Philopatry of winter moult area in migratory Great Reed Warblers *Acrocephalus arundinaceus* demonstrated by stable isotope profiles

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Abstract Stable carbon- ($\delta^{13}\text{C}$), nitrogen- ($\delta^{15}\text{N}$) and hydrogen (δD) isotope profiles in feathers of migratory Great Reed Warblers *Acrocephalus arundinaceus* recaptured for 2 or more years in 6 successive years were examined to test whether the isotope profiles of individual warblers appeared to be consistent between years. Similar isotopic signatures in successive years suggested that individual birds tended to return and grow their feathers in Afro-tropical wintering habitats that generate similar $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and δD signatures. Previous studies have shown that Great Reed Warblers exhibit strong natal and breeding philopatry, with most of the surviving birds returning to the breeding site. The present study of feather $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and δD isotopic values demonstrate the year-to-year fidelity might also include the African moulting sites in this migratory species.

Keywords Stable-isotopes · Nitrogen-15 · Carbon-13 · Deuterium · Philopatry

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Introduction

Many migratory bird species are known to exhibit strong philopatry to their breeding and wintering grounds (e.g. Nisbet and Medway 1972; Baker 1978; Curry-Lindhal 1981; Bibby and Green 1981; Greenwood and Harvey 1982; King and Hutchinson 2001; Holmes and Sherry 1992). The evolutionary advantage of being faithful to breeding and/or non-breeding grounds includes the use of acquired experience and prior knowledge to rely on potentially good breeding or wintering habitat, nesting or roosting territory, foraging sites, best mate choice and predatory avoidance (Greenwood and Harvey 1982).

Although numerical data confirming these are rather scarce, it has been shown that some *Acrocephalus* species exhibit strong site fidelity and return to same area in Afro-tropical wintering grounds both between and within winters (e.g. Ash 1981; Hanmer 1986; King and Hutchinson 2001). A total of about 60 migrant species are reported to return to a general area in Africa in subsequent winters (Salewski et al. 2000). Earlier, using winter grown feather stable-isotope profiles, we showed site fidelity and habitat in nine migratory species moving through sub-Saharan Africa (Yohannes et al. 2007). However, no study (at least to our current knowledge) has confirmed habitat choice (trophic-level fidelity) and philopatry in individually marked migratory birds at Afro-tropical moulting grounds and northern latitude breeding sites between and within years using stable-isotope techniques.

To date, studies on site fidelity have largely depended on scattered observations and ringing recoveries. The low probability of ringing recoveries between regions visited by Palearctic-African migratory birds, challenge the functional use of the method on migration studies. Hence, several other approaches, such as measurement of tissue

stable-isotope values (reviewed by Hobson 2005), are being applied in animal ecology studies. The technique is based on the application of natural abundance and composition of stable-carbon, nitrogen and hydrogen isotope values in food webs and avian tissues to provide information on habitat and trophic level use, diet and geographical origin (e.g. Lajtha and Michener 1994; Hobson and Wassenar 1997; Kelly 2000).

We applied both stable-isotope and ringing recoveries approach on a breeding population of the Great Reed Warbler *Acrocephalus arundinaceus* in a study site in Sweden to study the extent of moulting area philopatry during non-breeding season. The Great Reed Warbler is a small long-distance migratory passerine that breeds in reed-filled water areas throughout the Palearctic (Cramp 1992). Ringing recoveries of birds ringed in our population indicate that our study population migrates to tropical West Africa; one ringing recovery is from the Ivory Coast in November and one from southern Tchad in December (Bensch 1993). However, whether this population is also crossing the equator in mid-winter to reach areas in Congo is possible but not known (De Roo and Dehegher 1969; Hedenström et al. 1993).

Using individually marked birds at a breeding ground, our main objective was to assess whether there exists habitat philopatry in the non-breeding season by comparing stable-isotope profiles in feather (grown in the non-breeding season) samples collected from birds that are captured and recaptured in six successive breeding years. In our study species, the timing of autumn migration towards the south differs between the age classes. Hence, adult birds might start migration, reach their wintering ground and commence moulting earlier than juveniles. The moult-schedule difference might affect the stable-isotope signatures. Age effect was tested using birds that were caught as yearlings and re-caught as an adult in subsequent year(s).

Methods

Sample collection

Between spring 1999 and 2004, we collected the second outermost tail feathers from most Great Reed Warblers breeding at Lake Kvismaren (59°10'N 15°25'E), located in south central Sweden. Details about the study population and the field methods can be found in Bensch et al. (1998). Within the 6 consecutive study years, a total of 65 feather samples were collected and analysed from 29 individual birds that were re-caught for at least ≥ 2 years.

Briefly, in spring 2000, we re-captured seven individuals which were first caught in 1999 and feathers samples were

taken; and two other individual birds were re-caught in 2001 and further feather samples were collected. In 2001, we also re-caught and collected feather samples from one other bird that was first caught in 2000. In 2002, we re-captured a total of ten birds, which were first caught in 1999 ($n = 4$), 2000 ($n = 2$) and 2001 ($n = 4$). One of these individual birds was repeatedly caught three times: first in 1999 and then in 2001 and 2002. In spring 2003, we caught seven individuals that were first caught in previous years (among which one bird was also caught in 1999) and one other bird which was first caught in 1999 and another in 2000. In 2004, we caught three birds from the year 2003 and one bird from 2002. A single individual bird was controlled and feather samples were collected for the three consecutive years between 1999 and 2001. Two other birds were also monitored for three consecutive years between 2002 and 2004.

Stable-isotope measurements

Carbon and nitrogen

For the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements, cleaned sub-samples of approximately 0.5 mg of feather were weighed into small tin cups and combusted in a Eurovector (Milan, Italy) elemental analyzer. The resulting N_2 and CO_2 gases were separated by gas chromatography and admitted into the inlet of a Micromass Isoprime isotope ratio mass spectrometer (Manchester, UK) for determination of $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios. Measurements are reported in δ -notation relative to the PDB for carbon and atmospheric N_2 standard in parts per thousand deviations (‰). Typical precision of analyses was $\pm 0.5\%$ for $\delta^{15}\text{N}$ and $\pm 0.2\%$ for $\delta^{13}\text{C}$. The standard for $\delta^{15}\text{N}$ is atmospheric nitrogen and Peedee belemnite for $\delta^{13}\text{C}$. Egg albumin was used as daily reference material.

Deuterium

Deuterium measurements on feathers and standards were performed on H_2 derived from high-temperature flash pyrolysis of feathers and Elemental Analyser-Isotope Ratio Mass Spectrometry calibrated against standard reference material and quality control checks. Briefly, cleaned and dried feather samples were filled into silver capsules which were left open for a period of not less than 4 days to allow the exchangeable hydrogen in the sample chitin to fully equilibrate with the moisture in the laboratory air. In addition, we analysed multiple samples of BWB-II (whale baleen), with a known non-exchangeable $\delta 2\text{HV-SMOW}$ value, and our eggshell membrane standard, independently

measured by Len Wassenaar, NWRI, Saskatoon, Canada. Multiple keratin replicate standards, whose non-exchangeable δD values are known, were used for correcting uncontrolled isotopic exchange between samples and ambient water vapour (Wassenaar and Hobson 2000). Thus, values reported here are equivalent to non-exchangeable feather hydrogen (Wassenaar and Hobson 2003). Deuterium analyses of feathers were undertaken at the Iso-analytical laboratory, England.

Data analysis

Within-individual repeatability analyses were calculated according to Lessells and Boag (1987). Paired t -test was used to assess age-effect (first calendar year vs adults) on feather stable isotope signatures.

Results

Feather stable isotopes

There was a significant repeatability (Fig. 1) in feather $\delta^{13}C$ ($R = 0.61$, $F_{28,36} = 4.52$, $P < 0.001$) and $\delta^{15}N$ ($R = 0.33$, $F_{28,36} = 2.08$, $P = 0.02$), and δD values ($R = 0.28$, $F_{28,36} = 1.88$, $P = 0.04$). There was no significant age-related effect on the three elements analysed (paired t -test; $\delta^{13}C$: $t_9 = 0.72$, $P = 0.49$; $\delta^{15}N$: $t_9 = 0.23$, $P = 0.79$; δD : $t_9 = 0.25$, $P = 0.81$).

Discussion

The main finding of this analysis is that the 65 feather samples collected from 29 individuals in 6 different moulting years showed significant repeatability for feather $\delta^{13}C$, $\delta^{15}N$ and δD values. The strong between-year repeatability in feather isotope values of the Great Reed Warbler generally indicates that individual birds moult in similar habitats and depend on similar diets through the moulting period in different years. Although the observed isotopic homogeneity for feathers from the same individual does not guarantee that birds return to the exactly the same geographic spot in successive years, it clearly indicates that they selected habitats that generated similar isotope values within the potential moulting range.

We found no difference between yearlings and older birds in feather isotopic composition. Although we have a restricted data from first calendar year birds ($n = 9$), the mean $\delta^{13}C$, $\delta^{15}N$ and δD feather values were relatively consistent among age-groups. In addition to the spatial similarity of the moulting ground, these results imply that

Fig. 1 Winter grown feather $\delta^{13}C$, $\delta^{15}N$ and δD values in 29 individual Great Reed Warblers *Acrocephalus arundinaceus*. Within the 6 consecutive study years, a total of 65 feather samples were collected (1999 = 12, 2000 = 9, 2001 = 9, 2002 = 16, 2003 = 13 and 2004 = 6). Solid and dashed lines connect individual data points with consecutive and non-consecutive years, respectively

age-related moult schedule did not affect the isotope signatures, at least for the nine individuals monitored here.

Ecosystems with C3 and C4 plant photosynthetic pathways produce different $\delta^{13}C$ signatures (Smith and Epstein 1971; Koch et al. 1995). Feather $\delta^{13}C$ values of Great Reed Warbler reflect utilization of C4 biome during moult. Stable isotope analyses of feathers from three adult Great Reed Warblers collected at breeding grounds in Portugal (Neto et al. 2006) showed a higher $\delta^{13}C$ values (ca. + 6‰), but a similar $\delta^{15}N$ values (mean $\pm$ SE: 10.3 ± 0.74) than

the birds in our study. Though the Portuguese sample size is small, it suggests that these birds moult in an even more C4 extreme dependent biome than the birds from Sweden.

Consumer tissue stable-nitrogen isotopes ($\delta^{15}\text{N}$) are mainly used to infer food web trophic levels (e.g. Minagawa and Wada 1984; Kelly 2000). The significant repeatability $\delta^{15}\text{N}$ between years and age groups would suggest a similar diet between winter moult seasons (Hobson 1999; Kelly 2000).

In North America, δD values of feathers closely reflect that of the growing season average precipitation of the locations where the feathers were grown (Chamberlain et al. 2000; Hobson and Wassenaar 1997; but see also Langin et al. 2007). Based on this, and our results that showed a significant repeatability in feather δD , the birds are predicted to have returned to the same geographic location for moulting.

As shown by Hansson et al. (2002), the species is characterized by a high level of philopatry to natal and breeding sites. In a previous study comparing philopatric and immigrant Great Reed Warblers in Sweden, we have observed that the lifetime fitness of philopatric birds was higher than for immigrants (Bensch et al. 1998). Philopatry is generally believed to help maintain the adaptation of a population to a very specific environment (e.g. Clobert et al. 1988; Pärt 1994). Provided that one advantage of philopatry is based on acquired experience for better feeding sites and avoidance of predators, Great Reed Warblers that depend on similar winter habitat may benefit from that. However, our results do not exclude the alternatively hypothesis that high philopatry to wintering habitat could partly be due to the localised and uneven distribution of the preferred wintering habitats in Africa. This passerine bird mainly depends on large reed beds, often with some bushes around. These specific and preferred habitats are limited in their occurrence and distribution in Africa; the birds might show obligatory philopatry and a shift in habitat as a prevailing strategy to avoid unsuitable habitats.

Conclusion

The stable $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and δD isotope profiles in feathers of 29 Great Reed Warblers that were monitored for ≥ 2 years in 6 study years were consistent in their repeatability between years. In general, our results show some degree of year-to-year philopatry in selecting a moulting habitat that generated a similar $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and δD values outside the breeding season. Our use of stable isotope measurements on multiple elements, coupled with earlier studies using ringing recoveries and capture–recapture studies (e.g. Hansson et al. 2002), provides a strong basis to generally conclude

that the species shows a strong tendency to a year-round philopatry in both breeding and non-breeding seasons. Although with a smaller sample size, in a study of Willow Warbler *Phylloscopus trochilus* feathers presumably moulted in Africa and collected in Sweden in successive years (Bensch et al. 2006), there were high and significant repeatabilities for both $\delta^{13}\text{C}$ (0.71) and $\delta^{15}\text{N}$ (0.77).

Zusammenfassung

Untersuchung stabiler Isotope deutet auf Ortstreue von Wintermausergebieten beim Drosselrohrsänger *Acrocephalus arundinaceus*

Mit der Methode stabiler Isotope in Federn wurde bei Drosselrohrsängern, die innerhalb von sechs Jahren zweimal oder mehrfach kontrolliert wurden, untersucht, ob die Isotopenprofile der einzelnen Individuen konstant waren. Die Ähnlichkeit der Isotopensignaturen einzelner Individuen in folgenden Jahren legt nahe, dass sie regelmäßig zur Gefiedermauser zu den gleichen Winterbiotopen mit ähnlichen $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ und δD zurückkehren. Bisherige Studien am Drosselrohrsänger haben ausgeprägte Geburts- und Brutortstreue der zurückkehrenden Vögel gezeigt. Diese Studie unterstreicht die mögliche Bedeutung bestimmter afrikanischer Mausergebiete für diese Zugvogelart.

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