Bone collagen and muscle $\delta^{13}\text{C}$ in relation to the timing of the migration of Garden Warblers *Sylvia borin* during return migration from Africa

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Abstract In migratory birds, seasonal factors interacting at different stages of the annual cycle can influence individual life histories. These have been well documented for Nearctic-Neotropical birds. Yet, seasonal interactions between the wintering, migration, and breeding periods have not been demonstrated for Palearctic-African passerine migrants. We tested whether variation in long- and short-term dietary choice of the Garden Warbler *Sylvia borin* can influence events during the subsequent spring migration from the African winter quarters to the Palearctic breeding grounds. Using bone collagen and muscle carbon stable isotope ($\delta^{13}\text{C}$) analysis, we assessed the relationships between dietary history, body condition, and migration timing in the Garden Warbler during a return migration from Africa. We predicted that Garden Warblers arriving early

will have significantly different muscle and collagen $\delta^{13}\text{C}$ relative to those arriving later. Whereas muscle $\delta^{13}\text{C}$ (referring to events in the immediate past) was not related to body condition, we found a strong association between body condition and collagen $\delta^{13}\text{C}$ signatures (representing the integration of long-term events). Collagen and muscle $\delta^{13}\text{C}$ indicate that birds passing through later originated from moister or cooler geographic areas. The observed variation in isotope signatures might relate to differences in habitat and geographic/population origin, or in dietary intake.

Keywords Collagen · Pectoral muscle · Body condition · Avian migration

Zusammenfassung

$\delta^{13}\text{C}$ im Knochenkollagen und der Muskulatur von Gartengrasmücken *Sylvia borin* in Bezug zum Zugablauf während des Rückfluges aus Afrika

Wechselwirkungen saisonaler Faktoren während unterschiedlicher Etappen des Jahreszyklus können bei Zugvögeln deren individuelle Lebensgeschichte beeinflussen. Dies wurde für nearktisch-neotropische Vögel bereits gut belegt. Saisonale Wechselwirkungen zwischen dem Überwintern, dem Zug und der Brutperiode bei in der Paläarktis brütenden und in Afrika überwinternden Singvögeln sind bisher jedoch nicht bekannt. Wir überprüften ob die kurz- bzw. langfristige Nahrungswahl unter Gartengrasmücken *Sylvia borin* Ereignisse während des folgenden Frühjahrszuges aus den afrikanischen Winterquartieren in die paläarktischen Brutgebiete beeinflusst. Anhand des stabilen Kohlenstoffisotops ($\delta^{13}\text{C}$) im Knochenkollagen und in der Muskulatur untersuchten wir Zusammenhänge zwischen der vergangenen Nahrungsaufnahme, der Körperkondition und dem

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zeitlichen Ablauf des Zuges während des Heimfluges aus Afrika. Nach unserer Voraussage sollten signifikant unterschiedliche $\delta^{13}\text{C}$ Muster für Knochenkollagen und Muskulatur bei früh und spät ankommenden Gartengräsmücken auftreten. Die $\delta^{13}\text{C}$ Signatur der Muskulatur (zurückzuführen auf Ereignisse der unmittelbaren Vergangenheit) zeigte jedoch keine Beziehung zur Körperkondition, wohl aber die des Knochenkollagens (zurückzuführen auf Integration langfristiger Ereignisse). $\delta^{13}\text{C}$ Knochenkollagen und Muskulatur deuten darauf hin, dass später durchziehende Vögel aus relativ feuchteren oder kälteren Gegenden stammen. Die hier beobachtete signifikante Variation in der Isotopensignatur könnte auf Unterschiede in den Habitaten, im geographischen Ursprung (Populationen) oder in der Nahrungsaufnahme zurückgeführt werden.

Introduction

One of the key factors that determine the reproductive success and survival of individual animals is the quality of the habitat they occupy (e.g., Norris et al. 2004). In migratory species, individuals must select habitats in different geographic regions: breeding locations, and stopover sites during migration and at wintering quarters. In such a system, factors operating in one region are likely to affect an individual's subsequent success in another region (e.g., Marra et al. 1998; Norris et al. 2004; Bearhop et al. 2004; Norris 2005; Bauchinger et al. 2008, 2009). Events that result in a carryover effect on an organism's fitness in a subsequent season are generally termed seasonal interactions (Myers 1981; Webster et al. 2002).

Recent advances in the analysis of intrinsic markers, such as stable isotopes, have allowed great insight into the importance of seasonal interactions in migratory birds (e.g., Bearhop et al. 2004; Norris et al. 2004). In particular, stable carbon isotope ($\delta^{13}\text{C}$) analysis has been applied as a routine tool for addressing how the influence of a habitat experienced during one stage of the annual cycle may influence events in a subsequent stage. $\delta^{13}\text{C}$ can vary both spatially and temporally based on the type of plant community and the proportions of C_3 , C_4 , and CAM plants at a given place (Lajtha and Marshall 2007). Due to their photosynthetic pathways, C_3 plants have more negative $\delta^{13}\text{C}$ values than C_4 plants. C_3 plants are often common in more mesic areas, where insect abundance is often high, and these areas are known as "high-quality" sites for wintering birds (e.g., Parrish and Sherry 1994; Studds and Marra 2005). C_4 plants are usually abundant in drier, arid areas. The $\delta^{13}\text{C}$ composition at the base of the food web (e.g., primary producers) determines the $\delta^{13}\text{C}$ of higher trophic level consumers, such as birds (Hobson 1999).

It has been found that American Redstarts (*Setophaga ruticilla*) that rely on mesic, high-quality wintering locations arrive at their breeding sites earlier than birds from more dry and $\delta^{13}\text{C}$ -positive wintering locations (Marra et al. 1998). In a more extended study with American Redstarts, Norris et al. (2004) demonstrated that wintering in a high-quality site might have an extended carryover effect, whereby birds from mesic areas (with more negative $\delta^{13}\text{C}$ values) fledge more offspring than individuals from relatively poor wintering sites. Additionally, using claw $\delta^{13}\text{C}$ in individual bird samples during migration in the Bahamas, Bearhop et al. (2004) showed that winter habitat selection can influence events during the spring migration of Black-throated Blue Warblers (*Dendroica caerulescens*). Individual birds of this species originating from high-quality wintering grounds were in better body condition when sampled during spring migration than birds from lower-quality grounds.

These results are relevant to migratory passerine ecology, life history, and conservation. Yet, the applicability of these intriguing findings has not yet been widely examined for other species. In particular, seasonal interactions between the wintering, migration, and breeding periods have not been demonstrated for Palearctic-African passerine migrants. We tested whether variation in long- and short-term dietary choice of the Garden Warbler *Sylvia borin* can influence events during the subsequent migration season. The Garden Warbler is a small passerine bird (with a body mass of 15–17 g) that breeds throughout the Palearctic and winters in tropical and southern Africa, between approximately 10°N and 30°S (Moreau 1972; Klein et al. 1973; Cramp 1988). It has been one of the model species used in migration research in Europe and Africa, and its migration physiology has been well studied at wintering, migration, and breeding grounds across the migration routes (e.g., Gwinner 1987; Bairlein 1991, 2002; Biebach 1990; Bauchinger et al. 2005, 2007; Fusani et al. 2009; Goymann et al. 2010). In the northern latitudes, the species mainly breeds in boreal habitat with thick undergrowth. Its African wintering grounds comprise a wide range of habitats, such as thick bush, forest edges across rivers and swampy habitats, and around drier savanna areas (for details, see Cramp 1988). These variations in wintering, stopover, and breeding areas are linked to moisture gradients, and allow the use of stable carbon isotope ($\delta^{13}\text{C}$) analysis to infer habitat differences.

We used $\delta^{13}\text{C}$ analysis in two different tissues: pectoral muscle and bone collagen, which substantially differ in their rates of isotopic incorporation. The isotopic composition of pectoral muscles in small-bodied passerines reflects the diet of the bird over the last few weeks; for example, the mean retention time τ ranges between 19 and 24 days for 15 g Zebra Finches subjected to different

treatments (Bauchinger et al. 2010). In contrast, collagen has a much slower carbon turnover rate, reflecting the lifetime average dietary integration in adults, and is biased toward the growth period in juveniles (Hobson and Clark 1992; Hobson 1999). In a diet-switch experiment using Japanese Quail, *Coturnix japonica*, Hobson and Clark (1992) reported half-lives of 173.3 and 12.4 days for collagen and muscle, respectively. These half-life values, when transformed to the now more commonly used parameter of carbon retention time τ ($\tau = 1/k$, where k is the rate of isotopic incorporation, see Carleton et al. 2008 and Bauchinger and McWilliams 2009), result in values for the mean retention time τ of 18 days for flight muscles of quails and 250 days for collagen. Carleton et al. (2008) also reported a slower carbon incorporation rate in House Sparrow (*Passer domesticus*) collagen compared to its pectoral muscles, although only slightly slower. This result seems to be biased by the more positive $\delta^{13}\text{C}$ signature (ca. -18‰) found after 120 days on a wheat (-25.4‰) diet, and the fact that the birds were shifted to a corn diet at the time (-11.3‰). By implication, 120 days of incorporating the initial diet was not enough to fully incorporate the signature into collagen. This suggests a very slow carbon turnover for collagen compared to other tissues, as demonstrated by Hobson and Clark (1992). Unfortunately, the study does not report the $\delta^{13}\text{C}$ collagen before the birds were switched to the initial wheat diet.

By using two tissues with different $\delta^{13}\text{C}$ turnover rates, we investigated the relationship between an individual's dietary history and other body- and migration-related estimates of its condition: arrival date and physical condition at a stopover site during spring migration. Specifically, we tested two hypotheses:

- (1) By measuring the muscle $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{muscle}}$), we tested whether individual variation in arrival date and physical condition during spring migration can be explained by variation in events (e.g., diet) that took place in the immediate past (e.g., couple of weeks, Hobson and Clark 1992; Pearson et al. 2003; Bauchinger and McWilliams 2009; Bauchinger et al. 2010). We predicted that Garden Warblers who arrived early and in good condition would have a significantly different $\delta^{13}\text{C}_{\text{muscle}}$ from Garden Warblers arriving later. This would suggest that Garden Warblers arriving early presumably originate from different wintering areas or belong to a different population or age group.
- (2) Using stable isotope analysis of collagen ($\delta^{13}\text{C}_{\text{collagen}}$), we tested whether the current physical condition of an individual reflects its longer-term history (Hobson and Clark 1992). Similar to $\delta^{13}\text{C}_{\text{muscle}}$, we predicted that early-arriving Garden Warblers and those in better condition would have a significantly different $\delta^{13}\text{C}_{\text{collagen}}$

from Garden Warblers arriving later. Additionally, applying tissue-specific discrimination factors, we investigated the dietary $\delta^{13}\text{C}$ signature from which $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{muscle}}$ could have been synthesized.

Methods

Field work

Between 27 April and 30 May 2004, we captured 24 male migratory Garden Warblers with mist nets upon their arrival at the Crimean Peninsula in Ukraine ($45^{\circ}45'\text{N}$ $32^{\circ}56'\text{E}$), Europe. We measured body mass to the nearest 0.1 g using a Kern TEB 200-1-S3 balance, tarsus length to the nearest 0.05 mm using vernier calipers, and wing chord to the nearest 0.1 mm using a stopped wing ruler (Svensson 1992). The study site is not positioned in the breeding area of Garden Warblers; birds pass through it during their northward migration after having completed the flight across the Black Sea. Birds were decapitated, their body cavities were opened, and their carcasses were stored in 4% paraformaldehyde. The muscle mass of the right pectoral muscles (*Musculus pectoralis major*) was removed in the laboratory and dried at 75°C to constant mass. At dissection in the field, the tibiotarsus and the adhering tissue were removed from the carcass and stored in liquid nitrogen (-80°C) until final storage at -20°C in the laboratory. Birds were sacrificed under license as part of another research project on phenotypic flexibility of organ size and protein metabolism (Bauchinger et al., unpublished data). This study used the opportunity to collect samples from the sacrificed birds.

Stable isotope analyses

The preparation of collagen samples followed the general procedure described by Lee-Thorp and van der Merwe (1991) and Koch et al. (1995). Briefly, a sample of approximately 5 mg of collagen was taken, and its inorganic matrix and carbonates were removed by immersing the sample in a 1.0 M phosphoric acid solution for about 24 h and in an NaOH bath for about 6 h. Lipid was removed from each homogenized muscle sample using a 2:1 chloroform methanol solution for 72 h. Extracts of collagen were centrifuged and filtered every 24 h. Tissue samples were dried at 80°C for 24 h.

Cleaned and dried collagen (ca. 0.5 mg); and muscle (ca. 0.25 mg) subsamples were weighed into 0.3×0.5 mm tin capsules to the nearest 0.001 mg using a microanalytical balance. Samples were then combusted in a Eurovector (Milan, Italy) elemental analyzer. The resulting CO_2 gases

were separated by gas chromatography and admitted into the inlet of a Micromass (Manchester, UK) Isoprime isotope ratio mass spectrometer (IRMS) to determine $^{13}\text{C}/^{12}\text{C}$ ratios. The standard used for $\delta^{13}\text{C}$ was Vienna Pee Dee belemnite (PDB), and one egg albumen was used as a laboratory standard for every 11 unknowns in sequence. Each unknown was run in replicates. Hundreds of replicate assays of internal laboratory standards (albumen) and unknowns indicated measurement errors (SD) of $\pm 0.1\text{‰}$, 0.1 and $\pm 0.2\text{‰}$, respectively. Measurements are reported in δ -notation relative to the PDB standard in parts per thousand deviations (‰).

Data analyses

Tissue preservation methods that use formalin fixation systematically affect the stable isotope signatures of sample materials (e.g., Hobson et al. 1997; Sarakinos et al. 2002). We adjusted for this preservation effect in our results by using an average depletion factor of 1.78‰ in $\delta^{13}\text{C}$ (Hobson et al. 1997) prior to interpreting the data.

In small passerine birds, muscle and collagen integrate the diet over a period of few weeks and several months, respectively (Hobson 1999). The isotopic discrimination factors that were experimentally determined for Japanese Quail (*C. japonica*) muscle and collagen indicate that carbon discriminates from diet to tissues by $+0.11\text{‰}$ for muscles and $+2.7\text{‰}$ for collagen (Hobson and Clark 1992). Thus, muscle and collagen carbon isotopic results are not directly comparable.

Residual body mass during migration was estimated from a least square linear regression model of body mass and tarsus length ($F_{1,22} = 1.99$, $r^2 = 0.09$, $P = 0.17$, Fig. 1). We used residual body mass as a proxy for body condition during migration. A general linear model (GLM) was used to investigate the relationship between tissue stable-isotope values, body condition, and arrival date. Stable-isotope values from collagen and muscle were used as dependent variables. $\delta^{13}\text{C}_{\text{muscle}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ were not intercorrelated (Pearson correlation: $r = 0.32$, $P = 0.14$, $N = 22$). All

Fig. 1 Relationship between body mass and tarsus length in male migratory Garden Warblers sampled during their migration at the southern range of the breeding area ($r^2 = 0.09$, $P = 0.17$, $N = 23$)

variables were normally distributed (Kolmogorov Smirnov test $P > 0.05$). Differences between $\delta^{13}\text{C}_{\text{muscle}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ were also evaluated using Student's t tests.

Results

Tissue isotope signatures, residual mass, and arrival date

In our study, the Garden Warblers had a body mass (mean $\pm$ SE) of $17.9 \text{ g} \pm 0.36$. Overall, the GLM ($\delta^{13}\text{C}_{\text{collagen}}$: $F_{2,21} = 28.247$, $r^2 = 0.75$, $P < 0.001$; $\delta^{13}\text{C}_{\text{muscle}}$: $F_{2,21} = 7.7182$, $r^2 = 0.57$, $P = 0.004$) indicated that $\delta^{13}\text{C}_{\text{collagen}}$ is significantly associated with body condition during migration ($F_{1,21} = 32.971$, $P < 0.001$, Fig. 2). However, there was no relationship between $\delta^{13}\text{C}_{\text{muscle}}$ and body condition ($F_{1,21} = 0.278$, $P = 0.60$, Fig. 2). Garden warblers with better body conditions had lower $\delta^{13}\text{C}_{\text{collagen}}$ values. Birds with a lower $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{muscle}}$ arrived later at the study site (Fig. 3, $\delta^{13}\text{C}_{\text{collagen}}$: $F_{1,21} = 9.133$, $P = 0.007$; $\delta^{13}\text{C}_{\text{muscle}}$: $F_{1,21} = 15.078$, $P = 0.001$). Arrival date at the spring stopover site was not associated with body condition ($F_{1,22} = 1.88$, $r^2 = 0.08$, $P = 0.18$, Fig. 4).

$\delta^{13}\text{C}_{\text{collagen}}$ showed a wider range of values (between -18.9 and -25.5‰) than $\delta^{13}\text{C}_{\text{muscle}}$ (between -20.5 and -23.4‰ , Fig. 2; gray-shaded areas indicate ranges); however, the mean $\delta^{13}\text{C}$ values of the two tissues were not significantly different from each other (Student's t test, $t_{44} = 0.12$, $P = 0.90$).

Discussion

Garden Warblers that synthesized their collagen in habitats dominated by lower $\delta^{13}\text{C}$ signatures were in better body condition during migration than individual Garden Warblers with higher $\delta^{13}\text{C}_{\text{collagen}}$ (Fig. 2). A similar

Fig. 2 Collagen and muscle carbon isotope signatures in relation to residual body mass for Garden Warblers sampled during their spring migration. Residual body mass was used as a proxy for body condition. Collagen: $r^2 = 0.63$, $P < 0.001$, $N = 23$; muscle: $r^2 = 0.01$, $P = 0.50$, $N = 22$ (gray shaded areas indicate the isotopic ranges of collagen and muscle samples)

Fig. 3 Associations of arrival date with collagen and pectoral muscle carbon isotope signatures. Garden warblers with lower $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{muscle}}$ arrived later at the spring stopover site. $\delta^{13}\text{C}_{\text{collagen}}$: r^2 0.32, P 0.004, N 24, $\delta^{13}\text{C}_{\text{muscle}}$: r^2 0.44, P 0.001, N 22

Fig. 4 Wing chord and residual body mass for male Garden Warblers in relation to arrival time at a stopover site during migration. Wing chord r^2 0.18, P 0.04, N 24; residual body mass: r^2 0.08, P 0.18, N 23

relationship between pectoral muscle tissue and body condition during migration was not apparent. The much slower rate of isotopic incorporation into collagen tissue (many months) compared to the relatively fast rate of isotopic incorporation into muscle tissue (a few weeks, Hobson and Clark 1992; see also Brocherens 2005; Hobson 2005; Bauchinger and McWilliams 2009; Bauchinger et al. 2010), strongly advocates long-term effects on body condition due to habitat choice and/or dietary intake. We found no indication of short-term effects of habitat choice and/or dietary intake on body condition. In contrast, collagen isotope signature has a significant association with body condition

during migration prior to breeding. Such associations could possibly have fitness consequences during the subsequent breeding seasons through body condition during breeding and/or timely arrival at the breeding ground (e.g., Sandberg and Moore 1996; Silverin 1998; Kokko 1999).

Our findings are in agreement with a study that demonstrates a carryover effect of wintering habitat quality on migration period body condition based on claw isotopic values in of migrating Black-throated Blue Warblers *D. caerulescens* (Bearhop et al. 2004). Considering the isotopic signature in claw and the slow turnover in collagen, the observed $\delta^{13}\text{C}$ values presumably result from the different habitat types used by and the feeding events of the birds. Thus, the months spent in the wintering areas in Africa can be assumed to have the highest impact on the $\delta^{13}\text{C}_{\text{collagen}}$ values. Previous studies from the Nearctic-Neotropical migration system (e.g., Marra et al. 1998) show that later-arriving redstarts had tissue $\delta^{13}\text{C}$ values that were greater than earlier-arriving birds. We found a significant but opposite result for the Palearctic-African migratory Garden Warbler. Furthermore, our study is in agreement with the general hypothesis that there is a significant relationship between wintering habitat and spring arrival time in the breeding habitat based on muscle $\delta^{13}\text{C}$ values (Marra et al. 1998). These findings indicate that wintering habitat affects not only body condition during migration (Bearhop et al. 2004; this study) but also the timing of migration or arrival at the breeding grounds of Nearctic-Neotropical and Palearctic-African birds (Marra et al. 1998; this study).

Habitat

Typically, C_3 plants have lower $\delta^{13}\text{C}$ values than C_4 plants (Lajtha and Marshall 2007; Cerling et al. 1998). Due to the need for water-use efficiency, higher $\delta^{13}\text{C}$ values can also be found in C_3 plants of drier areas and xeric conditions (e.g., Ehrlinger and Cooper 1988; Marra et al. 1998). This suggests that birds in better condition might have a long-term feeding history in (and hence originate from) ecosystems dominated by C_4 habitats (see also Pearson and Lack 1992).

Diet

Garden Warblers are insectivorous during the breeding season and are considered to be primarily frugivorous during winter and at other times of their annual cycle (Berthold 1976; Simons and Bairlein 1990).

Few studies have evaluated diet frugivory in migratory birds using stable isotope analysis in the tropics (Herrera et al. 2003, 2005) and in experimental birds (Hobson and Bairlein 2003; Pearson et al. 2003; Podelsak et al. 2005). These studies indicate that the stable isotope technique can

be effectively applied at a relatively small scale and to a restricted range of habitats (e.g., Herrera et al. 2001a, b; Procházka et al. 2010). Yet, caution may be required while applying the isotope technique to track frugivory in migrating birds (Gagnon and Hobson 2009). It is very difficult to say whether the observed relationship between isotope values and migration condition or arrival time in Garden Warblers is due to birds switching diets between fruits and insects outside of the breeding season; variations in diet and habitat choice could be linked to the spatio-temporal distribution of individuals and the pattern of “migratory connectivity” the extent to which individuals breeding in one area also spend the winter together at a particular location (Salomonsen 1955; Webster et al. 2002). In such situations, birds caught during spring migration could originate from a single (or closely related) geographic location (this hypothesis, however, can not exclude the possibility that these birds depend on different feeding habitats). Alternatively, a breeding population in one area could consist of birds from a number of separate wintering locations (or vice versa). In such cases, birds caught during migration may comprise individuals that originate from geographically separated wintering areas. Spatial separation during migration might also be related to variation in population origin, age, or dominance (e.g., Lockwood et al. 1998; Grattarola et al. 1999; Bearhop et al. 2004).

Population origin

Although assumed to be in East and Southeastern Africa, the specific wintering ground of the Garden Warblers caught in Ukraine is not known. Our capture site in the Crimean Peninsula is not within the breeding area for Garden Warblers, but the exact natal sites of individual birds are not known. In a study on spring migration across the Mediterranean Sea, Grattarola et al. (1999) showed that migratory Garden Warblers originating from the east and north of Europe have longer wing lengths. Studies of other species indicate an increase in wing length and wing pointedness with increasing distance of migration and flight (Rayner 1988; Winkler and Leisler 1992; Lockwood et al. 1998).

The temporal variation in the wing chord measurements of our birds suggests that they might have originated from different breeding populations. We found a decline in wing chord with capture date ($F_{1,23} = 4.69$, $r^2 = 0.18$, $P = 0.04$, Fig. 4), indicating a high likelihood that they originated at different breeding grounds.

Age

First-year wintering Garden Warblers undergo a complete post-juvenile molt after arrival at African winter quarters,

and acquire plumage similar to that of adults (Cramp 1988). Adult birds also undergo a winter molt in Africa. As a consequence, it is difficult to detect and classify individuals into specific age groups using plumage and/or other morphological parameters during the field study period.

Collagen $\delta^{13}\text{C}$ in short-lived birds is likely to be biased towards the period of collagen growth in younger individuals (Hobson 2005). In an annual cycle, a Garden Warbler would spend approximately three months on the breeding grounds, 5–6 months on its wintering grounds, and three or more months migrating between breeding and nonbreeding locations (e.g., Bairlein 1997; Yohannes et al. 2009). Even if this species has spent most of its annual cycle in nonbreeding areas, the collagen $\delta^{13}\text{C}$ in the younger age class might have a $\delta^{13}\text{C}$ value that biases the collagen isotope values towards more of its natal or early developmental period signatures. This is mainly because tissues in these birds may hold a higher proportion of the natal-ground signature due to faster initial short-term isotopic incorporation from these areas during growth in the previous year, which is likely to be followed by slower equilibrium turnover after growth. This is shown, for instance, in young, captive, growing quail (Hobson and Clark 1992). Since older birds have spent most of their lives in an isotopically enriched environment compared to European breeding grounds, older birds should have relatively high $\delta^{13}\text{C}$ values. Currently, we still do not have a good measure of how metabolic rate changes, protein requirements, and switches in dietary source (e.g., omnivory during migration) during migration, molt, breeding, or other energetically demanding periods for migrating Garden Warblers influence these signatures in young and adult birds.

A final interpretation of the variation in tissue $\delta^{13}\text{C}$ between early- and late-arriving birds is that individuals originate from localities that vary in their relative proportions of C3 plants. Global carbon isotope maps exhibit a strong latitudinal decrease in vegetation $\delta^{13}\text{C}$, and continental terrestrial C3/C4 plant distributions in Africa exhibit a rapid gradient at approximately 5–15°N (Still et al. 2003). Mean $\delta^{13}\text{C}$ values of collagen (mean C:N = 3.8; range = 3.5–4.0) and pectoral muscles (mean C:N = 3.4; range = 3.2–3.6) in Garden Warblers were ca. −21.8‰ for each tissue.

Applying the diet tissue discrimination factor given by Hobson and Clark 1992, dietary values from which pectoral muscle and collagen stem would be ca. −22 and −25‰, respectively. Using the mean $\delta^{13}\text{C}$ values reported for C₃ (−27‰) and C₄ (−13‰) plants (Lajtha and Marshall 2007) as endpoints, this supports the absence of C₄-dominated carbon source incorporation from the food chain of the species. Higher $\delta^{13}\text{C}$ values can be due to C₃ plants of drier areas and xeric conditions, due to water-use efficiency (e.g., Ehleringer and Cooper 1988; Marra et al.

1998). The most parsimonious interpretation of our result is thus that birds might rely on drier habitats with relatively high $\delta^{13}\text{C}$ signatures. This has important implications for conservation. In recent years, local factors relating the rise in winter temperatures and decreased precipitation levels in sub-Saharan Africa have been widely discussed as factors that presumably affect migratory phenology and also link temperate breeding to tropical nonbreeding events (e.g., Cotton 2003; Sokolov and Kosarev 2003).

Tissue isotope signatures

The mean $\delta^{13}\text{C}$ values of the collagen (range: -18.9 to -25.5‰) and pectoral muscle (range: -20.5 to -23.4‰) samples did not differ from each other (Fig. 2).

Although similar isotopic signatures do not necessarily indicate the use of the same habitat or diet, the isotopic homogeneity of these tissues indicates that the birds tended to synthesize these tissues from habitats and/or diets that generated similar isotopic signatures. However, the wide range of $\delta^{13}\text{C}_{\text{collagen}}$ values shows that these tissues are synthesized from a relatively wide gradient of $\delta^{13}\text{C}$.

Since the tissue diet discrimination factors and the temporal periods of isotopic integration of the two tissues are different, these $\delta^{13}\text{C}$ values cannot be directly compared. Estimates of $\delta^{13}\text{C}$ discrimination factors between food and the collagen and muscles of birds (e.g., Japanese Quail) were about 2.7 and 1.1‰, respectively (Hobson 1999). The temporal periods of isotopic integration of these two tissues are different. The carbon half-life of bone collagen in growing Japanese Quail was 173 days (Hobson and Clark 1992). Flight muscles turnover more rapidly than bone collagen, and have half-lives of on the order of 12.5 for Japanese Quail and ~ 23.5 days for House Sparrow (Hobson and Clark 1992; Carleton et al. 2008). About half of the carbon in the flight muscles of Zebra Finch (*Taeniopygia guttata*)—a species which has a similar body size to our focal species, the Garden Warbler—turns over within two weeks (Bauchinger and McWilliams 2009; see also the erratum of Bauchinger and McWilliams 2010). Although data are missing for pectoral muscles, allometric scaling relationships between body size and turnover rates indicate that the fractional rate of $\delta^{13}\text{C}$ incorporation into blood cells, liver, and leg muscles is related to body mass with an exponent of approximately $-1/4$ (Carleton and Martínez del Río 2005; Bauchinger and McWilliams 2009).

If the estimates of the Zebra Finch tissue-specific turnover rates are generally applicable to the pectoral muscles of migrating Garden Warblers, then about half of the carbon in pectoral muscles from migrating Garden Warblers that have just arrived at the stopover site would have accumulated during the last 14 days of the migration period.

Residual body mass and arrival time

We found no strong association between body condition during migration and arrival time (Fig. 4). Contrary to our expectation, individuals with higher $\delta^{13}\text{C}_{\text{collagen}}$ and $\delta^{13}\text{C}_{\text{muscle}}$ values arrived earlier at the stopover site. There are at least two hypotheses that can explain this finding. Firstly, it is possible that high-quality individuals are able to sustain a lower body mass at arrival, and can quickly replenish their reserves. This is comparable to resident birds, where dominant individuals are lighter during winter than subdominants (e.g., Ekman and Lilliendahl 1993; Clark and Ekman 1995; Gosler 1996). Secondly, although we do not know the exact breeding location of each individual, it is possible that leaner, early-arriving individuals are closer to the end of their journeys, whereas birds in better condition may need to migrate further north. We found a decline in wing chord with capture date. If length of wing chord is associated with migratory distance, then short-winged birds might be closer to their breeding area (destination).

Conclusion

There is considerable interest in investigating the effect of seasonal interactions on life-history traits of migratory birds. Collagen has a slower turnover rate and its isotopic signature may represent the longer-term integration of several events and dietary sources. Stable isotope analyses of collagen have mainly been applied in archeological studies focusing on human and nonhuman faunal remains. Previous studies have applied collagen isotopic values to infer the diets, locations, and movements of living and extinct avian species (e.g., Hobson 1987; Hobson and Montevecchi 1991; Hobson 1993; Hobson et al. 1994; Braune et al. 2005). We used $\delta^{13}\text{C}$ in the collagen and muscles of migrating Garden Warblers to link habitat and diet use to condition and timing during migration. Our data showed that migrating birds with higher $\delta^{13}\text{C}_{\text{muscle}}$ and $\delta^{13}\text{C}_{\text{collagen}}$ values tended to arrive earlier than conspecifics with lower $\delta^{13}\text{C}$ values. Likewise, migrating birds with lower $\delta^{13}\text{C}_{\text{collagen}}$ values were in better condition. We believe that variations in condition may be a key factor governed by habitat choice, which in turn might be associated with geographic differences in population origin or age.

These hypotheses and speculations require future investigations of factors operating during the entire period of the annual cycle and across all geographic regions of the species distribution.

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