Scopus 32: 1–9, June 2013

Stable isotopes of soil collected from feet of two species of migratory *Acrocephalus* give clues to stopover sites

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Summary

Soil samples were collected from the feet of Marsh Warblers *Acrocephalus palustris* and Reed Warblers *Acrocephalus scirpaceus* caught soon after crossing the Sudan Red Sea coast of Africa. We measured carbon ($\delta^{13}C_{soil\_feet}$) and deuterium (δD_{soil_feet}) isotope ratios in these soils with the objective of identifying possible take-off sites of these birds. We collected soils from three sites in the Caucasus region, a potential refueling area for the flight to Sudan, and from the Sudan Red sea coast, and compared their deuterium (δD_{soil}) with δD_{soil_feet}. There was a strong relationship between the arrival date of the birds and the isotope signatures ($\delta^{13}C_{soil\_feet}$ and δD_{soil_feet}) of the soil they carried. Results suggest that warblers from different geographical regions or of different age groups might use different staging sites before reaching the Sudan. Data for precipitation deuterium (δD_{prec}) together with δD_{soil} and δD_{soil_feet} suggest that while early arriving birds had taken off from southeast Europe (picking up soil from this region), those arriving later had stopped off in Arabia. This indicates an intrinsic difference in strategy between birds migrating at different times of the season. The isotopic compositions of biological tissues such as feathers have commonly been applied to track animal movement. But this is the first report of analysis of soil from birds' feet: a novel approach to isotopic study based on material picked up and carried by an animal externally.

Introduction

The Marsh Warbler *Acrocephalus palustris* breeds across the temperate Western Palaearctic and migrates through the Middle East and Arabia to reach southern Africa (Cramp 1992). It enters Africa across the Red Sea and is one of the most common migrants on the Sudanese coast in August and September (Nikolaus 1983, Nikolaus unpubl. data). Its main route to southern Africa passes through central and southeast Kenya (Dowsett-Lemaire & Dowsett 1987). It is one of a number of passerine species whose overall southward autumn migration is known to occur in "two stages". Birds migrate rapidly to northeast Africa, then interrupt their journey from two to three months in a stopover area before continuing to the southern Africa wintering grounds (see Pearson *et al.* 1988, Pearson 1990).

Earlier, we applied feather stable isotope studies to identify the potential stopover area of Marsh Warblers in northeastern Africa (Yohannes *et al.* 2005, Yohannes *et al.* 2007). These studies were based on the rationale that the stable isotope ratios of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and non-exchangeable hydrogen (δD) become fixed in a feather during moult and should indicate the isotope composition of the area in which it grew. They were concerned specifically with the stopover area used during the partial moult in northeastern Africa.

During the autumns of 1981 to 1984 many migratory birds were caught and ringed on the Sudan Red Sea coast (Nikolaus & Pearson 1982, Nikolaus 1983), including almost 10 000 Marsh Warblers. Among these a number of individuals were found to be carrying mud/soil on their toes and feet. Samples of these soils were collected from 21 individuals (11 in 1983 and 10 in 1984), and also from two Reed Warblers *Acrocephalus scirpaceus* (in 1983). These birds were caught soon after crossing the Red Sea coast. Thus, it is likely that the soil had been carried from a take-off site used well before entering Africa, holding an isotope signal of this site.

We determined stable carbon ($\delta^{13}C_{soil\_feet}$) and deuterium (δD_{soil_feet}) isotope ratios for these soil samples. Values would be expected to reflect the elemental composition of the local environment from which they were picked up. Plants assimilate CO_2 from the atmosphere into their tissues through different photosynthesis systems. Subsequent microbial decomposition of organic compounds in plant detritus incorporates the fixed carbon into the soil organic matter (SOM; Ehleringer 2000, Garten *et al.* 2000, Powers & Schlesinger 2002). There is a strong correlation between ^{13}C to ^{12}C ratios (expressed as $\delta^{13}C$) in plant communities and the $\delta^{13}C$ in the SOM (Balesdent *et al.* 1993). The relative carbon isotope ratios of the SOM are commonly preserved for several years (Boutton 1996). The carbon isotope ratios can then be used to evaluate the composition of the plant species and the isotopic signature of a particular site, such as a stopover site along an avian migration route.

Methods

Study site and fieldwork

During August and September 1983 and 1984 migratory Marsh Warblers were caught for ringing at a transit site on the Sudan Red Sea coast, Khor Arba'at (19°48' N, 37°03' E, 100 m; Fig. 1). The site is located about 15 km inland, immediately east of the Red Sea Hills. Except for a few bushes scattered along the Khor, (a small seasonal stream), the Arba'at region is generally characterized by harsh terrain and a highly variable rainfall with recurrent drought spells (Osman-Elasha *et al.* 2006). Vegetation in the surrounding desert is rather scarce. A small area of cultivated land with lemon, guava, and date palm trees provided the only green vegetation for many kilometres around. This "oasis-like" watered garden site appeared to be the first potential refuge for migratory birds that had crossed the Saudi Arabian desert and Red Sea. For more details of ringing here see Nikolaus (1983).

Migrants were caught during the morning, shortly after their arrival, with mist-nets located in the garden. Soil samples from the feet of 21 Marsh Warblers and two Reed Warblers were stored in a cool dry place until analysed. In August 2004, we returned to Khor Arba'at to collect soil samples from the upper 10 cm of the site.

From empirical data on the speed of migration (Yohannes *et al.* 2009a) and body mass (Yohannes *et al.* 2009b) of Marsh Warblers along the migratory path, we anticipated that the last potential refueling area before reaching the Sudan Red Sea coast could be in the Caucasus region of southeast Europe. It had indeed been noted that according to the weather maps of the time the arrival of mud-carrying birds was usually preceded some three days earlier by heavy thunderstorms in the Caucasus. Therefore, in May 2008 we collected soils (upper 10 cm) from three different sites in Azerbaijan (Fig. 1): Candy Cane Mountains, a semi-desert landscape near Alti Agac

(40°51′36″ N, 48°55′48″E); Besh Barmag, located in Khizi Rayon on the Caspian Sea shore (40°55′52″N, 49°14′ 8″E); and Shirvan National Park (39°32′51″N, 49°00′56″E). We measured the deuterium (δD soil) isotope ratio in these soils.

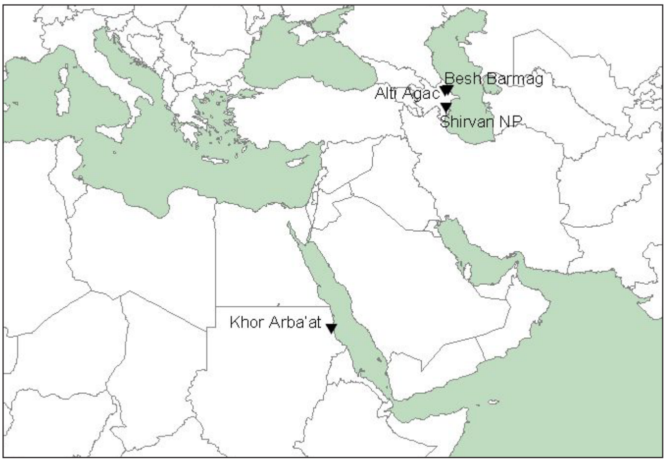

Figure 1. Study sites in the Caucasus and on the Sudan Red Sea coast.

Stable isotope analysis

Soil samples from the birds' feet were well mixed and ground on a roller mill to a fine powder and then dried at 550° C for 24 h. A sub-sample of approximately 5 mg was weighed into a capsule for determination of natural abundance $\delta^{13}C$ and non-exchangeable δD. We analysed soil samples from all 21 Marsh Warblers and the 2 Reed Warblers to determine their $\delta^{13}C$ values. We also analysed soil from 11 Marsh Warblers and one Reed Warbler for their δD values.

Stable isotope measurements are expressed in δ notation using the equation:

$$\delta X = [(Rsample/Rstandard\ ) - 1] * 1000$$

where R is the corresponding isotope ratio X ($^{13}C/^{12}C$ or D/H) of the sample and standard.

Carbon and Deuterium

For carbon isotope analysis, soil samples (*c.* 0.5 mg) were weighed into 0.3 × 0.5 mm tin capsules to the nearest 0.001 mg, using a micro-analytical balance and then combusted in a Eurovector (Milan, Italy) elemental analyser. The resulting CO_2 was separated by gas chromatography and admitted into the inlet of a Micromass (Manchester, UK) Isoprime isotope ratio mass spectrometer (IRMS) for determination of $^{13}C/^{12}C$. These measurements are reported in δ ($\delta^{13}C$) relative to an international standard Pee Dee Belemnite (PDB).

For deuterium, soil samples (*c.* 0.35 mg) were placed on silver capsules and left open for a period of 3 days to allow sample exchangeable hydrogen to equilibrate with the moisture in the laboratory air. Deuterium (δD) measurements were performed by applying high-temperature pyrolysis using the same elemental analyser interfaced to an IRMS as described above, calibrated against standardized keratin and hydrocarbon reference materials. Further technical details of carbon and deuterium isotope analysis are given in Yohannes *et al.* (2011).

Regional precipitation δD

We checked whether our δD measurements from soils collected from the Caucasus and Sudan corresponded with published annual δD values in precipitation for these areas. To do so, we consulted precipitation δD values from the GNIPS/ISOHIS database (IAEA 2001) and isocline interpolated maps developed by Bowen *et al.* (2005) for Azerbajan and north Sudan.

Data analysis

Differences in $\delta^{13}C_{soil\_feet}$ and δD_{soil_feet} between the two years' samples (1983 & 1984) were tested using Student's t-tests. Mean values of $\delta^{13}C_{soil\_feet}$ for the two years (including the Reed Warbler data) differ significantly (Student's t-test: $t_{21} = -2.58$, $p = 0.02$). Those of δD_{soil_feet} for the two years were different, but the difference was not statistically significant (Student's t-test: $t_{10} = -2.05$, $p = 0.07$). In subsequent analysis, we therefore pooled the δD_{soil_feet} data from the two years. The relationship between capture date (arrival date) and soil isotopic values ($\delta^{13}C_{soil\_feet}$ or δD_{soil_feet}) was explored using Pearson correlation coefficients and bivariate scatter plots. Stable isotope variables of soils from birds' feet were normally distributed (Kolmogorov-Smirnov test, $p > 0.05$).

Results

Carbon

The $\delta^{13}C_{soil\_feet}$ values for all samples taken from Acrocephalus warblers are shown as a scatter plot in Fig. 2. These ranged from *c.* -24 ‰ to *c.* -11 ‰, but increased significantly with capture date (1983: r = -0.64, $p = 0.02$, n = 13, and in 1984: r = -0.74, $p = 0.01$, n = 10). Analysis of 23 samples from 8 different days shows that values were significantly higher later in the season. Soils collected on 18 September 1983 are isotopically distinct (Mean $\delta^{13}C_{soil\_feet} \pm$ SE, -13.3 ‰ ± 0.86, n = 4) from those collected earlier that season (from both *Acrocephalus* species) between 22 August and 13 September (Mean $\delta^{13}C_{soil\_feet} \pm$ SE, -21.8 ‰ ± 0.66, n = 11). In 1984, soils collected between 7 and 26 September showed the higher $\delta^{13}C$ values (Mean $\delta^{13}C_{soil\_feet} \pm$ SE, -13.8 ‰ ± 0.48, n = 8) whereas the almost identical value from two birds on 27 August was relatively low. In 1983, soils from the two Reed Warblers in mid-September gave similar values ($\delta^{13}C_{soil\_feet} = c.$ -19.0 ‰) to those collected from Marsh Warblers at the same time, but higher than the samples from August.

Deuterium

δD_{soil_feet} values were lower in earlier arriving birds and became more enriched (higher) with later capture date (r = 0.64, $p = 0.03$, n = 12), Fig. 3. They showed a large variation, ranging from *c.* -80 ‰ to -46 ‰. Overall, the mean value for δD_{soil_feet} for the Marsh Warblers was -62.2 ‰, the result for the one Reed Warbler -73.7 ‰, Fig. 3. The corresponding mean δD_{soil} values for samples from the Caucasus and from Sudan were -65.2 ‰ and -20.3 ‰, respectively. Within the soil samples from birds' feet there was a strong correlation between δD and $\delta^{13}C$ (r = 0.90, $p < 0.001$, n = 12), Fig. 4.

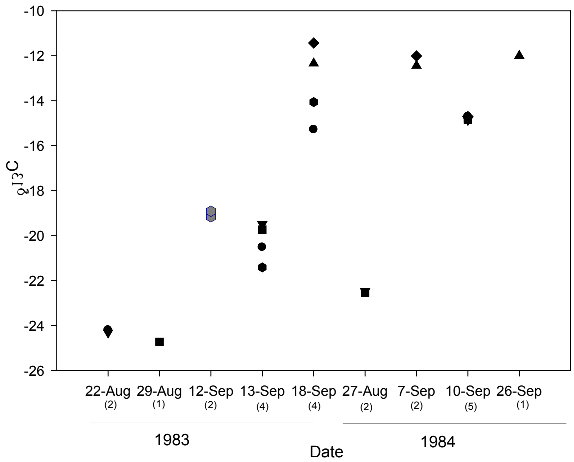

Figure 2. Carbon stable isotope ($\delta^{13}C_{soil\_feet}$) values of soils collected from 21 Marsh Warblers and 2 Reed Warblers (grey dots).

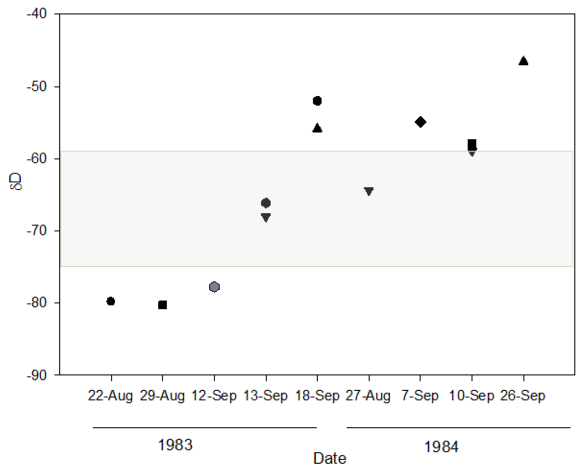

Figure 3. Hydrogen stable isotope (δD_{soil_feet}) values of soils collected from 11 Marsh Warblers and 1 Reed Warbler (grey dot) shown against capture date. The number of samples on each date is given in brackets.

The shaded region indicates the δD range of soils collected in Azerbaijan. NB: due to the limited amounts collected not all soil samples could be analysed for both elements.

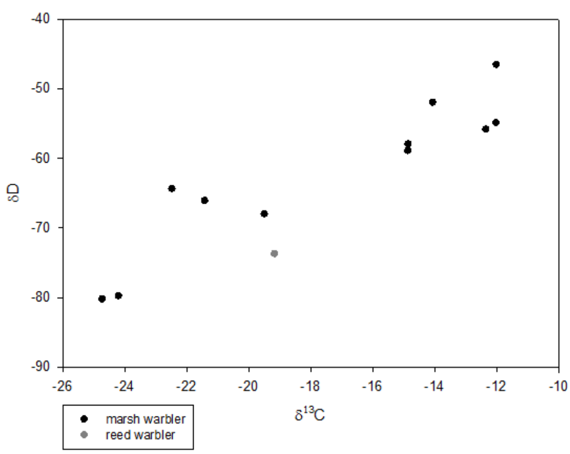

Figure 4. Relationship between soil carbon ($\delta^{13}C_{soil\_feet}$) and deuterium (δD_{soil_feet}) stable isotope values for samples collected from 11 Marsh Warblers and 1 Reed Warbler (grey dot).

Discussion

The $\delta^{13}C$ values of the soils collected from feet in August and early September are indicative of an ecosystem dominated by C3 plants, shrubs and grasses (mean approximately -27 ‰; Lajtha & Michener 2007). But the higher $\delta^{13}C$ values for the birds caught later in 1983 and all those caught in September 1984 suggest that these had stopped off in areas (Arabian Peninsula) with a more arid climate and/or a higher ratio of C4 to C3 plants. C4 plants have higher $\delta^{13}C$ values (mean approximately -13 ‰) than C3 plants (Smith & Epstein 1971). In addition, C3 plants have higher $\delta^{13}C$ values in arid areas (Ehleringer & Cooper 1988).

Global precipitation δD maps (Bowen *et al.* 2005) and predictions from the GNIPS/ISOHIS database (IAEA 2001) indicate expected δDprec values from the Caucasus region within the range -112 ‰ to -32 ‰. At sites on the Sudan Coast δD_{prec} ranges from approximately -31.0‰ to 32.0‰, and based on the maps of Bowen *et al.* (2005), values for the Red Sea coast overlap with those of the Arabian Pennisula. After controlling for fractionation of non-exchangeable hydrogen (Wassenaar & Hobson 2003), δD_{soil} values corresponding to these δD_{prec} data would be expected to range from -72.5 ‰ to -49.2 ‰ for soil from the Caucasus, and -53.95 ‰ to -12.3 ‰ for soil from Sudan. Our own δD soil measurements from Azerbaijan (mean -65.2) and Sudan (mean -20.3) are thus consistent with regional isotopic maps, and fall within the range predicted by the GNIPS/ISOHIS δD_{prec} database and the annual range given for these areas by Bowen *et al.* 2005. The δD_{soil_feet} of samples from the Marsh Warblers (Fig. 3) ranged from -80‰ (in August) to -42.2 ‰ (in September).

These δD results again suggest that Marsh Warblers staged at different areas along the migration route to Sudan at different times. While early arriving birds had probably used a site in southeast Europe later birds had probably stopped in the Arabian region. High seasonality and dynamic yearly and monthly values (in addition to the lack of standardized measurements from several sites) constrain our ability to use δD_{prec} to locate these staging sites more precisely. But although they lack the resolution required for accurate interpretation, values are based on ultimate isotopic data available for the Caucasus and Africa. Moreover, our determinations of δD_{soil_feet} and $\delta^{13}C_{soil\_feet}$ point to a similar conclusion regarding the birds' staging strategy. The similar values found for Marsh Warblers and the two Reed Warblers suggest that these species might have shared a common stopover area in mid-September 1983.

Observations and ringing recoveries have indicated that the entire Eurasian breeding population of the Marsh Warbler migrates through an Arabian route into Africa to reach a wintering range covering most of southeastern Africa (Dowsett-Lemaire & Dowsett 1987, Cramp 1992). But the different isotopic signatures found here presumably reflect a difference in strategy between birds crossing Arabia at different stages of the season. This might involve different breeding populations, and it is not known whether birds from eastern and western parts of the range reach Sudan at the same time. It might alternatively involve different age groups, for adults are known to pass through Khor Aba'at 7–10 days earlier on average than juveniles (Nikolaus unpubl. data). Eight of the birds caught between 27 August and 10 September 1984 were first year birds. The samples from 22 August 1983 and from late September 1984 were both from adult birds. Unfortunately, we do not have the ages of the other 1983 birds.

The difference found here between the earlier and later caught birds suggests two groups staging in isotopically distinct areas. But without regional isotopic soil maps from stations along the migration route, it is impossible to know exactly where these locations are. The ringing of Marsh Warblers in Africa, mostly at Ngulia, Kenya, has produced about 30 recoveries of birds on passage during August and September. One staging area in Saudi Arabia is indicated by no less than eleven recoveries of Ngulia ringed birds in the vicinity of Buraydah, at 26–27°N, 44–45°E, dated 15 August–1 September (G. Backhurst & D. Pearson unpubl. data). But this might of course just reflect a local concentration of hunting activity, and this area lies to the east of the likely route of birds heading for the Sudan coast.

Recent techniques, such as light-level geo-location (e.g., Bairlein *et al.* 2012) and satellite tracking will be of immense assistance in revealing migratory pathways. But these tend to be expensive, which seriously restricts their application to continent wide studies. Chemical signatures such as stable isotopes present an alternative approach for tracking migrating animals. This has recently been applied to several migrant birds (Hobson 2003, 2011) including Marsh Warblers (Yohannes *et al.* 2005, 2007). It is usually the isotopic composition of tissues such as feather, blood or claw which is determined. The investigation reported here is based on material picked up and carried by the birds externally. This appears to be the first time that isotopic analysis of soil from birds' feet has been used to study migration. It suggests a new approach for tracking routes and staging areas, particularly applicable to birds that feed and walk on wetland areas, or those that feed on pollen and plant fruits. During capture and handling it may prove useful to collect for analysis samples of soil adhering to feet, fresh pollen or plant materials around the bill, or defecated insect or nectar remains. Such materials can be easily recognizable in quantities sufficient for isotopic investigation.

Acknowledgements

This study applied information based on data collected from several study sites and years. We acknowledge all those involved and thank all authorities and persons who helped us during field work. Financial support was provided by the Max-Planck Research Centre for Ornithology, Andechs. We thank Profs. Ellen Thaler, Herbert Biebach and the late Eberhard Gwinner for their support during the study.

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Scopus 32: 1–9, June 2013
Received 8 November 2012