Stable isotope analysis of migratory connections in a threatened intra-African migrant, the Blue Swallow (*Hirundo atrocaerulea*)

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Abstract

The Blue Swallow (*Hirundo atrocaerulea*) is a threatened intra-African migrant with breeding populations in three geographically disjunct regions. We analysed stable hydrogen, nitrogen and carbon isotope ratios in feather keratin to determine whether these vary among breeding populations, and whether feathers can be used to infer migratory connections between breeding and non-breeding areas. Blue Swallows from the three major breeding populations differed significantly in terms of their feather δD and $\delta^{15}N$ values (South Africa / Swaziland: δD = -25.1 ± 6.7 ‰ VSMOW, $\delta^{15}N$ = 10.4 ± 1.0 ‰ AIR; Zimbabwe: δD = -59.9 ± 7.5 ‰ VSMOW, $\delta^{15}N$ = 10.1 ± 0.6 ‰ AIR; Malawi / Tanzania: δD = -43.2 ± 10.8 ‰ VSMOW, $\delta^{15}N$ = 11.7 ± 1.3 ‰ AIR), but not in terms of feather $\delta^{13}C$. We also analysed feathers from seven individuals caught in the non-breeding range on the shores of Lake Victoria in Uganda. A discriminant function analysis assigned four of these birds to the South Africa / Swaziland breeding population and two to the Malawi / Tanzania breeding population ($p > 0.997$), with the remaining individual not being unambiguously assigned. Our results reveal that migratory connections in this threatened species can be inferred from feather stable isotope analysis, and that there is overlap in the wintering ranges of at least two of the three major breeding populations.

Keywords

carbon conservation Hirundinidae hydrogen migration nitrogen

Introduction

Population declines in migratory birds can be caused by factors operating in breeding areas, non-breeding areas and/or along migration routes, and the development of effective conservation strategies for migrants can thus present significant challenges (Carlisle et al. 2009; Goodenough et al. 2009; Holmes 1997; Sherry and Holmes 1996). Among Nearctic-Neotropical migrants, for example, winter habitats as well as stopover sites along migration routes have been identified as critical determinants of population limitation (Carlisle et al. 2009; Sherry and Holmes 1996), and the mitigation of specific factors operating at a single stopover site can be instrumental in halting population declines (Baker et al. 2004). The conservation of migratory species requires knowledge of their ecology in breeding areas, non-breeding areas, as well as along migration routes (Goodenough et al. 2009; Holmes 1997; Sherry and Holmes 1996), and the challenges inherent in conserving migrants are exacerbated when connections between populations are not well understood.

The Blue Swallow (*Hirundo atrocaerulea* Sundevall) is an intra-African migrant whose recent population declines have led to it being red-listed as "Vulnerable" globally (BirdLife International 2008). The South African/Swazi breeding population, which has declined to approximately 80 breeding pairs, is thought to be in imminent danger of local extinction and has been red-listed as "Critically Endangered" (Evans and Barnes 2000). Factors implicated in the species' decline over the last two decades include habitat destruction through agriculture and forestry (Wakelin and Hill 2007), as well as its habit of nesting underground in sinkholes and aardvark (*Orycteropus afer*) burrows, where birds are vulnerable to flooding and disturbance (Spottiswoode 2005; Turner 2004).

The Blue Swallow's known core breeding range consists of three geographically disjunct areas, namely the mistbelt grasslands of eastern South Africa and Swaziland, the eastern highlands of Zimbabwe, and montane grasslands of northern Malawi and southwestern Tanzania (Figure 1, Spottiswoode 2005; Turner 2004). In South Africa and Zimbabwe, the swallows depart by the end of April, and return in August – October (Harrison et al. 1997). The non-breeding range includes southern Uganda, western Kenya, and northeastern Democratic Republic of Congo (DRC) (Figure 1, Spottiswoode 2005; Turner 2004). However, the migratory links between breeding and non-breeding populations and the routes followed by migrating birds remain almost entirely unknown.

The use of stable isotope analysis to infer migratory connections has increased exponentially in the last decade (Hobson and Wassenaar 2008; Rubenstein and Hobson 2004). The method relies on the fact that feathers are metabolically inert, and their isotopic composition thus reflects the area in which they were grown (Chamberlain et al. 1997; Hobson and Wassenaar 1997). This approach has proven particularly useful in the case of species whose non-breeding ranges and/or migration routes include remote areas with few active ringers (banders) (Chamberlain et al. 2001; Yohannes et al. 2007; Yohannes et al. 2005). In this study, we explore the potential of naturally-occurring stable isotope ratios in feathers to distinguish between various Blue Swallow breeding populations, with the goal of identifying migratory connections between breeding and non-breeding populations. Specifically, we ask a) whether population-specific stable isotope signatures exist in Blue Swallow feathers that can potentially be used to infer the origin of wintering individuals, and b) whether birds caught in the central African non-breeding range can be assigned to one of the breeding populations on the basis of feather stable isotope ratios.

Materials and Methods

Feather collection

We obtained primary feathers from Blue Swallows from each of the three major breeding populations. To ensure that these feathers were grown in the respective breeding ranges, we obtained feathers only from chicks and/or juveniles. Feathers were obtained between 1996 and 2009 from Blue Swallows at various sites in KwaZulu-Natal Province, South Africa (29° 45' - 30° 15' S, 29° 55' - 30° 15' E, n = 19), Kaapschehoop, Mpumalanga Province, South Africa (25° 37' S, 30° 45' E, n = 21), Malalotja Nature Reserve, Swaziland (26° 08' S 31° 07' E, n = 2), Nyanga National Park, Zimbabwe (18° 12' S, 32° 45' E, n = 11), and Nyika National Park, Malawi (10° 34' S, 33° 50' E, n = 9). Feathers were obtained either from chicks in nests, or from juveniles trapped with mistnets. In July 2005, we obtained feathers from seven Blue Swallows (five adult females and two first-year immature birds of unknown sex) mistnetted in the non-breeding range at Sango Bay, Uganda (00° 56' S, 31° 42' E), on the shores of Lake Victoria. The feathers we obtained from these birds were relatively worn and not recently grown, and we are confident that they were not grown on the wintering grounds. Although all the individuals we were able to sex were females, males also winter at Sango Bay, and non-breeding ranges do not differ between the sexes (S.W. Evans, *pers. comm.*).

Sample preparation and analysis

Feathers were cleaned in a 2:1 chloroform:methanol solution to remove surface oils and contaminants and then air-dried for at least 24 hr in a fume hood (Hobson et al.

2003). For $\delta^{13}C$ and $\delta^{15}N$ analyses, 2-3 mg feather samples were placed in tin capsules and then combusted at 1,020 °C in an Elemental Analyser (Flash EA, 1112 Series, Thermo Fisher Scientific, Waltham, MA, U.S.A.). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotope ratios were then determined using a Thermo Delta V Plus continuous-flow isotope ratio mass spectrometer (CFIRMS) (Thermo Fisher Scientific, Waltham, MA, U.S.A.) interfaced with the elemental analyzer using a ConFlo IV gas controller (Thermo Fisher Scientific, Waltham, MA, U.S.A.). Laboratory working standards (C and N: homogenized dried chicken blood calibrated against standards C652 ANU sucrose, NIST 1577b bovine liver and NIST 1547 peach leaves; H: NBS22, NBS30 and PEF1) were included at intervals (on average, after every 10 unknown samples) to correct for analytical drift. Measurement precision, based on repeated measurements of laboratory working standards, was ± 0.56 ‰ for δD, 0.15 ‰ for $\delta^{15}N$ and 0.05 ‰ for $\delta^{13}C$. It should be noted, however, that δD measurements for non-exchangeable hydrogen in feather keratin may be less precise than laboratory standards, with reported values of ± 4 ‰ (Wassenaar and Hobson 2000a; Wunder et al. 2005).

Analyses of δD in animal tissues are complicated by the exchangeable hydrogen fraction that equilibrates with water vapour in the environment in which the tissues are stored (Wassenaar and Hobson 2000a; Wassenaar and Hobson 2003). To correct for the exchangeable hydrogen fraction, we used the comparative equilibration approach where samples are equilibrated with the same ambient water vapour as keratin working standards with known non-exchangeable δD values (Wassenaar and Hobson 2003). Blue Swallow feather samples (2-3 mg) and three keratin working standards [BWB-II = -108 ± 4 ‰ Vienna Standard Mean Ocean Water (VSMOW), CFS = -138 ± 5 ‰ VSMOW, CHS = -187 ± 2 ‰ VSMOW (Wassenaar and Hobson 2003)] were weighed into silver capsules and then stored for 72 hr in a room prior to

analysis. The $^{2}H/^{1}H$ ratios of the feather samples, keratin working standards and laboratory working standards were measured using a high temperature TC/EA elemental analyzer with pyrolosis at 1,450 °C that was coupled to the CFIRMS as described above. Following analysis, feather δD values were corrected for the exchangeable hydrogen fraction by fitting a linear regression model to measured *vs* actual δD values for the three keratin working standards, and applying the correction derived in this way to the δD values of the feather samples, following Wassenaar and Hobson (2003).

Data analyses

Analyses of variance (ANOVA) were used to compare feather stable isotope ratios among populations, after checking assumptions of normality using Kolmogorov-Smirnov Tests (Zar 1999). In order to investigate the correlation between δD values of feathers and precipitation during the breeding season, we obtained predicted precipitation δD values for January [middle of the Blue Swallow breeding season (Turner 2004; Spottiswoode 2005)] corrected for altitudinal variation from the Online Isotopes in Precipitation Calculator (http://www.waterisotopes.org; Bowen et al. 2005; Bowen 2010) for each area where we obtained feathers. In the case of the South African / Swazi population, we used the average of the predicted δD precipitation values for Kaapschehoop and the approximate centre of the area in KwaZulu-Natal where feathers were obtained.

We used discriminant function analysis (DFA) to test the reliability of assigning birds to breeding grounds on the basis of feather δD and $\delta^{15}N$ (Wassenaar and Hobson 2000b), using the data for feathers collected in the three breeding areas, with breeding area as the independent (grouping) variable and δD and $\delta^{15}N$ as

dependent variables. We then used DFA to assign each wintering bird to one of the breeding populations. For all DFAs, we assumed equal *a priori* classification probabilities for each breeding population. All statistical procedures were carried out in Statistica 8.0 (StatSoft, Tulsa OK, USA).

Results

Feather δD values varied significantly among the three breeding populations (ANOVA, $F_{2,60} = 98.911$, $P < 0.001$, Table 1, Figure 2), with significant differences between all three populations (Tukey HSD post-hoc test, Table 1). Feather $\delta^{15}N$ also exhibited significant among-population variation (ANOVA, $F_{2,59} = 8.490$, $P < 0.001$, Table 1, Figure 2). Feather $\delta^{13}C$ did not vary significantly among populations (ANOVA, $F_{2,60} = 0.531$, $P = 0.591$, Table 1, Figure 2). A DFA correctly assigned 57 of 62 birds (= 92 %) to their breeding grounds. Within the South African / Swazi sample, feather δD values did not differ significantly between birds from KwaZulu-Natal and Mpumalanga/Swaziland (t-test, $t_{1,41} = 1.152$, $P = 0.255$), but birds from the latter sub-population had slightly but significantly depleted $\delta^{15}N$ values (t-test, $t_{1,39} = 2.170$, $P = 0.036$) and $\delta^{13}C$ values (t-test, $t_{1,41} = 2.543$, $P = 0.017$).

The δD values of feathers collected from Blue Swallows at Sango Bay (non-breeding range) varied from -44 to -11 ‰ VSMOW (Figure 2). The relatively wide ranges of δD and $\delta^{15}N$ values (Figure 2) for wintering birds provides further evidence against the possibility that these feathers were grown at Sango Bay. Discriminant function analysis assigned four out of seven wintering birds to the South African breeding population and two out of seven wintering birds to the Malawian / Tanzanian breeding population (Table 2, Figure 2). However, one wintering individual (ring J57764) could not be reliably assigned to a single breeding

population, with relatively high posterior probabilities of belonging to both the South African/Swazi and Malawian/Tanzanian populations (Table 2).

Discussion

Our data confirm that Blue Swallows originating from the three major known breeding ranges differ significantly in feather δD and $\delta^{15}N$ values, making it possible to infer the origin of wintering birds from their feather stable isotope ratios. Although there is some overlap in both δD and $\delta^{15}N$ between the various breeding populations, they are sufficiently isotopically distinct for at least some wintering individuals to be assigned to specific breeding populations. One limitation of this study concerns the fact that all the feathers we obtained from wintering birds were from females or unsexed juveniles, and our conclusions are based on the untested assumption that feather stable isotope ratios do not differ between sexes.

Typically, δD in precipitation and thus animal tissues decreases with increasing latitude (Bowen et al. 2005; Clark and Fritz 1997; Hobson and Wassenaar 1997), and the majority of isotopic studies of avian migration patterns have made use of this global pattern of variation in δD (Hobson et al. 2004; Hobson and Wassenaar 1997; Kelly et al. 2002; Meehan et al. 2001). In our study, however, the highest-latitude Blue Swallow population (South Africa / Swaziland) exhibited the most enriched feather δD values, with the population from intermediate latitudes (Zimbabwe) exhibiting the most depleted values. These differences are correlated with variation in precipitation δD (Bowen et al. 2005; Bowen 2010; Table 1), driven in part by altitudinal variation. Precipitation δD becomes progressively depleted with increasing altitude, with a corresponding depletion of feather keratin δD (Hobson et al. 2003). In South Africa and Swaziland, Blue Swallows breed at altitudes of 850 –

1,900 m, whereas the Zimbabwean population breeds at altitudes of 1,500 – 2,300 m (Spottiswoode 2005). The Malawian birds also breed at relatively high altitudes (> 2,000 m), but feather δD in the latter population may also reflect the influence of the nearby freshwater Lake Malawi on local precipitation. It should be noted, however, that the precipitation δD values for January provided in Table 1 are unlikely to be representative of the hydrogen sources used for feather synthesis by Blue Swallow chicks, and the δD of their food items likely integrates precipitation inputs over longer time scales.

We have assumed that the feather δD of adult Blue Swallows exhibits the same relationship with local precipitation δD as that of nestlings, and that adults and nestlings from each breeding range should exhibit similar feather δD values. This assumption, however, does not hold for all species; the feather δD values of primary feathers from adult Cooper's Hawks (*Accipiter cooperii*) were significantly enriched compared to feathers from nestlings (Meehan et al. 2003). These authors advanced three nonexclusive hypotheses as to the mechanisms underlying this variation, namely 1) adult feathers may contain hydrogen derived from migrant avian prey, 2) adult feathers, although grown on the breeding grounds, may be synthesized from body reserves laid down while wintering at lower latitudes, and 3) high heat loads experienced by incubating adults may lead to enrichment of body water, and growing feathers, because of a greater proportion of total water loss occurring via evaporative, and thus fractionated, pathways (McKechnie et al. 2004). It is very unlikely that similar differences exist between nestling and adult feather δD in Blue Swallows, since adults feed on non-migratory, aerial insects, and the species typically nests in cool, moist subterranean sites. Although we cannot exclude possibility 2) above, the

absence of wintering adult feather δD values that are outside the range for nestlings from known breeding sites argues against this possibility.

Diet-tissue discrimination factors for carbon in avian feathers vary from slightly negative values to approximately +7 ‰ (Bearhop et al. 2002; Hobson and Clark 1992). The feather $\delta^{13}C$ values of all three Blue Swallow populations suggest that the birds feed in food webs whose bases are dominated by plants with C_4 and/or crassulacean acid metabolism (CAM) photosynthesis (O'Leary 1998; Farquhar et al. 1989), an observation consistent with their mid- to high-altitude grassland habitats (Spottiswoode 2005; Turner 2004; Vogel et al. 1978). The feather $\delta^{13}C$ values of Blue Swallows from South Africa and Swaziland are within the range for Barn Swallow (*Hirundo rustica*) feathers moulted at various sites in the eastern half of South Africa, as are feather $\delta^{15}N$ values (Szep et al. 2009).

The feather δD and $\delta^{15}N$ values of wintering Blue Swallows caught at Sango Bay, Uganda, suggest that birds wintering at this site originate from at least two of the major breeding populations, and that the wintering ranges of the South African/Swazi and Malawian / Tanzanian populations overlap. One of the wintering individuals could not be unambiguously assigned to a breeding population, an observation that could reflect a) the overlap in δD and $\delta^{15}N$ values between South African/Swazi and Malawian swallows, b) the relatively small number of feathers we were able to obtain from Malawian birds, or c) an origin for this bird outside of the three major breeding areas. Additional small breeding populations occur in the southeastern DRC and southern Malawi (Mt. Mulanje) (Evans et al. 2002; Fishpool and Evans 2001). Moreover, a fairly large area of potentially suitable but as yet unexplored Blue Swallow habitat exists in the southern DRC (S.W. Evans, *pers. comm.*).

The use of stable isotopes to track the movements of intra-African migrants is complicated by the fact that the continent is situated over the equator, and thus lacks the uni-directional latitudinal gradients in precipitation δD that exist in North America and Eurasia (Bowen et al. 2005). Nevertheless, stable isotope-based approaches have proved useful in examining interspecific variation in stop-over site selection in Palearctic migrants that moult along their migration route (Yohannes et al. 2007; Yohannes et al. 2005). Our results reveal that this approach can also be used to infer migratory connections between populations that breed and winter in different parts of the continent. In the case of the Blue Swallow and other threatened species, this kind of information is vital for coordinating conservation activities between regions occupied by the birds at different times of the year.

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Table 1. Stable isotope ratios (mean ± SD) of feather keratin in Blue Swallows (*Hirundo atrocaerulea*) from each of the major known breeding areas. For each element, significant differences among populations are indicated by different superscript letters. δD values in square brackets are the predicted values for precipitation in January in each breeding area (Bowen et al. 2005; Bowen 2009).

Breeding population	δD (‰ VSMOW)	$\delta^{15}N$ (‰ AIR)	$\delta^{13}C$ (‰ VPDB)
South Africa / Swaziland	-25.1 ± 6.7[a] [-30]	10.4 ± 1.0[a]	-16.2 ± 1.7[a]
Zimbabwe	-59.9 ± 7.5[b] [-52]	10.1 ± 0.6[a]	-15.6 ± 1.1[a]
Malawi / Tanzania	-43.2 ± 10.8[c] [-44]	11.7 ± 1.3[b]	-15.9 ± 1.6[a]

Table 2. Posterior probabilities that Blue Swallows (*Hirundo atrocaerulea*) wintering at Sango Bay, Uganda belong to each of the three major known breeding populations, calculated using discriminant function analysis. Probabilities in bold font indicate instances where an individual could be unambiguously assigned to one of the three breeding populations.

Individual	**Breeding population**		
(ring number)	**South Africa / Swaziland**	**Malawi**	**Zimbabwe**
AM24856	**0.999**	0.000	0.000
J57760	0.033	**0.860**	0.107
J57761	**0.999**	0.000	0.000
J57762	**0.999**	0.000	0.000
J57763	**0.971**	0.025	0.003
J57764	0.554	0.433	0.013
J57765	0.069	**0.928**	0.003

Figure legends

Figure 1. Approximate breeding (black) and non-breeding (cross-hatched) ranges of the Blue Swallow (*Hirundo atrocaerulea*), redrawn from Spottiswoode (2005), Turner (2004) and BirdLife International (2008).

Figure 2. Feather δD and $\delta^{15}N$ for Blue Swallows (*Hirundo atrocaerulea*) from each of the three major breeding areas, as well as seven birds caught on the non-breeding range at Sango Bay, Uganda.

Figure 1.

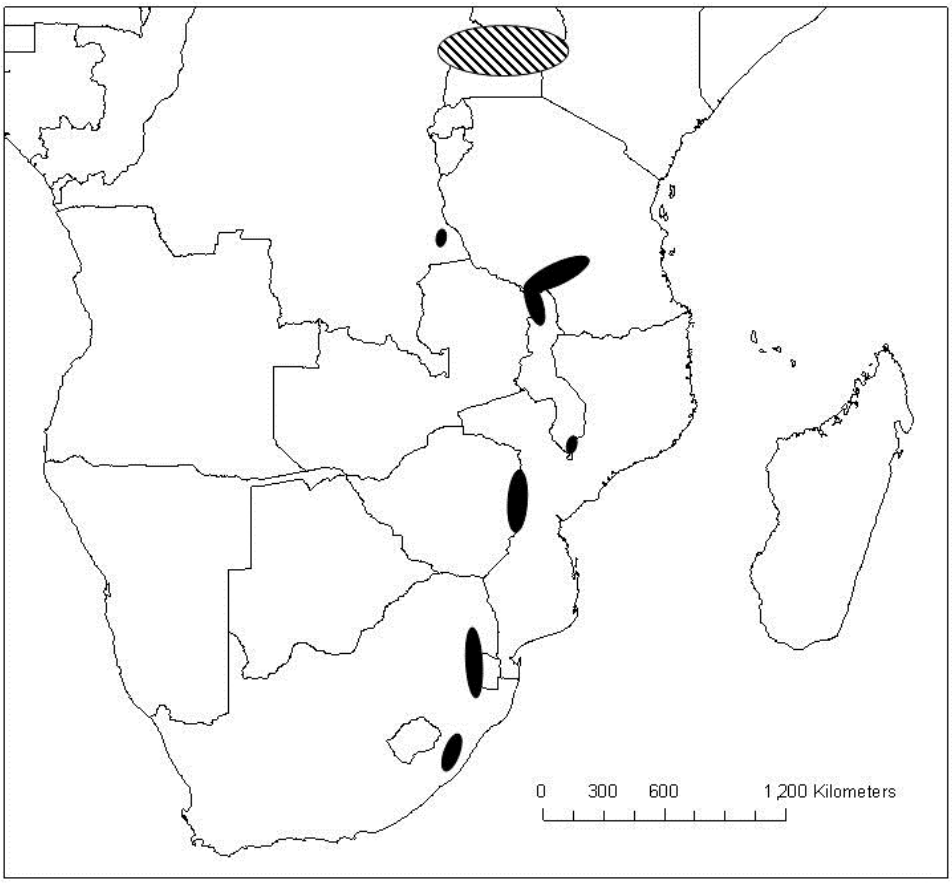
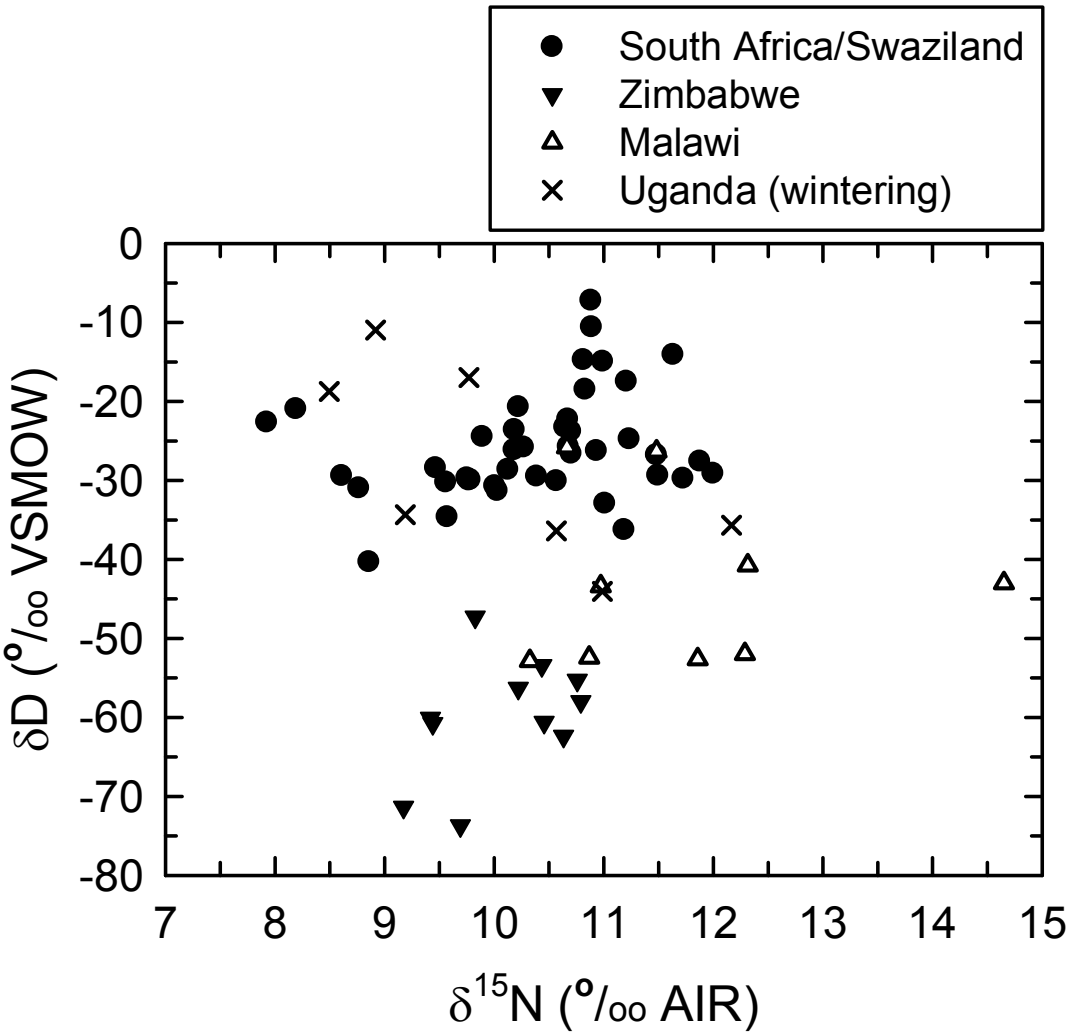


Figure 2.


South Africa/Swaziland
Zimbabwe
Malawi
Uganda (wintering)
0
-10
-20
-30
-40
-50
-60
-70
-80
δD (°/oo VSMOW)
7
8
9
10
11
12
13
14
15
δ15N (°/oo AIR)