CONSERVATION ECOLOGY - ORIGINAL RESEARCH

Conservation through connectivity: can isotopic gradients in Africa reveal winter quarters of a migratory bird?

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Abstract Conservation of migratory wildlife requires knowledge of migratory connectivity between breeding and non-breeding locations. Stable isotopes in combination with geographical isotopic patterns (isoscapes) can provide inferences about migratory connectivity. This study examines whether such an approach can be used to infer wintering areas in sub-Saharan Africa, where we lack such knowledge for many species, but where this method has not been used widely. We measured δ^2H, δ^{13}C and δ^{15}N in winter-grown feathers of a breeding Swiss and Spanish population of European hoopoe *Upupa epops*—a typical Palaearctic-Afrotropical migrant. δ^2H values predicted that $\sim$70 % of the hoopoes spent the non-breeding season in the western portion of their potential winter range. This was corroborated by a shallow east–west gradient in feather-δ^2H values of museum specimens from known African origin across the potential winter range and by the recovery of Swiss hoopoes marked with geolocators. Hoopoes categorized as from eastern versus western regions of the wintering range were further delineated spatially using feather δ^{13}C and δ^{15}N. δ^{15}N showed no trend, whereas adults were more enriched in ^{13}C in the western portion of the range, with eastern adults being in addition more depleted in ^{13}C than eastern juveniles. This suggests that eastern juveniles may have occupied more xeric habitats than sympatric adults. We demonstrated that stable isotopes, especially δ^2H, could only very roughly delineate the winter distribution of a trans-Saharan Palaearctic migrant restricted primarily to the Sahelian and savanna belt south of the Sahara. Further refinements of precipitation isoscapes for Africa as well the development of isoscapes for δ^{13}C and δ^{15}N may improve assignment of this and other migrants.

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Introduction

Defining the geospatial connectivity of different life-stages (i.e., breeding, stopover, and wintering sites) of migratory species is fundamental to understanding the different seasonal components of survival, which may provide crucial information to inform effective conservation efforts for endangered migratory species (Webster et al. 2002; Rubenstein and Hobson 2004; Webster and Marra 2005). Quantifying migratory connectivity is essential given the immense conservation challenge posed by the steady

decline of long-distance migratory birds in most parts of the world (Sanderson et al. 2006; Both et al. 2010). To unravel connectivity, individuals need to be tracked. Until now, the wintering quarters of migratory passerines have been inferred mostly from the recovery of ringed individuals. However, owing to exceptionally low ring recovery rates, especially in sub-Saharan Africa, knowledge of population-specific wintering grounds is scarce for many species (Hobson 2003; Reichlin et al. 2009). Satellite or GPS devices are too heavy to be carried by small animals (Webster et al. 2002; Hobson 2003). The recent development of small geolocators suitable for small passerines (Stutchbury et al. 2009; Bächler et al. 2010) offers considerable promise. However, the tags must be retrieved from the same individuals later, typically when they return to their breeding grounds the following year, with often low recovery rates, and the use of all exogenous markers is biased to the nature of the original marked population (Hobson 2011). Thus, there is still considerable interest in the use of intrinsic markers to infer origins since they require only a single capture and every individual sampled potentially provides information on origin.

The relative abundance of stable isotopes in tissues may provide information about the location where the tissue was grown (Hobson and Wassenaar 2008). The relative abundance of deuterium (^{2}H depicted as δ^2H) is especially promising because predictable large-scale geographical patterns of δ^2H in precipitation (δ^2H$_p$ isoscapes) are well documented and are transferred to tissues of animals via diet and drinking water (Bowen et al. 2005). Since feathers are inert after growth, the relative abundance of deuterium in feather keratin (hereafter δ^2H$_f$) is used as a geographic marker to indicate where the feather was grown (Hobson and Wassenaar 2008). For the Nearctic–Neotropical migration system, summer and winter quarters have been identified successfully using δ^2H$_f$ of feathers known to have been grown during the respective season (e.g., Rubenstein et al. 2002; Norris et al. 2006). In Palaearctic–Afrotropical migrants, the isotope method has been applied only rarely to differentiate or to geographically identify African winter grounds of Eurasian breeding migrants, and with mixed success (e.g., Evans et al. 2003; Pain et al. 2004; Yohannes et al. 2007, 2008a; Reichlin et al. 2010; Oppel et al. 2011).

Successful assignment of geographic origins using δ^2H$_f$ depends largely on a predictable and systematic geographical variation in δ^2H$_p$ and on the accuracy of the modelled isoscapes, which in turn depend on the number and spatial distribution of δ^2H$_p$ measuring stations. The spatial resolution of isotopic assignment to geographic origin of feathers can also be influenced by regional or temporal departures of δ^2H$_p$ from the decadal-scale estimates upon which isoscapes are based, variation in individual physiology, and analytical error (Hobson 2008;

Smith et al. 2008; Paritte and Kelly 2009). There is little information concerning these issues for Africa, and δ^2H$_p$ measuring stations are few, although the need to elucidate wintering quarters is most pressing for this region (e.g., Walther et al. 2010), especially from an integrated species conservation perspective.

Several studies have relied on δ^{13}C and δ^{15}N to infer additional information on location or type of wintering habitats (Marra et al. 1998; Rubenstein and Hobson 2004; Studds and Marra 2005; Bensch et al. 2006; Oppel et al. 2011). Values of δ^{13}C and δ^{15}N may be especially useful in Africa where large gradients in moisture occur, which affect vegetation physiological characteristics that are reflected in the isotopic composition of foodwebs (Koch et al. 1995; Still and Powell 2010). Population delineation may also be possible based on differences in isotopic composition of individuals even in cases where actual moult regions in Africa cannot be identified (Møller and Hobson 2004).

We used a multiyear data set of δ^2H in feathers from Swiss and Spanish breeding populations of the European hoopoe *Upupa epops* L. to delineate their likely African wintering grounds, which are currently unknown (there is only one recovery from sub-Saharan Africa of a hoopoe ringed in Europe; Reichlin et al. 2009), while winter-grown feathers can be sampled readily across European breeding grounds. We first investigated whether there were differences in δ^2H$_f$ values between years, age classes and sexes—factors that might confound the outcome of δ^2H$_f$ analyses for attributing wintering quarters. Second, we assigned winter-grown feathers of breeding hoopoes to their probable wintering quarters using a likelihood approach in order to account for potential sources of assignment error (e.g., Hobson et al. 2009). Third, we evaluated the overall applicability of δ^2H$_f$ for the delineation of sub-Saharan African winter grounds for European long-distance migratory birds in general; for this purpose, we cross-referenced hoopoe feathers of known African origin [museum skins and geolocated birds (Bächler et al. 2010)] to predicted δ^2H$_f$ isoscapes. The outcomes of these three approaches were then compared to evaluate the use of δ^2H$_f$ as a geographic marker in the assignment of feathers to sub-Saharan African wintering quarters. Finally, we used delineation of individuals to regions of Africa based on δ^2H$_f$ to investigate if any additional spatial or habitat information could be gleaned from feather δ^{13}C and δ^{15}N measurements.

Materials and methods

Study species, study sites and feather sampling

European hoopoes are assumed to winter mostly in sub-Saharan Africa (7°–16°N) although some may spend the

non-breeding season in southern Europe and North Africa (Cramp 1985; Fry et al. 1988). Yet, the wintering areas of specific populations remain largely unknown (Reichlin et al. 2009; Bächler et al. 2010). We analysed δ^2H values in hoopoe feathers sampled from populations in south-west Switzerland (46°14′N, 7°22′E, 460–520 m a.s.l.; Arlettaz et al. 2010) and southern Spain (37°18′N, 3°02′W, 800–1,100 m a.s.l.; Martín-Vivaldi et al. 1999).

Feather samples in Switzerland were collected from breeding birds caught at the nest box in 2005, 2006 and 2009 (140, 162 and 27 individuals, respectively; 48 birds from 2006 were recaptures from the 2005 sample; one and seven birds from 2009 were recaptures from the 2005 and 2006 sample, respectively). From the Spanish population, ten adult breeding birds were sampled in 2006. From adult birds (AD), we clipped a central piece (2.5–3 cm) of the inner vane of primary feather four (feathers are counted from the distal side; AD moult their primaries in winter), while from 2nd-year birds (first-breeders, SY) we clipped a secondary flight feather moulted during winter (SY do not moult their primaries in winter; Cramp 1985). The timing of moult within the wintering area is not known in detail for this species. From geolocator data it is evident that birds stay for some longer periods in one region, thus are likely to moult in one specific area (Bächler et al. 2010, and unpublished data from 14 individuals).

In order to calibrate the δ^2H isoscape [see Appendix S1 in the Electronic Supplementary Material (ESM)], we also clipped a piece of a primary flight feather (P4 or P5) from 42 SY hoopoes ringed as nestlings the previous year; these feathers were not moulted during winter and hence carried the isotopic value of the natal site.

Sex was determined from the size of the uropygial gland, which is conspicuous and filled with a dark and odorous liquid in breeding females, but small and empty in males (Martín-Vivaldi et al. 2009). Two females and one male of the Swiss birds recaptured in 2009 provided information from geolocators about the area where these individuals had spent the previous winter (Bächler et al. 2010).

Stable isotope analysis

Feather samples were cleaned of surface oils with a 2:1 chloroform–methanol solvent, and prepared for stable isotope analysis at the Environment Canada stable isotope laboratory in Saskatoon (SK, Canada). Feather material (350 ± 10 μg) was weighed into small silver cups, and the relative abundance of deuterium ($^2H/^1H$) was measured with the comparative equilibrium method (H_2 derived from high temperature flash-pyrolysis and CF-IRMS; described in detail by Wassenaar and Hobson 2003) which was referenced with three calibrated keratin isotope materials

(CFS = −147 ‰, CHS = −187 ‰, BWB II = −108 ‰). The keratin standards were used to control for the isotopic exchange between samples and ambient water vapor (Wassenaar and Hobson 2000). All results are expressed in the usual delta (δ) notation, in units of per mil (‰), and normalized on the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale (Wassenaar and Hobson 2003). The 6-month running error, based on control laboratory reference keratin was ±3.3 ‰.

For $\delta^{13}C$ and $\delta^{15}N$ measurements, 0.5 ($\delta^{13}C$) or 1.0 mg ($\delta^{15}N$) cleaned feather material was combusted online using a Eurovector 3000 (Milan, Italy, http://www.eurovector.it) elemental analyzer. The resulting CO_2 and N_2 analyte gas from the samples was separated by gas chromatograph (GC) and introduced into a Nu Horizon (Nu Instruments, Wrexham, UK, http://www.nu-ins.com) triple-collector isotope-ratio mass-spectrometer via an open split, and compared to a pure CO_2 or N_2 reference gas. Stable nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) isotope ratios were expressed in delta (δ) notation, as parts per thousand (‰) deviation from the primary standards: atmospheric nitrogen and VPDB (Vienna Pee Dee Belemnite carbonate) standards, respectively. Using previously calibrated internal laboratory C and N standards (powdered keratin and gelatin), within-run precisions for $\delta^{15}N$ and $\delta^{13}C$ were better than ±0.15 ‰.

Isotopic variation

For Swiss hoopoes, we tested the influence of age, sex, and collection year on the δ^2H values of winter-grown feathers with a linear mixed-effects model (lme with restricted maximum likelihood, R Development Core Team 2009), with individual as random intercept in order to account for the non-independence of samples from the same individual in two different years (34 %). To examine differences in δ^2H values of winter-grown feathers between the Swiss and the Spanish populations, we fitted fixed-effects models for feathers collected in 2006, with population being the independent variable.

Geographic assignment of feather origin

We used a likelihood approach to assign hoopoe feathers geographically to their probable place of growth on the wintering grounds (Wunder 2009). Samples were assigned to probable origins by comparing feather isotopic values with a δ^2H_f isoscape model calibrated for hoopoes. This isoscape model was derived by applying a GLM with known-origin (European) bird feather values (Bowen et al. 2005; P. Procházka, unpublished data; this study) regressed against mean annual δ^2H in precipitation (hereafter MAD; Bowen et al. 2005). The resulting calibration equation

($\delta^2 H_f = 19.53 + 1.27$ MAD), was used to create a hoopoe-specific $\delta^2 H_f$ model isoscape (see Appendix S1 Fig. S1, ESM).

For each feather sample, we assessed the probability that any given cell within the $\delta^2 H_f$ isoscape solution space represented a potential origin of an individual by using a normal probability density function. Birds were assigned singly to their likely origins based on reclassification of spatially explicit probability densities (see Appendix S1, ESM). The assignment was limited to the wintering area of Palaearctic hoopoes in Africa and Europe as given in the literature (Fry et al. 1988). The results of the individual assignments were then summed over all individuals by addition of the model surfaces for Swiss and Spanish birds separately. The resulting maps were expressed as percentages of the total sample assigned to cells within the isoscape for ease of comparison. It is important to realize that such an approach provides a probability-of-origin surface that is consistent with the nature of the isoscape and the odds ratio chosen and should best be interpreted as the most probable isotopic *solution space* rather than depictions of the definitive region occupied by the wintering individuals. Separate maps were constructed for each of the three tagged (geolocators) birds, with a 50 % Kernel of the assigned origins through geolocators.

Museum specimens

We sampled contour feathers from hoopoe museum specimens collected in the potential sub-Saharan wintering area of the European subspecies (14 African countries of the Sahel and North Africa, Appendix S2, ESM). Contour feathers are normally moulted during the moult of wing feathers. Most specimens had been assigned to various African subspecies and five to the nominate Palaearctic subspecies. Although African hoopoes are probably migratory (Morel and Roux 1966; Elgood et al. 1973), we presumed that such movements were comparatively limited and directed north–south following the rains, rather than east–west (P. Jones, personal communication). Six museum feather samples ($\delta^2 H_f$ between −81 ‰ and −58 ‰) were substantially more depleted in ^2H than the rest of the sample and did not conform to our African $\delta^2 H_f$ isoscape, and thus were omitted from further analysis as outliers. These outliers possibly represented retained body feathers grown in the Palaearctic breeding area (*U. e. epops*) and/or may reflect slight differences in moult scheduling among subspecies.

Feather $\delta^2 H$ values of museum specimens were compared with $\delta^2 H_f$ values predicted from their collection locality according to the $\delta^2 H_f$ isoscape map (Appendix S1, ESM) by Pearson correlation. The geometric mean regression slope was used to assess the correspondence

between measured and predicted $\delta^2 H_f$. To examine how well birds could be separated between origins in east ($\geq 20°$ longitude) versus west ($<20°$ longitude) Africa, we applied a likelihood assignment test. We used a normal probability density function to assess the likelihood that these bins represented an origin for individual samples given the observed $\delta^2 H_f$ and the expected mean and standard deviation for samples originating from that bin (sensu Royle and Rubenstein 2004). We estimated the mean and standard deviation of $\delta^2 H_f$ for these bins using (1) all museum samples within each bin, and (2) the mean and standard deviation of $\delta^2 H_f$ predicted from our isoscape east and west of 20° longitude, respectively. Samples were assigned to the bin for which the highest likelihood was obtained.

Feather $\delta^{13}C$ and $\delta^{15}N$ measurements

For individuals with data on all three isotopes ($n = 299$) we summarized $\delta^{13}C$ and $\delta^{15}N$ data in relation to samples assigned to east ($\geq 20°$ longitude) versus west ($<20°$ longitude) Africa (following the approximate migratory divide in Europe; Reichlin et al. 2009). Geospatial assignments result in individuals being assigned to "confidence" regions that can overlap east and west Africa (or have pockets on either side). To classify isotopic assignments into east versus west, we estimated the mean longitude to which individual samples would be assigned on the model isoscape. This was accomplished by overlaying a systematic grid of points (at every 1°) on the $\delta^2 H_f$ isoscape for which we summarized latitude, longitude and predicted $\delta^2 H_f$. We then regressed longitude against $\delta^2 H_f$ and $(\delta^2 H_f)^2$. The resulting equation [longitude = $16.147 + 0.852 \times \delta^2 H_f - 0.004 \times (\delta^2 H_f)^2$] explained ∼82 % of the variance, and was used subsequently to estimate the approximate longitude to which the unknown source birds would be assigned, and these estimates were reclassified to east versus west as per above. We then summarized and statistically compared $\delta^{13}C$ and $\delta^{15}N$ between samples assigned to east versus west Africa using separate one-way ANOVAs for each sex and age-class combination.

Results

Isotopic variation

Winter-grown feathers of Swiss birds differed in $\delta^2 H$ among years (2005: 1.0 ± 2.6 ‰ mean $\pm$ SD, 2006: -7.8 ± 2.1 ‰, 2009: -12.4 ± 4.7 ‰), while males showed higher $\delta^2 H_f$ values than females, and SY (first-year breeders) higher $\delta^2 H_f$ values than AD (adults) (Table 1). Winter-grown feathers of adult Swiss hoopoes collected in 2006 (sexes and ages combined) had significantly higher

Table 1 Effect of year, sex and age on δ^2H values of winter-grown feathers of Swiss birds, analyzed in a linear mixed-effects model with individual as random intercept ($n = 324$)

	Estimate $\pm$ SE	df	F value	P value
Intercept	1.00 ± 2.64	1, 270	9.20	0.003
Year		2, 49	15.42	<0.0001
2006	-8.75 ± 2.11			
2009	-13.40 ± 4.70			
Sex (females)	-5.18 ± 2.38	1, 49	5.30	0.026
Age (SY)	5.98 ± 2.30	1, 49	6.74	0.013

Effect sizes are given for the years 2006 and 2009 against 2005, for females against males and for 2nd-year birds (first-breeders, SY) against adult birds (AD) as a reference

δ^2H$_f$ values than corresponding feathers of Spanish birds from the same year (-8.1 ± 17.9 ‰ vs. -24.4 ± 29.66 ‰; ANOVA, $F_{1,78} = 6.11$, $P = 0.016$, effect of sex negligible $P = 0.37$).

Assignments to wintering areas

Because the differences between sexes and age classes were small, we pooled the data for further analysis. The geographic assignment of winter-grown hoopoe feathers of Swiss and Spanish breeding birds to the model isoscape suggested that the majority of the sampled wing feathers were grown in western Africa and the western Mediterranean region (Fig. 1). Differences in δ^2H$_f$ between birds sampled in Spain and Switzerland (see above) were reflected in assignment to moult origin (Fig. 1), though gross patterns of assigned origin were generally similar between populations. While both populations had the majority of samples assigned to origins in western portions of the wintering quarters, a greater proportion of the Swiss birds (~ 31 %, Fig. 1a) were assigned to origins in eastern Africa than for the Spanish sample (20 %, Fig. 1b).

Evaluation of assignments

The correlation between δ^2H$_f$ values of museum specimens and those predicted from their collection locality according to the isoscape model (Appendix S1 Fig. S1, ESM) was significant ($r^2 = 0.13$, $P = 0.019$). The resulting geometric mean regression slope was exactly 1.00 (± 0.148 SE) with an intercept of -9.1 ± 2.9 ‰ SE (Fig. 2a). Assigning the museum specimens to either west (<20°E) or east (>20°E) Africa with an aspatial analysis via Baye's theorem, resulted in 71 % classification accuracy based on means of museum feathers, or 69 % classification accuracy against our isoscape predictions. The δ^2H$_f$ values of the three birds equipped with geolocators compared with those predicted from their wintering locality according to the

isoscape map (Appendix S1 Fig. S1, ESM) also fell into the scatter of the museum specimens (Fig. 2a).

The geolocator kernels of two out of three birds placed well within the highest probability solution space predicted based on δ^2H$_f$ of winter-grown feathers assigned to the isoscape for these birds (i.e., with >67 % likelihood, Appendix S3 Fig. S2, ESM). The third individual wintered outside of our assumed winter distribution for hoopoe in Africa.

Typical for many Palaearctic–African migrant species, the wintering area of hoopoes in Africa has a much larger E–W than N–S extension. Because of the shallow variation of δ^2H$_p$, especially across the N–S extension of the wintering area, we were interested mainly in determining the wintering grounds from δ^2H$_f$ values along the E–W-axis. Body feather δ^2H$_f$ values of museum specimens showed a significant correlation with longitude (Fig. 2b; effect size 0.40 ± 0.16, $r^2 = 0.14$, $P = 0.015$, $n = 42$), but not with latitude ($P = 0.35$). However, the geographic precision of assignments to potential wintering quarters was limited due the combination of large confidence intervals and a shallow slope.

Feather δ^{13}C and δ^{15}N measurements

We found considerable variation in the birds examined for δ^{13}C and δ^{15}N (Appendix S4, ESM). For adults, δ^{13}C differed between eastern and western regions of the hoopoe wintering grounds with the east depleted in ^{13}C over the west. Adult females assigned to eastern winter origins were depleted by ~ 2.9 ‰ relative to females assigned to western origins, whereas for adult males, the depletion was ~ 2.1 ‰ (Appendix S4, ESM). Within birds assigned to eastern wintering regions, juveniles were more enriched in ^{13}C than adults by approximately 3 ‰. The only other effect we discovered was an enrichment of ~ 1.5 ‰ in ^{15}N for adult males from the east compared to the west (east 11.6 ± 2.4 ‰; west 10.1 ± 2.2 ‰).

Discussion

The primary motivation driving this study was the fact that population-specific winter quarters for most small Palaearctic-Afrotropical migrants remain largely unknown, and this is a major impediment to effective conservation and management of such species. Therefore, our general aim was to examine to what degree the wintering quarters of a typical migrant in sub-Saharan Africa can be delineated by the use of feather isotopic measurements. This approach has been used successfully in North America (Hobson 2008). The European hoopoe proved an excellent model for this objective as it has a winter range typical for many

Fig. 1 Likely wintering areas (moulting areas of winter-grown wing feathers) of **a** Swiss ($n = 325$) and **b** Spanish hoopoes ($n = 10$) predicted from the geographic assignment of $\delta^2 H_f$. The *colors* indicate the proportion of hoopoes that were isotopically consistent with a given cell in the isoscape representing a likely wintering place. The assignment is limited to the known wintering area of Palaearctic birds in Africa and Europe (Fry et al. 1988)
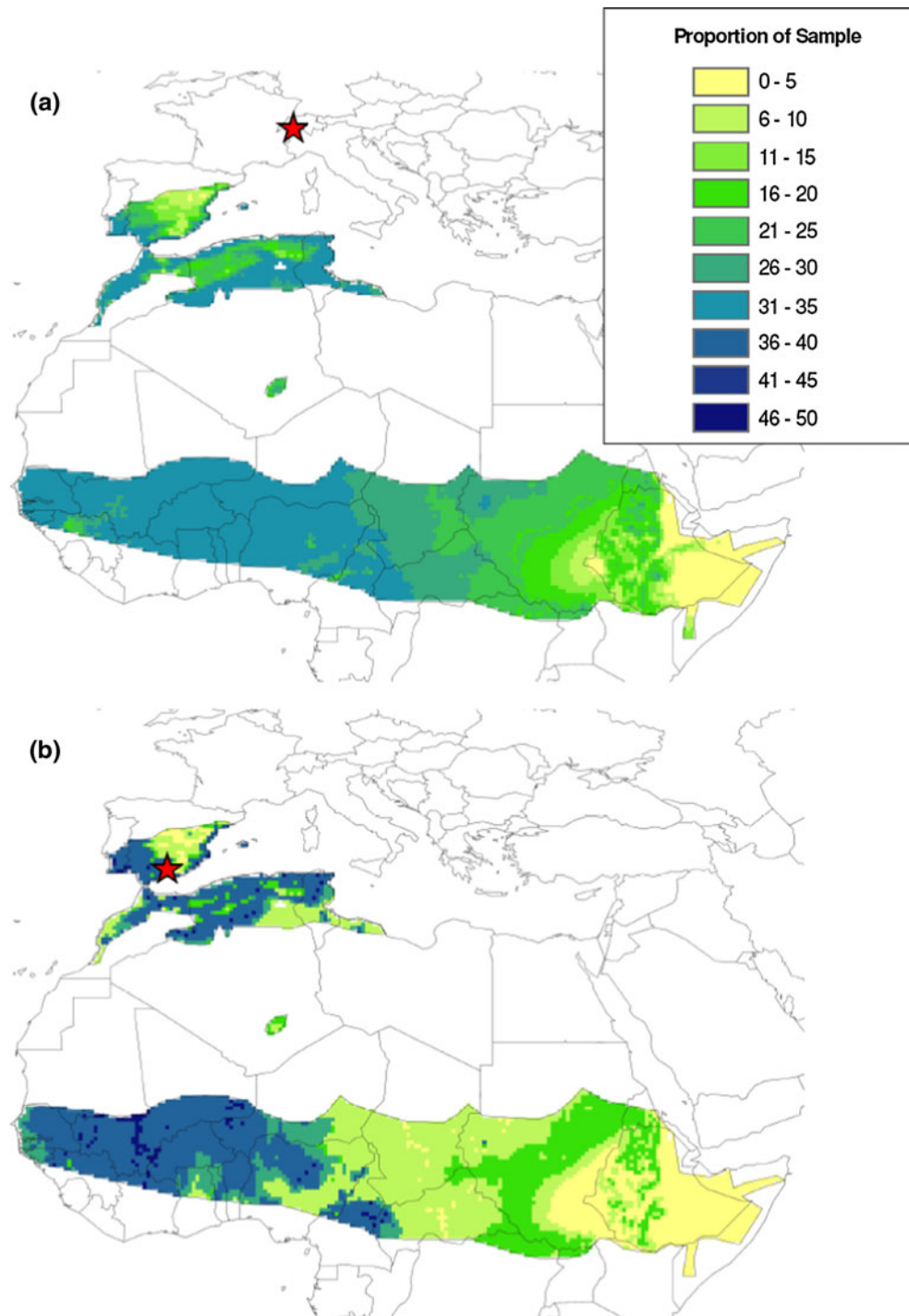
Palaearctic-Afrotropical migrants located in the Sahelian and savanna belt south of the Sahara (Moreau 1972). Therefore, we believe our findings may apply beyond our study model to many other Palaearctic-Afrotropical migrants. From the analysis of museum specimens and geolocated individuals, we showed that stable hydrogen isotopes can be used only to assign origin of feathers to broad E versus W regions of sub-Saharan Africa. The

nature of the $\delta^2 H$ model isoscape for this wintering area precludes any finer-scale resolution using this single isotope, although steps can be taken to improve the precision of future assignments.

At the population level, we found differences in $\delta^2 H_f$ values between years of ~ 13 ‰, which corresponds to a potentially large longitudinal range on the isoscape map within the winter range of the hoopoe (Appendix S1 Fig.
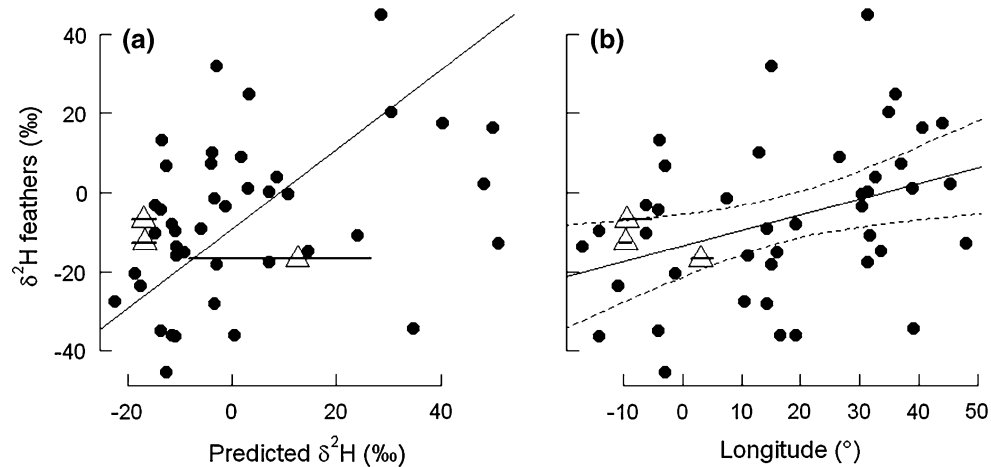
Fig. 2 a δ^2H values measured in body feathers from 42 museum specimens against the δ^2H values predicted from their collection locality according to the isoscape map [Appendix S1 Fig. S1, in Electronic Supplementary Material (ESM)]. The geometric mean regression slope of 1.00 ± 0.148 SE indicates an accurate relationship, but the relatively large SE indicates a low precision. **b** δ^2H values measured in body feathers from 42 museum specimens against the longitude of the collection site. The linear regression line with 95 % confidence limits is indicated. In **a** and **b**, the *triangles* indicate the three geolocated birds, with the *triangle* placed at the mean value of their predicted δ^2H values and longitudes, respectively, of their wintering kernel (Appendix S3 Fig. S2, ESM) and the *horizontal lines* indicating the range

S1, ESM). This is in contrast to two Palaearctic-African migrants, the great reed warbler *Acrocephalus arundinaceus* L. and the aquatic warbler *A. paludicola* Vieillot 1817, which showed no significant differences in δ^2H_f values among 5 (1999–2004) and 3 years (2007–2009), respectively, and consistent values within individual great reed warblers between years for birds breeding in south-central Sweden (Yohannes et al. 2008a, b; Oppel et al. 2011). Year-differences in hoopoe δ^2H_f values remain unclear, but could be due to (1) inter-annual differences in δ^2H_p compared to the long-term isoscape model for this region, (2) shifts in wintering distribution of the populations of hoopoes examined between years, (3) inter-annual variation in region-specific survival, or (4) changes in migration timing and moult scheduling. It is unlikely that hoopoes collectively changed winter quarters between years to such a large extent, because many migrants are faithful to their winter quarters, although this is not known for the hoopoe. Precipitation δ^2H is affected by several factors, including the amount effect (larger amounts of precipitation regionally cause more depleted δ^2H values; Hobson 2011), which may cause inter-annual variation in mean δ^2H_f (autumn 2004 and 2005 were rather dry, compared to 2008; data from Africa Rainfall Estimate Climatology, accessed July 2011). The invariant within-individual δ^2H_f values for the great reed warbler sample may have been due to a period of relatively invariant precipitation in Africa in those years. Survivorship of many migrants has been linked to rainfall in the non-breeding season (e.g., Sillett et al. 2000). Thus, it is conceivable that changes in average δ^2H_f reflect a shift

in the mean due to failure of individuals wintering in some portions of the non-breeding range to return.

Differences in δ^2H_f values between age classes and sexes were smaller (5–6 ‰) than differences between years and would not result in substantial differences in assigned wintering areas. Differences in δ^2H_f between age classes and sexes may be due to differences in wintering areas, food, physiology or habitat segregation on wintering locations (Marra and Holmes 2001; Meehan et al. 2003; Hobson 2011), although there is no a priori information that would support any of these (e.g., differential body size, migration pattern, phenology between sexes or age classes). Most likely, differences in δ^2H_f between age classes and possibly sexes are due to differences in the timing of moult in Africa since regions with highly pulsed seasonal rainfall are expected to show strong temporal dependence of foodweb δ^2H patterns (e.g., Wolf and Martínez del Rio 2000). Differences in δ^2H_f values between Swiss and Spanish birds may be due to differences in sub-Saharan wintering areas, but also to the fact that some Spanish birds (about 20 %; M.M.-V. unpublished data) are sedentary. In fact, in three Spanish individuals, δ^2H_f values of winter-grown feathers were very similar to those of summer-grown feathers (unpublished data), and as a result can be assigned to likely origins within Spain.

Comparing δ^2H_f values of museum specimens and geolocated birds with the isoscape model predicted values of the collection or wintering site revealed an accurate correspondence, however with poor precision (95 % confidence limits of the geometric mean regression slope were

0.73–1.41). The museum specimens showed the expected gradient in $\delta^2 H_f$ values, with longitude corresponding with the $\delta^2 H_f$ model isoscape. However, our museum sample was collected over 100 years and represented body feathers and not flight feathers as in our hoopoe sampling in Europe. Given spatial and temporal changes in rainfall throughout the Sahel (Lebel and Ali 2009), data from museum specimens must be interpreted with caution. In addition, the locality of moulting may be at a distance of several 100 km from the locality of collection. Despite these provisos, the congruence between museum samples and the $\delta^2 H_f$ basemap, as well as classification accuracy to east versus west, suggest that the east–west $\delta^2 H_p$ gradient in sub Saharan Africa is a robust phenomenon, although the gradient is shallow compared to the variation between $\delta^2 H_f$ of individuals of the same region. This may be one reason why no longitudinal gradient could be detected in $\delta^2 H_f$ among sedentary African wetland passerines over a restricted longitudinal range (17°W–14°E; Oppel et al. 2011). Wetland-associated species present additional challenges when using $\delta^2 H$ measurements due to the potential for evapotranspiration effects in shallow wetlands, potential recharge from groundwater sources and so on (Clark and Fritz 1997). The geolocated birds were also correctly assigned to the western part of the wintering range, but the broad range of likely origins based on the isotope solution space was obviously a less precise predictor of regions compared to the geolocator kernel estimates.

There are several challenges with using the $\delta^2 H_f$ isoscape to assign birds to sub-Saharan Africa. This is a region of highly pulsed seasonal precipitation (Lebel and Ali 2009), and so between-year $\delta^2 H_p$ is expected to be more variable than in temperate regions with more uniform rainfall patterns. This may be especially important for species of more xeric habitats, such as the hoopoe, compared to the two wetland warbler species (Yohannes et al. 2008a, b; Oppel et al. 2011). In addition, the foundational model isoscape we used from Bowen et al. (2005) is based on only 44 stations (clustered in Mali, Ethiopia and Uganda), and years of collection vary among stations. The deuterium signal in precipitation also varies between seasons. It is currently not clear which rainfall period matters in terms of the H supporting the foodweb that ultimately results in production of hoopoe feathers (Hobson 2011).

The main reason for the low precision of geospatial assignment using the $\delta^2 H$ approach is that the geographic gradient in $\delta^2 H_p$ across sub-Saharan Africa north of the equator (the wintering range of most Palaearctic-Afrotropical migrants) is very flat compared to North America and Europe, and compared to the variation between individuals collected at similar sites (Fig. 2a). A consequence is that the assigned wintering areas are very sensitive to the isotopic discrimination factor (difference between $\delta^2 H_p$

and $\delta^2 H_f$). In this study, it proved crucial to use a regression equation rather than a single discrimination factor (see also Hobson 2011). The use of a single discrimination factor determined from feathers grown on the breeding grounds (data not reported) would have shifted most wintering areas into east Africa, which proved to be erroneous, at least for the three geolocated individuals. Moreover, evaporative water loss and levels of hydration during feather growth can influence $\delta^2 H_f$ values (McKechnie et al. 2004; Hobson 2011) and these factors may be particularly significant for this wintering region.

Although $\delta^{13}C$ and $\delta^{15}N$ provided little additional information concerning E–W delineations of hoopoes on the wintering grounds, they provided insights into potential habitat segregation. The higher $\delta^{13}C$ values of juveniles in east Africa suggest that they occupied more xeric regions than adults. Possibly, juveniles were excluded from optimal habitats occupied by resident species or adult migrant hoopoes, as has been recorded for American redstart (*Setophaga ruticilla*) wintering in Jamaica (Marra et al. 1998; Studds and Marra 2005).

Conclusions and perspectives

In this study, stable isotopes could be used only to assign the origin of hoopoe feathers to broad E versus W regions of sub-Saharan Africa. This may be useful in delineating migratory divides in Europe for other species migrating in a southwest direction to western regions of sub-Saharan Africa and in a south-eastern direction to east Africa. Ring recoveries for hoopoes also suggested such a divide (Reichlin et al. 2009). Some of the individuals of the Swiss hoopoe population showed less depleted $\delta^2 H_f$, suggesting more eastern wintering areas (see also Bächler et al. 2010).

Several refinements should be possible to the isotope approach. The systematic collection of feathers across Africa of species with known food sources would improve the $\delta^2 H_f$ isoscape for sub-Saharan Africa, especially for upland birds. For wetland-associated species this exercise showed that variation in $\delta^2 H$, $\delta^{13}C$ and $\delta^{15}N$ at the same site was large compared to any systematic geographical variation (Oppel et al. 2011). Better knowledge of the moult period, water balance and of how and when birds in this region incorporate deuterium from precipitation into feathers will help to choose the appropriate seasonal period of integration of $\delta^2 H_p$. Other stable isotopes may improve the geographical assignment of winter quarters of migrant birds using this region of Africa, as has been shown for $\delta^{15}N$ and $\delta^{13}C$ when assigning winter origins of two subspecies of willow warbler (*Phylloscopus trochilus*) (Bensch et al. 2006). Recent advances in modeled plant $\delta^{13}C$ isoscapes for Africa is a promising development (Still and

Powell 2010). However, our study indicates that these isotopes may be more useful as indicators of local habitats than of geographical regions. Finally, other data sources could refine assignments based upon the isotope approach, such as migration direction used in a Bayesian framework (Van Wilgenburg and Hobson 2011), a method that could be extended to data from geolocated birds. However, because of the very shallow geographic gradients and large within-site variations in sub-Saharan Africa of the stable isotopes examined in this and published studies, it remains doubtful that these refinements would improve the spatial resolution to the precision desired for conservation and many ecological insights. This is in contrast to the situation in North America, where steep gradients run perpendicular to the main migration direction (Hobson 2008, 2011).

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