Sr isotope analysis of bird feathers by TIMS: a tool to trace bird migration paths and breeding sites

Laura Font,*[ab] Geoff M. Nowell,[a] D. Graham Pearson,[a] Chris J. Ottley[a] and Stephen G. Willis[b]

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Here we present a methodology to analyse $^{87}Sr/^{86}Sr$ isotope ratios in bird feathers with very low Sr concentration using ultra-low blank ion-exchange chemistry combined with thermal ionisation mass spectrometry. For this study, Sedge Warbler (*Acrocephalus schoenobaenus*) feathers were used from four different locations within Europe. Prior to analyses, dust particles from the feathers' surface were removed with nitrogen gas. The shaft and the vane parts of the feather were analysed separately. Generally, the vane had higher trace element abundances compared to the shaft. The vane contained between 3 ng and 12 ng of Sr and the shaft between 0.5 ng and 3 ng of Sr. Due to the small amount of Sr in the feathers, small loads (0.5–12 ng Sr) of international standard NBS 987 were analysed for $^{87}Sr/^{86}Sr$ isotope ratios giving an average of 0.710263 ± 0.000013 (2σ) ($n = 177$) and an external reproducibility below 0.002%. The average ^{88}Sr beam intensities for all the shaft analyses were 0.79 V while for the vane analyses it was 2.7 V, consistent with the measured Sr contents of the feather shafts and vanes. The $^{87}Sr/^{86}Sr$ isotope ratios of the vane were more precise than the shaft with 2 SD internal precision of 0.0026% and 0.053%, respectively. However, the precision was adequate for resolving Sr isotope variations between localities. The $^{87}Sr/^{86}Sr$ isotope ratios of the cleaned Sedge Warbler feathers varied geographically and were indicative of the different geology in the locations where the feathers were grown.

1. Introduction

Studies to determine the routes of migrant bird species have been undertaken using a variety of techniques to date, usually involving marking individuals with unique metal leg-rings, radio or satellite tags or simply by censusing at migratory stop-over or bottleneck areas. These methods have the disadvantage that they generate relatively little data for the amount of effort required or, in the case of censusing, that they cannot determine the origin of individuals. Such information is vital to effectively conserve migratory species, yet even today the migratory routes and wintering grounds of most European migrant birds are poorly understood, despite Europe having some of the most intense and longest-running studies of migration. In addition, with the dissemination of vector-borne diseases such as avian bird flu and with threats such as habitat destruction and global climate change increasing, there is an increased necessity to determine the origin and migratory paths of birds. Although trace element geochemistry has been applied to these problems,[1] this approach has limitations in discriminatory power and there are complexities due to physiological fractionation of certain "essential" trace elements over others. For these reasons it is usually better to employ an isotopic tracer system that has good discriminatory properties and is unlikely to be subject to isotopic fractionation through the ecosystem involved. Analyses using C, N, H and O isotopes have been shown to allow broad discrimination of latitude and oceanicity gradients but are of limited use in further refining localities of samples.[2–4] The long-lived β-decay of ^{87}Rb to ^{87}Sr has been successfully used by the geological community as a chronometer and a tracer in Earth systems,[5] and the degree of fractionation of Rb from Sr can show pronounced differences between rock types. This means that the system has the ability to differentiate between different lithologies and between rocks of different ages and so may be a useful tracer of the breeding and residential sites of animals.[6]

Sr isotope variation, measured as $^{87}Sr/^{86}Sr$ has already been documented in parts of birds that naturally contain high Sr concentrations, *i.e.*, bone, claws and egg shells[7,8] and these studies have concluded that the measured $^{87}Sr/^{86}Sr$ isotope ratios reflect those of their natural environment.[4,7,8] The Sr taken up by the birds through their diet does not appear to fractionate through the food chain. From a geological perspective, the main caution that needs to be applied, in areas of complex silicate bedrock, is the possible difference between bedrock $^{87}Sr/^{86}Sr$ and the likely bioavailable Sr in soils derived from weathering of this material. This is because some rocks undergo incongruent weathering reactions that may preferentially release radiogenic Sr into the soil system.[9] In such situations there will not be a direct correspondence between the Sr isotope composition of the bedrock and the composition of bioavailable Sr.

[a] *Northern Centre for Isotopic and Elemental Tracing, Department of Earth Sciences, University of Durham, UK DH1 3LE*
[b] *School of Biological and Biomedical Sciences, University of Durham, UK DH1 3LE*

Previous studies of Sr isotope variations in adult birds show that bones integrate the Sr isotope signature of the wintering and breeding areas,[7] which can be difficult to correlate with a particular location. Feathers offer several advantages over bone and claw analyses. Firstly, obtaining feather samples is much less invasive; loose feathers can be collected from nesting sites or small samples collected from birds trapped, *e.g.* during migration, with negligible effects. Most importantly, because many species replace particular feather tracts at regular intervals, often pre-migration, and such feathers can be easily identified, these have much better potential for reflecting the isotopic signature of breeding or wintering areas prior to migration. This is important for studies of endangered species. One drawback of feather analysis that has prevented the previous application of Sr isotope tracing is that, unlike bones which contain stoichiometrically substituted Sr (for Ca), they are keratine-based tissues and hence contain very low Sr abundances.

Here, we present a methodology to determine $^{87}Sr/^{86}Sr$ isotope ratios in bird feathers with very low Sr concentrations. We use an ultra-low blank ion-exchange chemistry combined with thermal ionisation mass spectrometry (TIMS) since the amount of Sr that can be recovered from a typical feather (<12 ng) is too low to be analysed with any degree of precision by multicollector inductively couple plasma mass spectrometry (MC-ICPMS). The presence of Kr in the Ar plasma limits the precision and accuracy of Sr isotope ratio measurements for samples of <20 ng due to the presence of Kr isobaric interferences on ^{86}Sr and ^{84}Sr. The technique presented here, also permits determination of a suite of trace elements by inductively couple plasma mass spectrometry (ICPMS). We present a pilot set of data for a single species and demonstrate Sr isotope variation dependent on geographic location. The application of the methodology in a biological context will be presented in future papers.

2. Analytical requirements and sample description

Previous trace element determinations of the Sr abundance in bird bones and egg shells from warblers, for example, indicate concentrations of 23 to 265 μg g^{-1}, [7,8] respectively. In these studies[7,8] Sr isotope ratios of bones and egg shells on sample loads between 250 ng and 75 ng of Sr were analysed and blanks (total procedural determination levels) of 76 pg and 40 pg of Sr[7,8] were reported. Due to the relatively high concentration of Sr in material such as bone and egg shells the contribution from the blank is negligible. However, the small samples (0.2 mg to 1.5 mg) of a feather (shaft or vane) available from a small bird such as a Sedge Warbler (*A. schoenobaenus*), with Sr concentrations between 0.2 μg g^{-1} to 20 μg g^{-1}, provide only 0.5 ng to 12 ng of Sr for analysis. If we take the reported Sr blanks (total procedural contamination levels) of 40 pg to 76 pg,[7,8] the blank contribution to a feather analyses would be between 8% and 15%. Furthermore, >10 ng of Sr is typically required for a precise and accurate analysis using conventional Sr loading and mass spectrometry protocols. If a technique for feathers is to be of wide utility then it should be able to cope with the sometimes very small feather quantities available from conservation studies. Hence our goal was to develop a routine method for measuring 1 ng or less of Sr in keratine based materials such as feathers.

To evaluate the potential variation of Sr isotopes with geographic location, and hence show the potential as a tracer, we analysed $^{87}Sr/^{86}Sr$ isotope ratios in sixteen feather samples from Sedge Warblers from four different localities (Table 1) with contrasting geology (*e.g.* the Cretaceous chalk in the south of the UK and the Proterozoic–Archean basement rocks such as granite and gneiss from the east-European platform). This contrasting geology should be reflected in variable Sr isotope ratios in feathers. All the feathers were taken from juvenile birds (and hence were grown in the region of collection). Eight of the feathers were used to test the effectiveness of different cleaning methods (see Section 3.1). The remaining eight feathers, comprising two from each locality (labelled A and B), were analysed for isotopic and trace element composition. For each feather both the shaft and vane were analysed. Feathers of at least 3 mg provided enough material for Sr isotope and trace element determinations while samples in excess of 4 mg are required to yield additional material for stable carbon and oxygen isotope measurements (not reported here). These feather masses are equivalent to approximately 3 to 4 cm long Sedge Warbler feathers.

3. Sample preparation

3.1 Feather cleaning methods

The structure and large surface area of feathers makes them particularly effective particulate traps, as is clearly shown in Fig. 1a. This, together with some birds' habit of dustbathing means that most feathers have some level of particulate contamination. To measure the isotopic and trace element composition of the feather itself, it is therefore essential to ensure they are cleaned thoroughly, but, just as importantly, cleaned in such a way that does not partially dissolve or leach any cations from the feather.

Table 1 Feather (shaft and vane) weights and coordinates of the locations where the samples for this study were collected

		Coordinates		Shaft weight/mg		Vane weight/mg	
Location		Latitude	Longitude	Feather A	Feather B	Feather A	Feather B
1	Zvanets (Belarus)	52.08 N	24.83 E	1.82	1.72	1.93	1.81
2	Kliosiai (Lithuania)	55.52 N	21.22 E	2.1	1.81	1.89	1.64
3	Tuziya Valley (Ukraine)	51.42 N	24.5 E	1.5	1.59	1.71	1.7
4	Titchfield Haven NNR (UK)	50.49 N	1.14 W	1.02	0.91	1.73	1.34

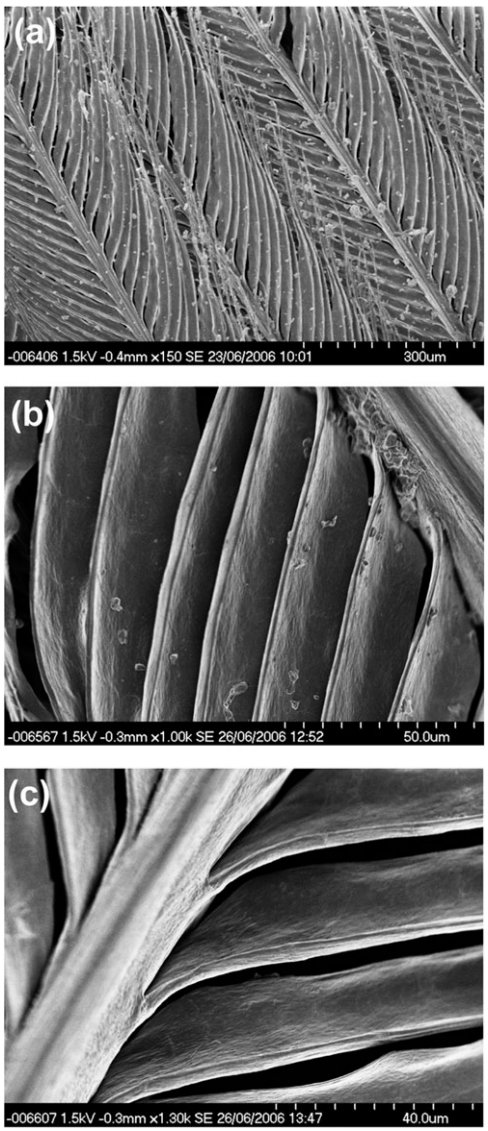

Fig. 1 (a) A large field of view of an unwashed bird feather after simulation of dustbathing. (b) Magnified view of a feather washed using Milli-Q water in an ultrasonic bath. Particles and waxy deposits on the surface of the feather remain after being washed. (c) Magnified view of a feather cleaned using nitrogen gas. All dust particles and waxy deposits are removed from the feather surface.

Two different cleaning methods were assessed as part of this study, the results of which are shown in Fig. 1b and c. Prior to the cleaning experiments the action of dustbathing was simulated by sweeping the feathers through dry sandy soil. This provided an extreme case for particulate contamination (Fig. 1a) and hence the most extreme test of a cleaning method. The first cleaning method comprised washing the feathers in ultrapure Milli-Q water (resistivity of 18.2 MΩ at 25 °C). This was carried out in an ultrasonic bath to encourage the dislodging of particles from the feather. Fig. 1b shows the results after two steps of 10 min of cleaning and although this method is quite effective, approximately 5–10% of particulates still remain. None of the waxy deposits seen at the intersections of vane and shaft (top right of Fig. 1b) are removed. The effectiveness could be improved by washing the feather for longer but in practice the feather begins to dissolve if exposed to Milli-Q water in an ultrasonic bath for any longer than 20 min. Furthermore, since Milli-Q water is ion deficient it is actually very efficient at leaching trace elements from the feather and so is best avoided for this purpose. Weyers *et al.*[10] carried out similar washing experiments in an ultrasonic bath using a mix of propanone and water but also found particulates remained after cleaning.

The second method involved exposing the feather to several 30 s blasts of high pressure dry nitrogen gas. The feather was secured in a small plastic container through which the nitrogen was passed. Nitrogen gas was used as a convenient and clean source of compressed gas for this purpose. Fig. 1c shows the effectiveness of this cleaning method; 100% of particulates and waxy deposits were removed. The advantages of this gas-phase cleaning method are that it is quick, introduces almost no contamination, and does not leach or damage the feather as with liquid-based methods. Following cleaning, the feather is weighed and placed into a pre-weighed Teflon beaker in preparation for dissolution.

3.2 Sample dissolution procedure

Organic compounds must be decomposed during the feather dissolution step since they interfere both with the column chemistry and mass spectrometric analysis, so the feather was repeatedly attacked with nitric acid and hydrogen peroxide. The initial sample digestion was achieved with the addition of 1.5 mL of Ultra purity acid (UpA, Romil, Cambridge, UK) 16 M HNO_3 + 0.5 mL of UpA 12 M HCl to the beaker, which was sealed and placed on a hot plate at 110 °C for 2–3 h until the sample was completely digested. Longer digestion times may be necessary depending on the size of feather sample and/or part of feather being digested, *i.e.* vane *versus* shaft. Once the sample was dissolved it was evaporated down at 105 °C, and the dry residue was then taken up in 100 μL of 35% H_2O_2 (w/w) (Tamapure™) before being evaporated down at 105 °C. The final dissolution step was a combined nitric acid plus hydrogen peroxide attack where the sample was taken up in 100 μL UpA 16 M HNO_3 + 100 μL of 35% H_2O_2, agitated, allowed to sit on a hotplate at 105 °C for 10 min before being evaporated down at 105 °C. At this stage, the solution should be free of organic components that could interfere in the column chemistry. Following column chemistry (see Section 3.4) a final nitric acid–hydrogen peroxide step was repeated to ensure that any organic components were oxidised before the analysis. Our initial experiments conducted without the use of H_2O_2 resulted in final post-column dry residues that were not suitable for loading on TIMS filaments because of residual organic material. The problem ceased once H_2O_2 addition was included in our procedure.

3.3 Aliquoting procedure

To aid in the interpretation of Sr isotope variations in feathers and to allow accurate blank corrections to be made, the trace element composition was also determined for each feather. To avoid issues of sample heterogeneity, isotope and trace element compositions were determined on the same dissolution

by removing a 10% aliquot of the sample for trace elements prior to Sr separation chemistry.

Aliquoting of the sample was done on the basis of weight rather than volume. 200 to 400 μL UpA 3 M HNO_3 acid was added to the dry sample residue in a pre-weighed sample dissolution beaker and the weight of acid added was recorded. The beaker was then tightly sealed and placed on a hot plate at 50 °C for 15–20 min or until such time as the sample was completely dissolved. The beaker was then allowed to cool to room temperature and agitated several times, to make sure that any sample condensate on the sides and cap of the beaker were collected into the bottom of the beaker. A 10% aliquot of the acid, which contained the sample, was removed (approximately 20 to 40 μL) and placed into a micro-centrifuge tube, previously leached using UpA 6 M HCl, ready for trace element analysis. The beaker containing the remaining sample was weighed a final time, and knowing the original weight of acid added to the beaker, the amount of acid (and hence sample) removed for the trace element analysis was calculated. All weighing steps were carried out with the beaker lid in place to avoid any errors arising from sample evaporation and the lid only removed for as long as it took to pipette out the 20–40 μL aliquot. The remaining sample solution (approximately 180 to 380 μL) was then ready to go through the column chemistry procedure of Sr fraction separation.

3.4 Column chemistry: Sr separation

The Sr separation procedure used in this study was based on the micro-Sr column chemistry method described by Charlier *et al.*,[11] designed for samples with small amounts of Sr available.

The micro-columns are disposable and made up from 1 mL pipette tips fitted with a pre-cleaned frit (pore-size of 30 μm: see Charlier *et al.*[11] for further details). Prior to use, the columns were cleaned by placing them in a 1 L Teflon beaker and immersing them in dilute (<1 M) UpA HCl. To facilitate the cleaning procedure, the beaker was placed on the hotplate at 60 °C for several hours, and thereafter, left at room temperature until required. Immediately prior to chemistry the columns were removed, placed in column racks and rinsed twice with Milli-Q water and UpA 6 M HCl. All column elutions were gravity assisted only. Then approximately 60–70 μL of pre-cleaned Sr Spec resin (see Charlier *et al.*,[11] for resin cleaning protocol) was pipetted onto each column. The resin was cleaned further using one column volume (CV) of UpA 6 M HCl and 2 CVs of Milli-Q water. The resin was finally preconditioned with two volumes of 100 μL of UpA 3 M HNO_3. The sample was loaded onto the micro-column in approximately 180 μL UpA 3 M HNO_3. Several washing steps of UpA 3 M HNO_3 followed (approx. 400 μL), to remove matrix elements such as Rb and Ca. The Sr fraction was then eluted in two stages of 100 μL and 200 μL of Milli-Q water and collected in pre-cleaned 3 mL beakers. The Sr fraction was then dried down in preparation for TIMS analysis.

3.5 Total procedural Sr blanks

Bird feathers have very low concentrations of Sr (from 0.2 μg g^{-1} to 20 μg g^{-1}) which combined with the low sample mass make it imperative to monitor both the size of the blank contribution from the dissolution and column chemistry procedure and the isotopic composition of the blank. Thus, with each batch of samples, 2 total procedural blanks (TPBs) were also carried out to determine the average size of the blank. The TPBs were prepared following the same sample digestion procedure used for the feather samples. The same acids and volumes used to dissolve the feather samples were added into a clean empty beaker and were placed on the hot plate for times that accord with the steps of the digestion procedure. When sample digestion was completed, the TPB was loaded onto a Sr-spec micro-column and the Sr TPB fraction was collected. Then the Sr fraction was analysed by ICPMS to determine the Sr concentration in the TPB. This indicated the level of Sr contamination during the total dissolution and column chemistry procedure. The Sr TPBs during this study averaged 11.7 ± 3 pg (2 SD; $n = 5$). The isotopic composition of the lab blank was determined periodically by combining the equivalent of 60 TPBs to yield sufficient Sr (>500 pg) for a precise and accurate TIMS analysis. The average $^{87}Sr/^{86}Sr$ composition of the lab blank was 0.710853 ± 0.000194. Although the average TPB was very low, the feather samples were all, nevertheless, blank corrected since half of the samples analysed in this study had <1 ng Sr and therefore a blank component of 1% or more. Table 2 lists the non-blank-corrected and blank corrected $^{87}Sr/^{86}Sr$ compositions for each feather analysis. The blank correction had most effect (Δ_{BC} between ~0.0012% and 0.0065%, where Δ_{BC} is the difference between the blank corrected and non-blank corrected $^{87}Sr/^{86}Sr$ ratios) for the shaft analyses, which is simply due to the lower Sr contents of the feather shaft (see Table 2 below). This level of blank correction was within the analytical uncertainty for the amounts of Sr typical of a shaft sample (see 0.5 ng to 3 ng standard reproducibility in Table 3). For the vane analyses the effect of the blank correction was <0.001% in all cases and this is well within the uncertainty of the analytical method for Sr samples of this size (see 3 ng to 12 ng standard reproducibility in Table 3).

4. Analytical methods

4.1 Trace element analysis: inductively couple plasma mass spectrometry (ICPMS)

Aliquots of the dissolved feather solutions were analysed for trace element concentrations on the ThermoElectron Element II ICPMS at Durham University, UK. The aliquots were taken up in 1 mL 3% UpA HNO_3 and introduced into the ICPMS using a 100 μL min^{-1} microflow nebuliser and a dual cyclonic scott double pass spray chamber. A 1 ng mL^{-1} In solution was used to tune the instrument for sensitivity until the ^{115}In peak yielded $>10^6$ counts s^{-1}. A 1 ng mL^{-1} Ce solution was then used to optimise the oxide production rate by adjusting, primarily, the nebuliser gas-flow rate to give a Ce/CeO ratio of between 0.03 and 0.05. See Table 4 for general instrument parameters. The elements of interest, and isotopes of each element used for concentration measurement, are shown in Table 4. Other isotopes of the elements measured were used for background interference assessments.

Table 2 Table illustrating the magnitude of corrections made for the total procedural blank for the different Sedge Warbler feather samples. The $^{87}Sr/^{86}Sr$ isotope ratios before (Raw) and blank corrected (BC) are given. $^{87}Sr/^{86}Sr$ isotope ratio of the total procedural blank = 0.710853 ± 0.000194. A and B represent 2 different feathers taken from different birds from the same location (1–4). Data are given for the vane and the shaft of each individual feather

Location	Feather	Part of the feather	^{88}Sr average intensity/V	$^{87}Sr/^{86}Sr$ (Raw)	2 SD	$^{87}Sr/^{86}Sr$ (BC)	2 SD
1	A	Shaft	1.45	0.709604	0.000082	0.709573	0.000083
		Vane	1.76	0.709441	0.000021	0.709435	0.000021
	B	Shaft	0.33	0.709519	0.000082	0.709479	0.000083
		Vane	2.56	0.710235	0.000022	0.710232	0.000022
2	A	Shaft	0.52	0.710298	0.000072	0.710289	0.000072
		Vane	3.01	0.710229	0.000016	0.710227	0.000016
	B	Shaft	0.38	0.710407	0.000091	0.710397	0.000091
		Vane	1.03	0.710571	0.000035	0.710570	0.000035
3	A	Shaft	0.41	0.708873	0.000070	0.708827	0.000071
		Vane	0.69	0.708979	0.000045	0.708972	0.000045
	B	Shaft	0.39	0.709163	0.000079	0.709118	0.000080
		Vane	2.03	0.708952	0.000019	0.708948	0.000019
4	A	Shaft	0.91	0.709104	0.000033	0.709091	0.000033
		Vane	5.62	0.709023	0.000011	0.709021	0.000011
	B	Shaft	1.97	0.708031	0.000020	0.708022	0.000020
		Vane	5.26	0.707957	0.000012	0.707956	0.000012

Concentration calibration was established using multi-element solutions of (50–1000 pg mL^{-1}) made up from Romil™ (Cambridge, UK) 1000 μg mL^{-1} standards.

The TPBs for the analysed elements were <25 pg. The trace element concentrations of the sample solutions were corrected for blank and instrument drift. The trace element concentrations of the feather fragments were calculated using the aliquot weight and original feather mass.

4.2 $^{87}Sr/^{86}Sr$ ratio analysis: thermal ionisation mass spectrometry (TIMS)

4.2.1 Sample loading. Sr samples were loaded onto Re filaments that were pre-outgassed at 3.9 A for 20 min. When analysing sub-ng sized samples it is essential that the load is concentrated in a very small area in the centre of the filament and this was done by running a 1 A current through the filament and melting two narrow strips of parafilm onto the filament surface to define a small central loading space. To enhance ionisation, small Sr samples/standards were loaded with a TaF_5 activator.[12] The activator was cleaned of Rb following the ammonia precipitation method outlined in Charlier *et al.*[11]

The purified Sr was taken up in 1 μL UpA 16 M HNO_3 and it was loaded onto the Re filament together with 0.5 μL of TaF_5 activator. The sample and the activator were then dried down slowly at 1.5 A. When the sample was dry the current was increased to 2 A to burn off the parafilm. The current was then increased to about 2.2–2.4 A for several seconds causing the filament to glow orange/red. Finally, a small amount of TaF_5 activator (0.2 μL) was loaded on top of the dried sample to avoid any small amounts of sample falling off the filament. The activator was dried down for few seconds at a current of 1 A. The filament was then loaded onto the sample magazine ready for analysis.

4.2.2 Mass spectrometry. Sr isotope ratios were measured on a ThermoElectron Triton TIMS at the Arthur Holmes Isotope Geology Laboratory, Durham University, UK. Standard and sample filament currents were both turned up at a rate of 50 mA min^{-1} until a Rb signal was observed (typically 750 mA) at which point the current was held constant while the Rb was allowed to 'burn off'. While Rb was burning off, the line of sight valve was closed and a constant current gain calibration was conducted to determine the amplifier efficiencies. This gain calibration took approximately 20 min by which time the ^{85}Rb intensity was usually below 1 mV (using 10^{11} Ω resistors). The filament current was increased at the same rate until an ^{88}Sr intensity of ~10 mV was obtained. Using the ^{88}Sr beam in the high mass 3 (H3) Faraday cup as the control isotope, the filament was manually focussed and a peak-centre routine performed. A 30 s instrument baseline was then performed, with the beam deflected out of the Faraday

Table 3 $^{87}Sr/^{86}Sr$ and $^{84}Sr/^{86}Sr$ average ratios and external reproducibility of different loads of standard NBS 987 analysed during this study. The arrows indicate the range of ng of Sr in the shaft and vane parts of the feather analysed in this study

NBS 987	^{88}Sr intensity range/V	$^{87}Sr/^{86}Sr$ average	2 SD abs.	2 SD (%)	$^{84}Sr/^{86}Sr$ average	2 SD abs.	*n*	Sr ng shaft	Sr ng vane
0.5 ng	1.1–1.3	0.710260	0.000005	0.0007	0.056491	0.000019	4	↕	
1 ng	1.1–4.7	0.710260	0.000025	0.0036	0.056482	0.000030	15		↕
3 ng	3.2–12.2	0.710264	0.000012	0.0017	0.056490	0.000007	36		
6 ng	4–18.6	0.710264	0.000011	0.0015	0.056490	0.000007	57		
12 ng	5–31	0.710264	0.000012	0.0017	0.056489	0.000005	38		
600 ng	16.8–35	0.710263	0.000008	0.0011	0.056490	0.000003	27		
All loads	1.1–35	0.710263	0.000013	0.0019	0.056489	0.000012	177		

Table 4 Inductively couple plasma mass spectrometry (ICPMS) instrument setting conditions for trace element analysis

Incident RF power:	1300 W
Plasma cool gas (Ar) flow rate:	16 L min^{-1}
Auxiliary gas (Ar) flow rate:	1 L min^{-1}
Nebuliser gas (Ar) flow rate:	~0.95 L min^{-1}
Resolution:	300 (low)
Method	
Isotopes measured:	^{49}Ti, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{90}Zr, ^{93}Nb, ^{137}Ba, ^{139}La, ^{140}Ce, ^{141}Pr, ^{143}Nd, ^{147}Sm, ^{151}Eu, ^{157}Gd, ^{161}Dy, ^{166}Er, ^{172}Yb, ^{175}Lu, ^{159}Tb, ^{179}Hf, ^{208}Pb, ^{232}Th, ^{238}U
Sample time:	10–30 ms dependent on isotope
Samples per peak:	20
Mass window:	60
Runs:	4
Passes:	3
Total time per sample:	52 s

cups using the *x*-symmetry lens, prior to the start of an analysis. Sr isotopes were measured using a static multicollection routine and the Faraday cup configuration given in Table 5. Each measurement consisted of 180 ratios with an integration time of 4 s per ratio; total analysis time approximately 12 min. $^{87}Sr/^{86}Sr$ and $^{84}Sr/^{86}Sr$ ratios were corrected for mass fractionation using an exponential law and a value for the $^{86}Sr/^{88}Sr$ ratio of 0.1194.

The average ^{88}Sr beam intensities during analysis of each of the feather samples are presented in Table 2. The overall average ^{88}Sr beam intensity, for all the shaft analyses, was 0.79 V while for the vain analyses was 2.7 V, consistent with the measured Sr contents of the feather shafts and vanes (see Section 5.1). Fig. 2a shows the measured ^{88}Sr beam intensity and mass bias corrected $^{87}Sr/^{86}Sr$ ratios as a function of time (expressed as number of ratios) for the analysis of the vane portion of feather A from location 4, while Fig. 2b shows the same data for the shaft portion of feather B from location 1. These two analyses represent the largest and smallest Sr samples analysed during this study. Since the range on the *y*-axis scale is the same for each plot (0.8%) it is clear that the vane portion of a feather yields far more precise data than the shaft. On average the 2 SD internal precision of a vane analysis was a factor of 2 better than a shaft analysis at 0.0026% and 0.053%, respectively, which is entirely consistent with the relative Sr beam sizes. Although the shaft $^{87}Sr/^{86}Sr$ isotope ratio measurements were less precise than the vane measurements (Table 2), the precision was still more than adequate for resolving Sr isotope variations between the 4 localities (see Section 6 for discussion). The small amount of Sr that was recovered for a typical feather shaft resulted in low ^{88}Sr beam intensities during the analysis (*e.g.*, Fig. 2b). The Sr loaded on the filament was therefore almost exhausted near the end of an analysis and was, as a result, reasonably fractionated relative to longer loads from the vane. The measured $^{86}Sr/^{88}Sr$ ratio for several of the smaller shaft analyses dropped below 0.1190, whereas for the vane analyses the ratio never dropped below 0.1194.

To ensure that any slight differences in the measured $^{87}Sr/^{86}Sr$ ratio between shaft and vane were not due to the more fractionated nature of the shaft analyses, we inspected

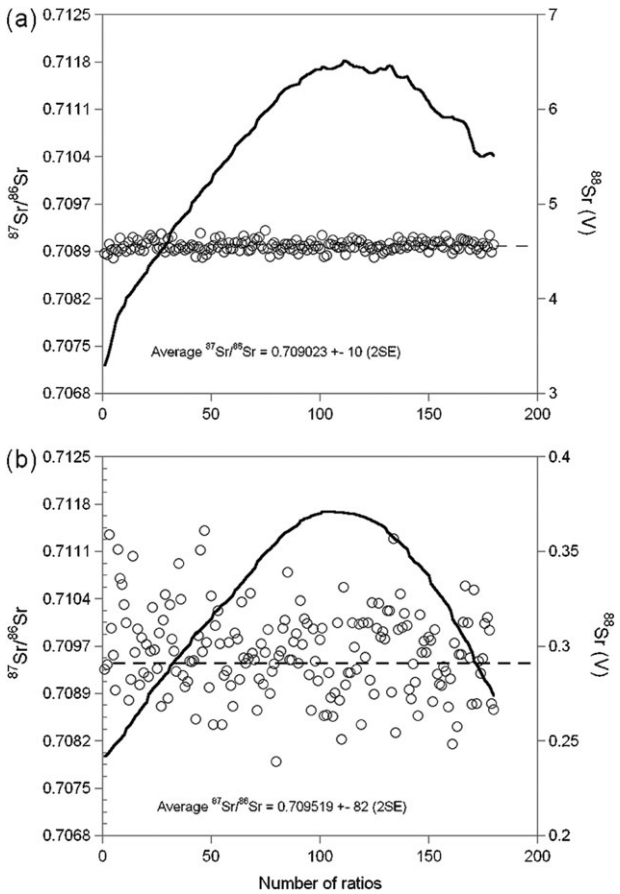

Fig. 2 (a) Time resolved variation (expressed as a number of ratios) in $^{87}Sr/^{86}Sr$ isotope ratios and ^{88}Sr intensity of the vane analysis of feather A from location 4. (b) Time resolved variation (expressed as number of ratios) in $^{87}Sr/^{86}Sr$ isotope ratios and ^{88}Sr intensity of the shaft analysis of feather B from location 1. Open circles correspond to $^{87}Sr/^{86}Sr$ isotope ratios and the black thick line indicates the ^{88}Sr intensity. Dashed line corresponds to the average $^{87}Sr/^{86}Sr$ isotope ratio.

Table 5 Cup configuration used for the analysis of Sr on the AHIGL Triton TIMS. Species in italics is the monitor isotope used for the correction of ^{87}Rb on ^{87}Sr

Cup	L2	L1	Axial	H1	H3
Analyte	^{84}Sr		^{86}Sr	^{87}Sr	^{88}Sr
Interference		*^{85}Rb*		^{87}Rb	

the raw data of all samples and standards for mass fractionation behaviour and checked that it closely approximated to one of the commonly used mass fractionation laws, either power or exponential. Fig. 3 shows the natural logarithms of the average measured $^{84}Sr/^{86}Sr$ *versus* $^{88}Sr/^{86}Sr$ ratio for all the feather samples, together with a representative NBS 987 standard from each size analysed. The shaft analyses can be easily distinguished from the vane analyses by the larger error bars and because they tend to have the higher $\ln(^{88}Sr/^{86}Sr)$ values due to their more fractionated state. The same also applies to the 0.5 ng *versus* >1 ng NBS 987 analyses. Despite the more fractionated nature of the small 0.5 ng NBS 987 and feather shaft analyses, they nevertheless, define linear trends together with the larger >1 ng standards and vane analyses. They are clearly not lying along different trends or have a different slope to the larger Sr analyses. The slopes of these trends are related to the difference in mass of the two isotopes on each axis and to the mass bias behaviour and can be compared to the theoretical slopes for the exponential and power law mass fractionation models (shown as solid and dashed lines, respectively, on Fig. 3). The calculated slope for the NBS 987 standards from 0.5 ng to 600 ng in size is -1.03533 ± 0.0506. This value is within error of that predicted for exponential mass fractionation (Fig. 3) and differs by only 1.2%. In contrast to the standards, the calculated slope of the feather samples (-1.00196 ± 0.0278) more closely approximates, to within 0.2%, the theoretical value for power law mass fractionation, but is nevertheless also, within error of the exponential model used in this study. Since the shaft and vane analyses for all the feathers lie along the same trend in Fig. 3, and this overlaps that of the NBS 987 standards which have the same average $^{87}Sr/^{86}Sr$ ratio irrespective of size, we can be sure that any $^{87}Sr/^{86}Sr$ variations between shaft and vane are not simply due to the more fractionated nature of the shaft analyses.

During the period of this study (February–July 2006) 177 analyses of the international Sr standard NBS 987 were carried out on load sizes from 0.5 ng to 600 ng to monitor and document the TIMS performance. The average $^{87}Sr/^{86}Sr$ and $^{84}Sr/^{86}Sr$ ratios and reproducibility for the NBS 987 analyses are summarised in Table 3 as a function of load size, from 0.5 ng to 600 ng, together with the number of analyses for each size. The average $^{87}Sr/^{86}Sr$ ratio for NBS 987 standard loads of 3 ng, typical of Sr abundances in shaft and vane, is 0.710264 ± 0.000012 (2 SD, $n = 36$). This value is in excellent agreement with the average $^{87}Sr/^{86}Sr$ of much larger Sr loads (600 ng: 0.710263 ± 0.000008 2 SD). The agreement in the average $^{87}Sr/^{86}Sr$ and $^{84}Sr/^{86}Sr$ ratios across the range of standard size is excellent (<0.0006% and 0.016%, respectively) and the values for each load size are also well within error of the TIMS values for this standard reported and recommended by Thirwall.[13] Furthermore, there is minimal degradation in the 2 SD external reproducibility of the $^{87}Sr/^{86}Sr$ ratio as load size decreases from 600 ng to 0.5 ng. The only exception to this are the 1 ng standards which seem to show more than a factor of 2 worse reproducibility at 0.0036%. The reproducibility of the $^{84}Sr/^{86}Sr$ ratio does degrade slightly with decreasing load size but this is to be expected since ^{84}Sr is the least abundant Sr isotope (0.56%) and for standards <1–3 ng would be no bigger than 5 to 10 mV.

Table 3 also shows the typical Sr load for feather shaft and vane analyses to give an estimate of the external reproducibility for samples by comparison to appropriately sized standards. For vane analyses the external reproducibility is expected to be better than 0.002% while for shaft analyses it should be better than 0.0035%.

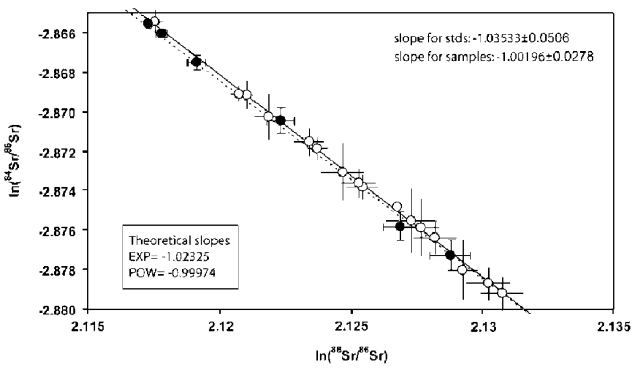

Fig. 3 Natural logarithms of the average measured $^{84}Sr/^{86}Sr$ *versus* $^{88}Sr/^{86}Sr$ ratio for all the feather samples (open circles) together with NBS 987 standard average values from each size analysed (0.5 ng, 1 ng, 3 ng, 6 ng, 12 ng and 600 ng of Sr) (filled circles). Black and dashed lines are the expected slopes for exponential and power law mass fractionation, respectively (actual slopes for each law are given in text box). The calculated slope for the NBS 987 standards, from 0.5 ng to 600 ng in size, is -1.03533 ± 0.0506. The calculated slope for all feather samples is -1.00196 ± 0.0278 (see text for further explanations). The $\pm$ is the standard uncertainty on the slope that is calculated using the software package Isoplot (by Ken Ludwig of the Berkley Geochronology Centre, http://www.bgc.org/isoplot_etc/software.html).

5. Results

5.1 Trace elements

Differences in trace element concentrations between a feather's vane and shaft were initially observed in preliminary tests carried out using Sedge Warbler feathers washed using the Milli-Q water rinsing method. These differences between vane and shaft were first thought to be due to contamination of the feather surface by dust particles and prompted the development of the gas-phase cleaning method. However, even with gas-cleaned feathers the same trace element compositional difference between the shaft and the vane of Sedge Warbler feathers was observed (Table 6), suggesting that this difference is due to other processes rather than contamination (see Section 6). The vane of the gas-cleaned Sedge Warbler feathers had higher trace element concentrations compared to the shaft (Table 6). Shaft and vane of feathers from location 4 (UK) showed the highest concentrations in trace elements compared to feathers from the other three locations. Feathers A and B from each location (taken from different individuals) had variable concentrations of trace elements. For example, the vane of feather A from location 4 has ~ 7 μg g^{-1} Sr, ~ 3.6 μg g^{-1} Ba and ~ 0.05 μg g^{-1} Ce; and the vane of feather B from

Table 6 Trace element concentrations (ng g^{-1}) of Sedge Warbler feathers analysed in this study. A and B represent 2 different feathers taken from different birds from the same location (1–4). Data are given for the vane and the shaft of each individual feather. The dash indicates the concentrations are below or at the detection limit (< 1 pg g^{-1})

Location	1						2						3						4					
	A			B			A			B			A			B			A			B		
Feather Part of the feather	Shaft	*SD*	Vane	Shaft	*SD*	Vane	Shaft	*SD*	Vane	Shaft	*SD*	Vane	Shaft	*SD*	Vane	Shaft	*SD*	Vane	Shaft	*SD*	Vane	Shaft	*SD*	Vane
Ti	—		0.20	—		0.96	—		0.07	—		0.36	—		0.38	—		0.31	—		0.17	—		0.75
			0.07			*0.08*			*0.06*			*0.03*			*0.08*			*0.04*			*0.01*			*0.02*
Rb	79.5	*1.57*	288	98.7	*13.43*	233	161	*9.43*	415	135	*5.04*	373	187	*28.7*	1650	152	*14.2*	461	45.9	*9.84*	254	117	*0.91*	672
			36.8			*20.6*			*90.6*			*7.4*			*190*			*25.5*			*22.1*			*47.7*
Sr	250	*6.74*	1383	218	*23.05*	1302	345	*15.4*	1949	290	*9.42*	2543	325	*41.6*	1789	269	*21.8*	3523	1544	*151*	7261	3788	*55.1*	19 687
			140			*73.9*			*357*			*27.1*			*175*			*151*			*526*			*1233*
Ba	—		1037	—		1056	—		808	—		1567	299	*35.7*	1151	10.23	*7.53*	2994	341	*63.1*	3545	135	*13.1*	1595
			79.8			*70.5*			*125*			*36.7*			*111*			*123*			*188*			*86.5*
La	—		3.05	—		26.73	—		4.95	—		11.1	—		9.74	—		19.9	—		27.9	2.59	*0.59*	18.2
			0.58			*1.21*			*0.92*			*2.47*			*0.90*			*1.47*			*0.81*			*1.35*
Ce	—		3.32	—		24.39	—		10.72	—		15.9	—		18.8	—		38.5	—		46.9	—		32.6
			0.25			*0.78*			*2.08*			*0.78*			*1.43*			*2.37*			*3.24*			*2.66*
Pb	—		213	—		481	—		431	—		383	—		524	—		948	—		921	—		1634
			12.8			*15.1*			*34.5*			*6.33*			*24.2*			*18.3*			*27.2*			*43.2*

Fig. 4 $^{87}Sr/^{86}Sr$ isotope ratios of Sedge Warbler (*A. schoenobaenus*) feathers (A and B) from 4 different locations (see Table 1 for names of locations and coordinates). 2σ uncertainties are smaller than or equal to the symbol size.

the same location has ~20 μg g^{-1} Sr, ~1.6 μg g^{-1} Ba and ~0.03 μg g^{-1} Ce.

5.2 Isotopes

The $^{87}Sr/^{86}Sr$ isotope ratios (Table 2) of the nitrogen gas-cleaned Sedge Warbler feathers varied geographically (Fig. 4). Feathers from location 2 (Lithuania) were the most radiogenic ($^{87}Sr/^{86}Sr$ ~ 0.7102–0.7105) followed by feathers from location 1 (Belarus) ($^{87}Sr/^{86}Sr$ ~ 0.7094–0.7102), location 3 (Ukraine) ($^{87}Sr/^{86}Sr$ ~ 0.7088–0.7092) and location 4 (UK) ($^{87}Sr/^{86}Sr$ ~ 0.7079–0.7092). The $^{87}Sr/^{86}Sr$ isotope ratios of feathers A ($^{87}Sr/^{86}Sr$ ~ 0.708) and B ($^{87}Sr/^{86}Sr$ ~ 0.709) from location 4 (UK) are noticeably different and lie outside of analytical uncertainty from one another (Fig. 4). In contrast, each pair of feathers (A and B) from locations 1–3 shows similar $^{87}Sr/^{86}Sr$ isotope ratios (Fig. 4). The shaft and vane for each feather from all the locations show similar $^{87}Sr/^{86}Sr$ isotope ratios, except for the vane of feather A ($^{87}Sr/^{86}Sr$ ~ 0.7102) in location 1 (Belarus), which is more radiogenic than the corresponding shaft ($^{87}Sr/^{86}Sr$ ~ 0.7096) (Fig. 4).

6. Discussion

The scanning electron microscope (SEM) images (Fig. 1) demonstrate that dust particles in the bird feathers can be very effectively removed with a gas-phase cleaning procedure. This implies that the compositional scatter in trace element concentrations and the compositional difference between shaft and vane are due to processes other than secondary contamination of the feather. The trace element composition of the feathers is related to the composition of the environment where the birds grew their feathers. However, the variations in trace element concentration observed between the shaft and vane of the feathers suggest that other factors, such as physiological processes determine the uptake of trace elements in the birds and hence limit their usefulness as tracers on their own. Otherwise, we should find similar trace element concentrations in all parts of the feather. In previous studies of trace element concentrations in bird feathers,[1,14,15] it has been observed that differences exist between feather samples from

individuals within a single population and among populations of the same bird species. In some of these studies it was claimed that the trace element variations observed in the bird feathers reflect the micro-geology of the breeding area of the birds.[1,16] However, these studies were inconclusive and were unable to demonstrate correlations between the trace element compositions in feathers and that of the local soil. In contrast, other studies have related these elemental differences to physiological mechanisms related to sex, age or nutritional status[14,15] and our results support the latter interpretation.

The $^{87}Sr/^{86}Sr$ isotope ratios of the Sedge Warbler feathers vary geographically and are indicative of the different geology in the locations where the juvenile birds had grown the feathers (Fig. 4 and Table 1). The small range in $^{87}Sr/^{86}Sr$ isotope ratios between feathers (A and B) in a single location (for instance in location 2 and 3) suggests the different individuals grew their feathers in an area where the $^{87}Sr/^{86}Sr$ isotope ratio was relatively homogeneous. By contrast, the large difference in $^{87}Sr/^{86}Sr$ isotope ratios between feathers (A and B) from location 4 suggests the opposite; the $^{87}Sr/^{86}Sr$ isotope ratio of the habitat where the individuals grew their feathers was more heterogeneous. The origin of this isotopic heterogeneity could be related to simple geological factors such as a variable sub-surface geology with rock types having different Sr isotope ratios, or due to complex and variable local drift geology (*e.g.* a glacial boulder clay veneer or loess), with variable Sr isotope compositions. It is also possible for isotopic heterogeneity to be evident within a soil from a single underlying lithology because of the operation of more complex processes such as differential weathering of primary minerals. In this case, a rock such as granite, with minerals that have radically different Rb/Sr ratios will develop variable $^{87}Sr/^{86}Sr$ and if these minerals weather differentially, this can lead to an isotopically heterogeneous soil. These factors show that the isotopic composition of all the sources of Sr to the system and the extent of isotopic variation within individual sources must be determined to be able to use $^{87}Sr/^{86}Sr$ isotope ratios as an ecosystem tracer.[17] For example, to characterise a soil–vegetation system, it is necessary to determine the isotopic composition of the soil parent material, of local rain and dust and of groundwater and surface waters that come in contact with the soil.[17] If the soil–vegetation system is then linked to the bird feathers, it is necessary to determine the $^{87}Sr/^{86}Sr$ isotopic composition of bioavailable Sr in the form of prey items from within the same ecosystem. The $^{87}Sr/^{86}Sr$ isotope ratios do not fractionate through the food chain,[4,7] but to determine the range in Sr isotopes of the ecosystem, it is important to analyse as many components as possible. At this stage of our research we can not explain the causes of the heterogeneity in $^{87}Sr/^{86}Sr$ isotope ratios observed in the feathers of location 4 compared to the other locations since we do not have information on the $^{87}Sr/^{86}Sr$ isotope ratios of the ecosystem where the individuals grew the feathers. However, we have demonstrated that we can determine $^{87}Sr/^{86}Sr$ isotope ratios from bird feather samples. We have shown that the Sr isotope composition of feathers of birds from different locations varies in the way we would expect from even a basic understanding of bedrock geology. We are currently taking this research to a proof of method stage by studying the composition of bird feathers from different localities together with samples of the soil and vegetation from the moult area.

7. Conclusions

A methodology to analyse $^{87}Sr/^{86}Sr$ isotope ratios in Sedge Warbler feathers by TIMS has been presented in this work. The feathers have very low Sr concentrations (from 0.2 μg g^{-1} to 20 μg g^{-1}). Feathers were cleaned prior to analysis by nitrogen gas blasting to remove surface contamination, which would otherwise compromise the feather composition. The SEM images of the feathers cleaned using different methods proved that multiple blasts of nitrogen gas was the most effective method to remove particles, rather than cleaning the feathers with Milli-Q water in an ultrasonic bath. After cleaning, the feathers were analysed for trace elements by ICPMS. The trace element results showed that the vane was generally more concentrated in trace elements than the shaft. For vane and shaft parts of feathers in the 1–2.2 mg mass range, the vane contained between 3 and 12 ng of Sr and the shaft between 0.5 and 3 ng of Sr. The Sr fraction of the feather samples was separated using a Sr-spec micro-column method. The $^{87}Sr/^{86}Sr$ isotope ratios of the feather samples were analysed by TIMS. The vane and shaft portions of the same feather showed similar Sr isotope ratios. However, the $^{87}Sr/^{86}Sr$ isotope ratios of the analysed feathers varied geographically and were related to the different geology of each locality. Due to the small size of some of the feather samples, particularly when measuring the shaft part (<6 ng of Sr), the data were collected very early as a security measure in case of sample failure. The data was evaluated offline in a time-resolved sense for the ^{88}Sr and ^{87}Rb intensities and $^{87}Sr/^{86}Sr$ ratios. The raw data was also inspected to check the mass fractionation behaviour. The results for standards and samples were within error of the values predicted by the exponential mass fractionation model and the predicted power law model, respectively.

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