0.001$) and positively correlated with UV chroma ($R^2 = 0.86, N = 109, P < 0.001$).

Thus, PC1 from both tail feathers and flank feathers was used to describe “redness”, where “redder” birds have more negative tail and flank PC1 values. Following Lemon et al. (1992) bib size was ranked in hand on ASY males on a scale from 1-5, where 1 = small amount of black and 5 = large amount of black, with intermediates given half-points. See Lemon et al. (1992) for illustrations of bib size characteristics.

**Statistical Analysis**

All statistical analyses were performed in JMP 5.1 (SAS Institute 2006). For individuals that bred on the site more than one year of the study, data from one year only was randomly selected to avoid pseudoreplication. We used stepwise logistic regression with presence/absence of extra-pair offspring as the response variable to determine the factors that best predicted the occurrence of extrapair offspring, sequentially eliminating variables with $P < 0.05$ from the model. We also used stepwise logistic regression with polygynous/monogamous pairing as a response variable to determine the factors that best
predicted whether a male achieved polygyny. To determine the factors that best predicted the proportion of within-pair offspring and total fledging success, we used backward stepwise multiple regression. We used matched-pairs t-tests to compare the characteristics of extrapair and within-pair sires.

**Ethical Note**

Research was conducted under Queen’s University Animal Care and Use Committee guidelines (Protocol # Reudink-2005-007) and under Canadian Wildlife Service collection permit # CA 0154 and banding permit #10766C.

**RESULTS**

**Paternity Analysis**

Of the 44 nests from 2005 to 2007 included in this study, 22 (50%) contained one or more extrapair offspring, while 45 of the 135 (33%) offspring analyzed were extrapair. Of the 45 extra-pair offspring, we were able to assign paternity to 22. A subset of males for whom we collected paternity data were polygynous (10/44), though there was no difference in paternity between polygynous and monogamous males (1st females: 16/34 (47%) of nests contained EPO, 34/104 (33%) of offspring were EPO; 2nd females: 6/10 (60%) of nests contained EPO, 11/31 (35%) of offspring were EPO; all p > 0.9). We did not include nests of second females in subsequent analyses.

**Colour Analysis**
Pairwise correlations between plumage variables revealed that bib size was not related to any measure of colour; however, tail brightness and tail redness (PC1) were negatively correlated ($r^2 = 0.54$, $N = 73$, $P < 0.001$). There were no relationships between any of the other colour variables (all $P > 0.21$).

When we examined individuals recaptured in subsequent seasons, we found that tail feather redness (PC1) decreased with age (matched-pairs t-test, $t_{17} = -2.15$, $P = 0.046$) while flank brightness increased and redness (PC1) decreased marginally, but not significantly (flank brightness: $t_{14} = -1.96$, $P = 0.07$; flank redness (PC1): $t_{15} = 1.81$, $P = 0.09$). Tail brightness and bib size did not change between year X and year X+1 ($t_{18} = -0.71$, $P = 0.50$; bib size: $t_{16} = -0.39$, $P = 0.70$).

**Polygny and colour**

Results of a backward stepwise nominal logistic regression with polygny as a binary response variable (polygynous/monogamous) and tail brightness, tail redness (PC1), flank brightness, flank redness (PC1), bib size, year, arrival date and body size as main effects revealed that males with higher tail brightness were more likely to be polygynous ($\chi^2 = 6.15$, $N = 73$, $P = 0.01$).

**Paternity and colour**

Stepwise nominal logistic regression with presence/absence of extrapair offspring in the nest (yes/no) and tail brightness, tail redness (PC1), flank brightness, flank redness (PC1), bib size, year, arrival date, and body size as main effects revealed that increased flank redness best predicted whether individuals sired all their own offspring ($\chi^2 = 5.15$, $N = $
Backwards stepwise multiple regression revealed that the proportion of extrapair offspring in a males’ nest was significantly predicted by tail brightness (positive), tail redness (PC1; negative), flank redness (PC1; positive), and arrival date (negative; early arriving males sired more offspring) (whole model: $F = 6.63$, $N = 40$, $P = 0.0004$; Table 4.1). None of the variables significantly predicted whether males were extrapair sires. Pair-wise comparisons of within-pair and extrapair sires revealed that extrapair sires had less red tail and flank feathers than the males they cuckolded (Table 4.2). Increased realized fledging success (actual within-pair offspring plus extrapair offspring sired in other nests) was significantly predicted by early arrival date ($F = 4.77$, $N = 42$, $P = 0.03$; Table 4.1).

**DISCUSSION**

Our results indicate that the carotenoid-based patches on the tail and flanks of American redstarts significantly predict polygyny and extrapair paternity (Figure 4.2). Tail feather brightness, which is associated with territory quality during the non-breeding season, may also serve a signaling function during the breeding season. When we examined the plumage correlates of monogamous and polygynous males, only tail brightness predicted whether individuals achieved polygyny. Because achieving polygyny is highly dependent on males securing and defending multiple territories (Secunda & Sherry 1991), our results support the idea that tail brightness may function in male-male competition and agonistic interactions. However, because tail brightness also significantly predicts paternity, we suggest that it may serve as a dual-utility signal for both inter- and intra-sexual interactions. Interestingly, bib size, a trait commonly associated with dominance
and male-male competition (Senar 2006), was not related to polygyny or any of the other reproductive variables we measured. This result is somewhat surprising, as Lemon et al. (1992) previously reported that males with smaller bibs had higher reproductive success in a New Brunswick, Canada population of American redstarts. Our previous work on an over-wintering population of American redstarts also failed to find any evidence for plumage acting as a social signal during the non-breeding season. It is possible that bib size is related to other aspects of male quality (e.g., parental care) that were not measured in this study.

The relationship between extrapair paternity and plumage ornamentation was more complicated. Males with redder flanks were most likely to sire their own offspring, while the proportion of offspring sired was predicted by increased flank redness (PC1), decreased tail redness (PC1), increased tail brightness, and early arrival date. These results suggest that carotenoid-based patches on both the flanks and tail of redstarts may act as signals of male quality. Previous research on our study system indicates that early arrival, driven by conditions experienced during the non-breeding season, significantly predicts realized fledging success (Reudink et al. submitted). The results of this study are consistent with our previous work and suggest that while flank and tail colouration are important for achieving polygyny and securing paternity, ultimately, arrival date best predicts realized fledging success.

Extrapair sires, compared to the social males at each nest, had significantly less red tail and flank feathers. These results are inconsistent with the finding that males with redder flank feathers were less likely to lose paternity. However, the finding that reduced redness (PC1) appears to be beneficial runs contrary to our common understanding of
carotenoid-based plumage colouration. Generally, plumage redness (PC1) is associated with increased carotenoid deposition and thus birds with higher redness (PC1) scores would be assumed to be of higher quality (Saks et al. 2003a). Interestingly, our results show a strong association of increased plumage brightness and decreased plumage redness (PC1) with reproductive success (i.e., polygyny, paternity). Plumage colouration, however, is a result of multiple processes that influence a given signal and is not necessarily as straightforward as carotenoid acquisition/deposition increasing feather redness (PC1) and thus indicating individual quality. Plumage colouration is influenced both by feather structure and pigment deposition (Saks et al. 2003a, Shawkey & Hill 2005) and can also be influenced by feather abrasion (Blanco et al. 2005) and moult location or carotenoid availability (McGraw 2006). Redstart feathers contain the carotenoid pigments canary xanthophylls A and B and cathaxanthin (McGraw et al. 2005), and it remains unknown how the ratio and bulk depositions of these pigments, as well as variation in feather microstructure, contribute to the overall colour display exhibited by these birds. It will be informative to learn if redness (PC1) decreases due to decreased levels of the red pigment cathaxanthin, or if decreased redness (PC1) is actually the result of increased concentrations of the yellow pigments, canary xanthophylls A and B.

The finding that increased tail feather brightness is correlated with achieving polygyny and the proportion of sired offspring is not surprising given that higher plumage brightness in redstarts is correlated with higher territory quality in winter (Reudink et al. accepted). In other species, plumage brightness has also been shown to
predict mating success (golden-collared manakins; Stein & Uy 2006) and body condition (Saks et al. 2003b).

While more study is needed, the finding that males that were more red were better able to retain paternity while males that were less red were better able to sire extrapair offspring may reveal opposing selective pressures. For example, Delhey et al. (2003) showed that male blue tits (Parus cyanistes) with more UV-shifted crown hue were less likely to lose paternity, while older males and males with less UV-shifted crown hue were more likely to sire extrapair offspring. Unlike our study, however, Delhey et al. (2003) did not find a difference in UV ornamentation between within-pair males and the extra-pair sires. The authors suggest that this could be due to a trade-off between UV ornamentation and traits that optimize extrapair success. While we do not currently have the data to address this question, it is possible that these results reflect alternative mating tactics whereby males with redder flanks increase success through within-pair paternity while less ornamented males increase success through extrapair paternity.

With respect to the evolution of multiple ornaments in American redstarts, our results suggest that tail and flank colouration may have signaling functions on the breeding grounds; however, we found no relationships with bib size and any of the reproductive variables we measured. Our finding that tail brightness predicted polygyny and flank redness (PC1) predicted the presence of extra-pair offspring suggests that tail brightness and flank redness (PC1) may be signals intended for different receivers (Pryke et al. 2001). Needed still are aviary-based experimental studies. It is possible that because polygyny is highly dependent on securing multiple territories, tail feather brightness may serve as a signal of competitive ability, while flank redness (PC1) functions in female
choice. Furthermore, our data support the idea that tail brightness has a dual function both during the breeding and non-breeding period (Reudink et al. in press). By expanding the study of plumage colouration in migratory birds to events and selective pressures occurring throughout the annual cycle, we can gain insight into the function and evolution of ornamental traits.

We find it unlikely that females are using plumage redness (PC1) as a direct indicator of male quality when deciding whether to pursue extrapair fertilizations or in choosing extrapair sires. Rather, this pattern may arise because tail redness (PC1) is negatively correlated with male characteristics that are related to female choice or a male’s ability to retain paternity and sire extrapair offspring. Indeed, by examining males captured in year x and recaptured in year x + 1, we found that tail feathers became significantly less red in year x + 1. In their first breeding season, males have female-like plumage and suffer highly-reduced reproductive success, indicating there is a clear premium for males to acquire ASY plumage. Given that so few SY males successfully pair or raise offspring, ASY plumage, in and of itself, does not indicate that males are experienced breeders. Our results suggest that because tail plumage decreases in redness (PC1) as ASY males age, plumage colour has the potential to signal male age and previous breeding experience once males acquire ASY plumage. Longitudinal studies examining individuals recaptured in multiple seasons could reveal if patterns of paternity and polygyny co-vary with changes in plumage colouration.

In this study, we drew on previous work conducted on plumage colouration during the non-breeding period (Reudink et al. in press) to investigate potential signaling roles of multiple plumage traits during two phases of the annual cycle. We suggest that
further work, addressing the role of plumage in mediating access to resources at migratory stopover sites, may provide further evidence that plumage can act as a signal throughout the entire annual cycle. Furthermore, such research could help elucidate if different traits function in discreet or overlapping periods of the annual cycle. This study reveals that traits implicated as signals in agonistic interactions during the non-breeding period may also serve to enhance reproductive success on the breeding grounds. Our results support the idea that American redstarts display multiple plumage-based signals intended for different receivers. Tail feather brightness appears to function as an intra-sexual signal, both during the breeding and non-breeding seasons, and potentially as an inter-sexual signal during the breeding season, while flank redness (PC1) appears to be important for inter-sexual signaling. A potential, but as yet untested, hypothesis is that because tail brightness appears to signal an individual’s ability to obtain a high-quality territory during the non-breeding season, individuals may use that information, obtained thousands of miles from the breeding grounds, to inform mate choice decisions and whether to engage in agonistic interactions. If so, this would suggest a carry-over effect of signal efficacy from the non-breeding season to the breeding season, a pattern thus far only observed in temperate residents (e.g., Mennill et al. 2003, Doucet et al. 2005).

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Thank C. Scott for molecular assistance. Funding was provided by the Natural Sciences and Engineering Research Council of Canada, the Canadian Foundation for Innovation, the National Science Foundation grant to PPM (0085965), the Smithsonian Institution, Queen’s University, Ontario Innovation Trust, Sigma Xi, the American Ornithologists’ Union, the Society of Canadian Ornithologists, and the American Museum of Natural History. All methods in this study complied with the laws of Canada.

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**SAS Institute.** 2006. JMP Statistical Discovery 6.0.2. Duxbury, Pacific Grove, California.


Table 4.1. Significant predictors of proportion of within-pair offspring and total fledging success from a stepwise backwards multiple regression procedure.

<table>
<thead>
<tr>
<th>reproductive variable</th>
<th>predictors</th>
<th>$N$</th>
<th>$\beta$</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>proportion WP offspring</td>
<td>whole model</td>
<td>40</td>
<td></td>
<td>6.63</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>tail brightness</td>
<td>40</td>
<td>-0.10</td>
<td>8.28</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>tail redness</td>
<td>40</td>
<td>0.06</td>
<td>6.77</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>flank redness</td>
<td>40</td>
<td>0.04</td>
<td>14.87</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>arrival date</td>
<td>40</td>
<td>-0.02</td>
<td>7.26</td>
<td>0.0002</td>
</tr>
<tr>
<td>total genetic fledging success</td>
<td>arrival date</td>
<td>42</td>
<td></td>
<td>4.77</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 4.2. Comparisons of social males and the extrapair sires at their nest.

<table>
<thead>
<tr>
<th>all EP sires</th>
<th>tail brightness</th>
<th>tail redness</th>
<th>flank brightness</th>
<th>flank redness</th>
<th>bib size</th>
<th>body size</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP male</td>
<td>17.56</td>
<td>-0.4</td>
<td>15.32</td>
<td>-2.07</td>
<td>2.43</td>
<td>63.19</td>
</tr>
<tr>
<td>EP male</td>
<td>17.13</td>
<td>1.43</td>
<td>17.91</td>
<td>2.16</td>
<td>2.77</td>
<td>62.95</td>
</tr>
<tr>
<td>mean difference</td>
<td>0.42 ± 0.55</td>
<td>1.83 ± 0.82</td>
<td>-2.59 ± 1.39</td>
<td>4.22 ± 1.54</td>
<td>-0.33 ± 0.33</td>
<td>-0.24 ± 0.44</td>
</tr>
<tr>
<td>df</td>
<td>19</td>
<td>19</td>
<td>17</td>
<td>17</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>T</td>
<td>0.77</td>
<td>2.25</td>
<td>-1.86</td>
<td>2.74</td>
<td>-1.01</td>
<td>-0.55</td>
</tr>
<tr>
<td>P</td>
<td>0.45</td>
<td><strong>0.04</strong></td>
<td>0.08</td>
<td><strong>0.01</strong></td>
<td>0.32</td>
<td>0.59</td>
</tr>
</tbody>
</table>
Figure 4.1. Average reflectance spectra of adult male flank (gray line) and tail feathers (black line).
Figure 4.2. Significant relationships between potential signals/predictor variables and reproductive variables. Solid lines represent positive relationships, while dashed lines represent negative relationships. Thin lines represent $P < 0.05$, while thick lines represent $P < 0.01$. For the reproductive variables: polygyny = whether males were polygynous or monogamous; secured offspring = whether the within-pair male sired all offspring at his nest; % paternity = the proportion of within-pair offspring a social male sired; extra-pair sires = the difference between within-pair males and the extra-pair sires; fledging success = # of within-pair offspring fledged at primary and secondary (if polygynous) nests + # extra-pair offspring fledged.
Moult-migration in the American Redstart re-visited: explaining variation in feather δD signatures
INTRODUCTION

At least once a year, birds face the energetically demanding task of moulting all their flight and body feathers. As a result, most birds avoid an overlap between moult and other costly activities during the annual cycle (e.g., raising young, migrating). Most Nearctic–Neotropical migratory birds undergo an entire prebasic moult at the end of the breeding season, before fall migration, and some also go through a second, pre-alternate moult of body feathers on the wintering grounds to refurbish their breeding plumage before reproduction (Pyle 1997, Froehlich et al. 2005). Even so, some birds employ a strategy of moult-migration, delaying some or all of their prebasic moult until after fall migration begins (Stresemann and Stresemann 1966). This phenomenon is relatively common in shorebirds, and nearly half the Neotropical migrants moult at least some feathers south of the breeding grounds (Leu and Thompson 2002). However, moult-migration is not nearly as frequent in passerines (e.g., only 7 of 53 wood warblers are reported to complete at least some of their pre-basic moult outside of the breeding grounds (Vermivora peregrina, V. celata, V. luciae, Dendroica petechia, D. pensylvanica, D. kirtlandii, Protonotaria citrea); Pyle 1997), and it also appears to vary geographically, occurring more commonly in western North America (Rohwer et al. 2005).

In a recent study, Norris et al. (2004b) suggested that moult-migration is a conditional strategy in American Redstarts (Setophaga ruticilla) whereby individuals investing in late-season parental effort (i.e., birds fledging more young late in the breeding season) delay part of their moult until sometime during migration. Using stable-hydrogen isotopes (δD) in tail feathers to infer the latitude of moult, Norris et al. (2004b)
showed that reproductive effort was positively correlated with \( \delta D \) values the following year. Additionally, birds that fledged young had tail feathers with more positive \( \delta D \) values (indicative of moult at a southerly stopover site) than individuals that did not fledge offspring. Birds presumed to be moult-migrants also had less red plumage (lower red chroma values), which suggests a tradeoff between current and future reproduction, if plumage is an important sexual signal.

Since the publication of Norris et al.’s (2004b) paper, studies examining isotopic variation have determined that \( \delta D \) is more variable within populations than was previously assumed (Wunder et al. 2005, Powell and Hobson 2006, Rocque et al. 2006, Langin et al. 2007). For example, in the same American Redstart population studied by Norris et al. (2004b), Langin et al. (2007) reported 22‰ variation in \( \delta D \) (first primary [P1], range: –92 to –70‰, mean = –82 ± 4‰ SD, \( n = 42 \)) among feathers grown by different individual birds that bred within 2 km of each other during the year of moult, suggesting that some birds Norris et al. (2004b) presumed to be moult-migrants may have grown their tail feathers locally using hydrogen sources with more positive \( \delta D \) values than expected (Norris et al. [2004b] estimated a range of –88 to –72‰).

In the present study, we report additional data on the relationship between reproductive effort, moult location (as inferred by \( \delta D \) analysis), and feather colour to test the robustness of these relationships and to further document the frequency of moult-migration. We also test an alternative hypothesis that a high degree of variation of \( \delta D \) on the breeding grounds, coupled with inadvertent feather loss during the non-breeding period, can explain the occurrence of individuals returning to the breeding grounds with more positive \( \delta D \) signatures and tail feathers with lower red chroma.
METHODS

Breeding population.—Work on the breeding grounds was conducted May—July, 2004–2007, at the Queen’s University Biological Station, Chaffey’s Lock, Ontario (44°34′N, 76°19′W), using the same study population of American Redstarts as Norris et al. (2004b) and Langin et al. (2007). All birds were captured and processed following Norris et al. (2004b). One difference, however, is that none of our nests was experimentally depredated as was done with a subset of birds by Norris et al. (2004b; see Langin et al. 2006). We also collected the first primary (P1) from all individuals and a second rectrix from individuals with regrown tail feathers (i.e., feathers that appeared less orange than the rest of the feathers were unworn and had narrow growth bars).

Overwintering population.—American Redstarts were also studied at a wintering site consisting of high-quality (Black Mangrove (Avicennia germinans) dominated) and low-quality (second-growth scrub dominated by logwood trees (Haematoxylon campechianum)) habitats from January to March, 2004 and 2005, at Font Hill Nature Preserve, Westmoreland Parish, Jamaica, (18°02′N, 77°57′W; see Marra 2000). We studied birds overwintering in Jamaica because populations breeding in the northeastern United States and southern Ontario appear to overwinter in the Caribbean (Norris et al. 2006), which suggests that conditions in Jamaica should be similar to those experienced by our breeding population during the non-breeding season.

We tested the hypothesis that birds previously reported as moult-migrants may have lost and subsequently regrown tail feathers on the wintering grounds and that
feather regrowth may be condition-dependent. To do this, we captured birds in Jamaica during January–February, weighed them to the nearest 0.1 g, and plucked a single tail feather (R3). Before spring departure, we recaptured the same individuals to determine overwinter change in body mass and whether they regrew the plucked feather. Feathers were defined as “regrown” if they extended >1 cm from the feather sheath. For a subset of birds \((n = 10\) ASY males), we then plucked the regrown feather for comparisons of \(\delta D\) and colour between the original and regrown feathers.

**Stable-hydrogen isotope and colour analyses.**—Details of our stable-hydrogen isotope analysis are reported in Langin et al. (2007) and are the same as those employed by Norris et al. (2004b). Reflectance spectra from tail feathers were obtained by measuring percent reflectance from 320–700 nm using an Ocean Optics USB2000 spectrometer attached to a PX-2 xenon pulsed light source. The sheathed probe was held at a 90° angle to the feather surface. All feathers were mounted on minimally reflective (<5% reflectance) black paper (Colorline no. 142 Ebony). To standardize our measurements, we took dark and white (spectralon) standard readings between each measurement and the next. Twenty-five measurements were taken haphazardly within the yellow–orange region of each tail feather, avoiding the rachis. We then averaged across the 25 reflectance spectra and quantified plumage colouration by calculating standard measures of brightness, hue, and chroma (Montgomerie 2006). Our measure of red chroma is the same as that reported by Norris et al. (2004b): brightness = mean \(B_{320-700}\), Hue = \(\arctan\left(\frac{(B_{415-510} - B_{320-415})/B_{320-700}}{(B_{575-700} - B_{415-515})/B_{320-700}}\right)\), UV chroma = \(B_{320-415} / B_{320-700}\), and Red chroma = \(B_{575-700} / B_{320-700}\).
Data analysis.—Following Norris et al. (2004b), reproductive effort was calculated as a combination of the number of young fledged and fledging date, where birds with reproductive effort scores of 0 did not fledge offspring and birds with the highest reproductive effort scores fledged more offspring later in the season. All statistical analyses were performed in JMP 7.0 (SAS Institute 2007).

RESULTS

Isotopic signatures.—Adult males known to have bred at our study site in 2004 and 2005 returned the next year with tail feather δD values that ranged from –92 to –64‰ (n = 26), with an average value of –78 ± 7‰ SD. Two of these males had δD values that fell just outside the previously reported range of variation at our study site (–92 to –70‰; Langin et al. 2007), with δD values of –64‰ and –69‰. We found no difference in δD between the first primary (P1), which should always be moulted first on the breeding grounds (Pyle 1997), and tail feathers (R3; t15 = –1.59, P = 0.13).

Reproductive effort.—There was no relationship between reproductive effort in year x and δD the following year (r2 = 0.02, P = 0.58, n = 16), no difference in δD between birds that did and did not fledge offspring the previous season (t14 = –0.71, P = 0.49; 7 no, 9 yes), and no relationship between fledging date in year x and δD the following year (r2 = 0.22, P = 0.29, n = 7). We also found no relationship between reproductive effort in year x and red chroma (r2 = 0.0005, P = 0.91, n = 26), UV chroma (r2 = 0.001, P = 0.86, n = 26), hue (r2 = 0.0006, P = 0.90, n = 26), or brightness (r2 = 0.08, P = 0.16, n = 26). Finally, there was no relationship between δD and red chroma (r2
= 0.02, \( P = 0.38, n = 50 \), UV chroma \( (r^2 = 0.06, P = 0.08, n = 50) \), hue \( (r^2 = 0.05, P = 0.14, n = 50) \), or brightness \( (r^2 = 0.03, P = 0.23, n = 50) \).

**Feather regrowth.**—From 2005 to 2007, 7\% (4 of 56) adult males captured at our study site in Ontario had one or more tail feathers that were clearly regrown, which also had more positive \( \delta D \) values \((-42, -48, -49, \) and \(-54\%)\) in relation to mean \( \delta D \) value of locally grown adult flight feathers \((-82 \pm 4\%; \text{Langin et al. 2007}) \). In the feather regrowth experiment on the wintering grounds, the \( \delta D \) of the original feather plucked from adult males averaged \(-73 \pm 3\% \) \( (n = 10) \). By contrast, \( \delta D \) of regrown feathers from the same individuals plucked when recaptured later in winter averaged \(-35 \pm 2\% \) \( (n = 10; t_8 = 11.68, P < 0.0001; \text{Fig. 5.1A}) \). Those same feathers had significantly lower red chroma values \( (t_8 = -3.70, P = 0.005; \text{Fig. 5.1B}) \) and hue \( (t_8 = -6.61, p < 0.0001) \), but not UV chroma \( (t_8 = 1.13, P = 0.29) \) or brightness \( (t_8 = 0.61, P = 0.56) \).

The probability of regrowing an experimentally plucked tail feather during the non-breeding season in Jamaica was higher among American Redstarts that maintained or gained mass between captures \( (\text{Wald’s } \chi^2 = 6.42, P = 0.01) \). Variation among birds in the time elapsed between capture and recapture did not influence the probability of feather replacement \( (\text{Wald’s } \chi^2 = 0.00, P = 0.98) \).

**DISCUSSION**

Over the two years of the present study, we found no substantive evidence of moult-migration in American Redstarts and no support for a reproductive tradeoff. Only 2 of 26 return breeders had feathers with \( \delta D \) values \((-64 \) and \(-69\%)\) that were marginally
outside the known range of variation at our study site (i.e., values greater than –70 ‰; Langin et al. 2007). Furthermore, no American Redstarts that returned to breed had tails with highly positive δD signatures and low red chroma.

Unlike the previous study by Norris et al. (2004b), which suggested that moult-migration could be driven by reproductive effort, we found no difference in δD between males that did and did not fledge offspring the previous season and no relationship between reproductive effort and δD. One of the major implications of Norris et al.’s (2004b) study was that late-season parental effort could result in a tradeoff between current and future reproduction, given that birds with more positive δD signatures (presumed moult-migrants) also had feathers with lower red chroma values. We found no relationship between δD and red chroma and no relationship between reproductive effort in year $x$ and red chroma the following breeding season.

Results from our feather regrowth experiment demonstrated, as expected, that feathers regrown in Jamaica have significantly more positive δD signatures and lower red chroma values (Fig. 5.1). This pattern is consistent with observations from our breeding population and from the Powdermill Avian Research Center (Rector, Pennsylvania), where a few individuals arrive with one or two yellow tail feathers that are clearly distinguishable from the orange tail feathers (R. S. Mulvihill pers. comm., M. W. Reudink and K. M. Langin pers. obs.). During the present study, 7% of the birds had clearly regrown a tail feather (see above), which also had highly positive δD signatures, which is consistent with overwinter regrowth. We suggest that the pattern observed by Norris et al. (2004b) was more likely driven by adventitious moult during the overwintering period in a few individuals, resulting in feathers with highly positive δD
signatures and low red chroma (consistent with the regrown feathers in our feather regrowth experiment).

Regardless of the mechanism that results in highly positive δD signatures and low red chroma, the fact that Norris et al. (2004b) detected a relationship between reproductive effort and δD–feather colour is intriguing. One explanation for this result may be condition-dependent feather regrowth on the wintering grounds. In Jamaica, individuals that maintained or gained mass during winter were more likely to regrow feathers than individuals that were unable to maintain mass in winter. Previous research indicates that differences in mass-change during the overwintering period are driven by habitat quality (Marra and Holmes 2001, Studds and Marra 2005). Furthermore, birds in high-quality habitats have lower corticosterone levels (Marra and Holberton 1998), depart the wintering grounds earlier (Marra and Holberton 1998, Studds and Marra 2005), arrive on the breeding grounds earlier (Marra et al. 1998, Norris et al. 2004a, Reudink et al. unpubl. data), and ultimately fledge more offspring and have higher reproductive success (Norris et al. 2004a, Reudink et al. unpubl. data). Therefore, these birds would have been more likely to be classified as moult-migrants according to Norris et al. (2004b). We suggest that birds in good condition during winter are more likely to fledge offspring the following breeding season and are also more likely to have regrown any feathers lost during winter on the wintering grounds (as opposed to not regrowing lost feathers). On the other hand, birds that do not regrow lost feathers during the overwinter period are likely to have been in poor condition during winter and are less likely to fledge offspring. Thus, individuals arriving with adventitiously moulted feathers are more likely to be high-quality birds that fledged offspring.
Finally, the results in the Norris et al. (2004b) study were driven primarily by three individuals with extremely high δD values and low red chroma. It is possible that the observed relationship with reproductive effort may be a spurious result driven by a small number of birds with adventitiously moulted feathers that happened to fledge young late in the season.

Our inability to find evidence of moult-migration in our breeding population of American Redstarts, combined with the results of our feather regrowth experiment, suggest that moult-migration is unlikely to play a role in structuring American Redstart life history. Furthermore, our results are consistent with a lack of evidence of moult-migration at banding stations. In nearly five decades of capturing fall-migrating American Redstarts at both the Powdermill Avian Research Center and Long Point Bird Observatory (Long Point, Ontario), investigators have not detected a single moult-migrant among the thousands of American Redstarts captured (R. S. Mulvihill pers. comm., D. J. T. Hussell pers. comm.).

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LITERATURE CITED


Fig. 5.1. (A) δD signatures and (B) red chroma of original (open circles) and re-grown (closed circles) feathers of adult males originally captured in January-February on the wintering grounds in Jamaica and subsequently recaptured prior to spring departure (n = 10).
Chapter 6

General Discussion
## Foundations

While on a winter field course in Mexico, I was observing over-wintering migratory birds and began thinking about how events and pressures experienced by migratory birds in the tropics influence mate choice or other aspects of their behaviour during the breeding season. Do events during the breeding season influence behaviour during the winter or during the following breeding season? Is selection on traits complementary or antagonistic during different phases of the annual cycle? While these questions seemed straightforward, I soon realized how little we know about the year-round ecology of migratory birds and how events occurring throughout the annual cycle interact to influence their behaviour.

Asking questions about the year-round ecology of migratory birds requires two major pieces of information. First, we need to have a strong baseline understanding of the ecology and behaviour of birds during different phases of the annual cycle. This is often a major obstacle, given the relative paucity of studies on the non-breeding season ecology of migratory birds (Greenberg and Marra 2005). Second, once we pinpoint factors that may carry-over to influence events in subsequent seasons, we need a way to track individuals or the impacts of these carry-over effects (Webster et al. 2002).

American redstarts are an ideal species for asking the questions about the year-round ecology of migratory birds, due in large part to two decades of continuous study on an over-wintering population of redstarts in Jamaica. This long-term study has led to a preponderance of papers detailing some of the major selective forces acting on redstarts during the non-breeding season (e.g., Holmes et al. 1989, Marra et al. 1993, Marra and Holberton 1998, Marra 2000, Studds and Marra 2005, Studds et al. 2008), allowing us to
make specific predictions about the factors that influence the behaviour and ecology of
over-wintering redstarts. Perhaps more importantly, it has also allowed us to identify
potential carry-over effects that can influence individuals and populations in subsequent
seasons (Marra et al. 1989, Norris et al. 2004a, Norris 2005, Norris and Marra 2007,
Studds et al. 2008).

The difficulty, however, is tracking the impact of carry-over effects from the
overwintering period through migration to the breeding grounds. To this end, the use of
biochemical markers such as stable-isotopes has greatly expanded our ability to draw
inferences about animal movements and trace the impacts of carry-over effects (Hobson
isotope analysis has revealed that the quality of an individual’s winter territory can
influence condition during migration (black-throated blue warbler: Bearhop et al. 2004),
timing of arrival on breeding areas (American redstarts: Marra et al. 1998, Norris et al.
2004a), apparent reproductive success (Norris et al. 2004a), and patterns of polygyny and
paternity (Chapter 3). Furthermore, utilization of stable-hydrogen isotopes has revealed
that winter territory quality can influence patterns of natal dispersal (Studds et al. 2008)
and has identified potential carry-over effects due to late-season reproductive effort
(Norris et al. 2004b, but see Chapter 5).

**Insights and implications**

My research presented in this dissertation highlights the importance of understanding
behavioural and evolutionary processes in light of events and pressures occurring
throughout the annual cycle (Fig. 6.1). In Chapters 2 and 4, I investigated if tail feather
colouration was related to performance on both the breeding and overwintering grounds. I chose to examine tail colouration because the carotenoid-based yellow-orange tails have the potential to honestly signal individual quality and tail fanning is a display used during foraging, courtship, and agonistic interactions. I demonstrate that tail feather brightness is associated with performance during both the breeding and non-breeding seasons, suggesting that this trait functions as a dual-utility signal. Furthermore, another aspect of male colouration, tail feather redness, is associated with extrapair paternity (and potentially female mate choice) on the breeding grounds, but is not related to winter habitat quality. This finding suggests that some signals may function only during one portion of the annual cycle. Important to note is that the Colour variables used differed in Chapters 2 and 4. This is in part a historic artifact – Chapter 2 was the first chapter completed and accepted for publication and used a traditionally employed approach of examining 4 standard colour variables. In Chapter 4; however, we took a more refined approach that allowed us to better characterize the colour curves using a smaller number of variables (2 rather than 4).

My research also highlights the importance of expanding our view of the evolutionary process of sexual selection to incorporate events outside the short period of time surrounding courtship, including the non-breeding period. In Chapter 3, I demonstrate that arrival date, driven by conditions on the wintering grounds, can influence rates of extrapair paternity, polygyny, and genetic success. These carry-over effects then have the potential to dampen the strength of sexual selection on a preferred trait. For example, though we have strong evidence for directional selection for tail feather brightness, achieving polygyny is still highly dependent on arrival date. Even if a
male arrives on the breeding grounds with traits favored by females, if he arrives in late May, he will not achieve polygyny and has a high probability of losing paternity. Thus, though there is directional selection on plumage colouration, the strength of selection on that trait can be greatly reduced due to non-breeding season events. Many studies have failed to find consistent patterns of female mate choice for phenotypic traits (Chaine and Lyon 2008). It is possible that carry-over effects from the non-breeding season could confound relationships between male phenotype and reproductive success as well as patterns of female choice.

New techniques, such as genetic analysis or stable-isotope analysis, can revolutionize a field of study, but in Chapter 5, I present a cautionary note about the interpretation of stable-isotope data. A recent paper by Norris et al. (2004b) that suggested moult-migration in redstarts could represent a clear trade-off between current and future reproduction – a concept supported by theory, but lacking in empirical evidence for vertebrates. This work has been highly cited and has nearly entered into the scientific dogma. However, my research (Chapter 5; Langin et al. 2007) shows that moult-migration has a much simpler and far less exotic explanation that relies on understanding the underlying sources of isotopic variation in redstart feathers. However, if moult-migration was indeed indicative of a trade-off, it could represent a crucial carry-over effect that could influence behavioural dynamics during the non-breeding season and following breeding season. While it appears moult-migration is not an aspect of redstart life history, research that explores sources of potential carry-over effects is critically important. By exploring potential carry-over effects, we gain a clearer understanding of the factors that shape the life-history of migratory birds.
Future directions

1. *Studying selection on traits throughout the annual cycle.* Selection can act on phenotypic traits, such as plumage, under varying conditions and during different phases of the annual cycle. These selective pressures can be complementary (Mennill et al. 2003, Doucet et al. 2004), opposing (Delhey et al. 2003), or act on different aspects of the male phenotype (Pryke et al. 2001; Doucet and Montgomerie 2003, Evans and Hatchwell 1992a, 1992b). However the vast majority of research has focused on selective pressures facing birds during the breeding season with the presumption that if an ornamental plumage trait is not related to reproductive success, that trait is not currently under selection. However, those traits may be under selection during phases of the annual cycle that have traditionally been overlooked, such as migration and the over-wintering period (Marchetti 1998). Finally, my research was limited to examining the function of plumage during the stationary phases of the annual cycle, yet plumage ornamentation could also function to mediate access to food resources during migration, an idea that has not yet been tested.

2. *Role of plumage during the non-breeding season.* My work on plumage colouration during the non-breeding season (Chapter 2) was the first to find a potential signaling function for plumage during the non-breeding season in a long-distance migratory bird. Only one other study has investigated non-breeding season signaling in a long-distance migrant (hooded warblers: Stutchbury 1994), yet no relationship was found between the extent of male-like plumage in females
and habitat quality. Intraspecific competition for food resources is ubiquitous amongst over-wintering Neotropical migrants, but a role for plumage in mediating access to food resources has been largely ignored. It is highly likely that plumage ornamentation serves a signaling function during the overwintering period in many migratory birds.

3. **Influence of carry-over effects on evolutionary processes.** Sexual selection has traditionally been viewed as being influenced only by events immediately surrounding courtship and insemination. However, events temporally and spatially separated from breeding can have a significant impact on this evolutionary process. Events occurring both during the over-wintering period and during migration may have significant impacts on the opportunity for selection. For example, the choice of migratory routes and stopover sites during spring migration, or prospecting prior to breeding could have a significant impact on rates of polygyny and paternity through variation in arrival dates. Furthermore, it remains unclear how factors occurring during the first breeding season (while males remain in sub-adult plumage and generally do not breed) influence success in subsequent seasons. The challenges of tracking the influences of these carry-over effects are not insubstantial, but it is essential to begin incorporating carry-over effects into studies of sexual selection.

4. **Understanding variation in $\delta D$.** The use of stable isotopes has revolutionized the study of avian ecology and allowed us to make connections between breeding and wintering populations and trace the impacts of carry-over effects (Webster et al. 2002, Rubenstein et al. 2004, Hobson 2005). However, as we have become
increasingly aware, there are many sources of variation that can influence stable-isotope signatures in bird tissues (Hobson and Wassenaar 1999, Meehan et al. 2003, Smith and Dufty 2005, Wunder et al. 2005, Powell and Hobson 2006, Rocque et al. 2006, Langin et al. 2007). A recent paper I co-authored (Langin et al. 2007) as well as Chapter 5 present cautionary notes on the extent of that variation. Indeed a number of studies have recently documented age- and tissue-specific variation in δD (e.g, Rocque et al. 2006, Langin et al. 2007), yet the sources of that variation remain the subject of speculation. We desperately need rigorous studies that address the ecological, behavioural, and physiological sources of isotopic variation to ensure the correct interpretation of isotopic data and help us identify both the opportunities and limitations of isotopic studies.

Conclusions

Migratory birds experience events and pressures during each phase of the annual cycle that together shape their behaviour and ecology. A wealth of studies on the breeding grounds have informed much of what we know about the life history of migratory birds, yet we are only now beginning to understand the impact of events occurring during migration and the over-wintering period. My thesis demonstrates that events interacting throughout the annual cycle can shape the behaviour and ecology of migratory birds. Expanding our study of migratory birds to include events occurring during the non-breeding season and incorporating carry-over effects will greatly advance our understanding of avian life history, ecology, and evolution.
References


Marra, P. P. 2000. The role of behavioural dominance in structuring patterns of habitat occupancy in a migrant bird during the nonbreeding season. — Behav. Ecol. 11: 299–308.


Figure 6.1. Schematic representation of the data chapters in this thesis. Bold lines represent confirmed relationships, and dashed lines represent relationships I was unable to detect. Numbers in orange circles represent chapter numbers.