Inferring the links between breeding and wintering grounds in a Palearctic–African migratory bird, the Great Reed Warbler, using mitochondrial DNA data

Naglaa El-Arabany¹,², Marjorie Sorensen³ and Bengt Hansson*³

¹ Department of Biology, Lund University, Lund, Sweden
² Department of Zoology, Faculty of Science, Damietta University, Damietta, Egypt
³ Department of Zoology, University of Cambridge, Cambridge, UK
* Corresponding author, e-mail: bengt.hansson@biol.lu.se

Understanding spatial connections between breeding and wintering populations is critical for developing sound conservation plans in migratory animals. However, for long-distance migratory songbird species wintering in sub-Saharan Africa, many of which are in a state of population decline, information on migratory connectivity is especially lacking. We used mitochondrial DNA data from wintering populations of the Great Reed Warbler (*Acrocephalus arundinaceus*) in western (Nigeria), southern (Botswana and Zambia) and eastern (Kenya) Africa, as well as from several Eurasian breeding populations, to compare genetic differentiation and haplotype sharing between non-breeding and breeding populations. We found that the population in Nigeria had the best genetic match to breeding populations in West and Central Europe. In contrast, Botswana matched with West, Central and East Europe, and Zambia with Central and East Europe and the Middle East. Finally, Kenya showed the most distinct connectivity pattern of the four analysed populations and matched with East Europe and, in particular, to the Middle East. Our results indicate clear but weak migratory connectivity in Great Reed Warblers, a pattern that should be considered in conservation strategies of Palearctic–African migratory passerines.

Keywords: bird, connectivity, distribution range, genetic similarity, migration, mitochondria, population differentiation

Introduction

Knowledge of migratory connectivity, the geographic links between breeding and non-breeding populations, is required for understanding the population dynamics of migratory animals (Webster et al. 2002; Hobson et al. 2014). Detailed evaluations of migratory connectivity in long-distance songbird migrants are mainly from Neartic–Neotropical species (Rubenstein et al. 2002; Boulet et al. 2006; Norris et al. 2006; Franks et al. 2012; Gratto-Trevor et al. 2012) and have relied largely on geographic variation in stable isotope signatures to assign birds to origins. However, for Palearctic–African migrants, stable isotope methods have been limited to distinguishing between subpopulations at a large scale (Evans et al. 2003; Bensch et al. 2006; Procházka et al. 2008) since poor predictability and high within-site variation have made assignment to African origins problematic (Oppel et al. 2011; Reichlin et al. 2013). Recently, satellite tracking has made it possible to reveal and understand migratory connectivity in larger birds, such as raptors (Klaassen et al. 2014; Trierweiler et al. 2014). Most information on connectivity in Palearctic–African migrants, and in particular for small passerine birds, comes from ringing recoveries, but due to very low recovery probability (in the order of 1 to 100 recoveries) on the African wintering grounds (Hedenström and Pettersson 1987; Yohannes et al. 2008) our understanding remains fragmented. Many long-distance songbird migrants to sub-Saharan Africa are in a state of severe population decline (Sanderson et al. 2006) and an understanding of migratory connectivity is essential to predict the consequences of habitat loss and develop effective conservation plans (Martin et al. 2007; Taylor and Norris 2010; Iwamura et al. 2013).

Utilising mitochondrial DNA (mtDNA) variation is considered one of the most promising molecular methods for determining migratory connectivity. This is because for many species the frequency of mtDNA haplotypes varies geographically over the breeding range, so that specific breeding populations, areas or regions can be identified (Wennerberg 2001; Hansson et al. 2008; Kraus et al. 2011). In such situations it is possible to determine the degree of migratory connectivity by evaluating from which populations wintering individuals originate. For example, mtDNA haplotype data has made it possible to determine the population origins and migratory routes used by migrating dunlins *Calidris alpina* (Wennerberg 2001).

In the present study, we focus on the migratory connectivity of the Great Reed Warbler (*Acrocephalus arundinaceus*). This is a long-distance migratory passerine bird species that breeds in Europe and Asia and winters in Africa. Previous work has documented substantial mitochondrial population differentiation over its breeding range, which has been strongly influenced by independent postglacial expansion events from two glacial refugia (Bensch and Hasselquist 1999; Hansson et al. 2008). The data suggest that birds from a refugium presumably located in western Europe colonised not only Europe, but also Asia, before birds from a refugium presumably located in the Middle East expanded north into central and eastern Europe (Hewitt 2000; Hansson et al. 2008).
The winter distribution of Great Reed Warblers is located throughout sub-Saharan Africa; the western subspecies (A. a. arundinaceus) overwinters mainly in West and Central Africa and the eastern subspecies (A. a. zarudnyi) mainly in East and South Africa (de Roo and Deheegher 1969; Backhurst and Pearson 1984; Kennerley and Pearson 2010). Recoveries of marked birds from a Swedish long-term study population of Great Reed Warblers (Bensch et al. 1998; Hasselquist 1999; Hansson et al. 2002) indicate a southward migration route via the central Mediterranean region (five recoveries in Italy, Slovenia and Croatia) and a wintering area ranging at least between the Ivory Coast in the west to southern Chad in the east (Yohannes et al. 2008). Recent results from a few Great Reed Warblers equipped with geolocators, in the same Swedish study population, confirm this general migration route and wide wintering area (Lemke et al. 2013). These recoveries and observational data represent the rudimentary information that is currently available for understanding migratory connectivity in Great Reed Warblers. The situation is similar or worse in most other passerines species (but see, e.g., Marra et al. 1998; Chamberlain et al. 2000; Stutchbury et al. 2009; Tøttrup et al. 2012).

In the present study, we use mtDNA to examine patterns of migratory connectivity in the Great Reed Warbler. The substantial differentiation in mtDNA haplotype frequencies across the breeding range in this species (Bensch and Hasselquist 1999; Hansson et al. 2008) makes the Great Reed Warbler highly suited to mtDNA methods for determining the origins of populations of wintering birds. In contrast, Great Reed Warbler populations are much less differentiated at microsatellite loci (Hansson unpublished data), which makes such markers less suitable for evaluating migratory connectivity. Detailed assessments of mtDNA population genetic patterns and processes over the breeding range, based on extensive population genetics, phylogenetic, network and isolation-by-migration analyses, can be found in previous publications (Bensch and Hasselquist 1999; Hansson et al. 2008). Here, our objective was to examine the patterns of connectivity between the Great Reed Warblers’ wide breeding range in Eurasia and non-breeding range in sub-Saharan Africa (Kennerley and Pearson 2010). In particular, the geographic setting of our study system with large breeding and overwintering ranges suggests that longitudinal connectivity may occur. For these reasons, we measured mitochondrial sequence variation of birds from four African localities – one in the western part, one in the eastern part and two in the southern part of the wintering range – and compared the genetic differentiation and haplotype sharing between non-breeding and breeding localities.

**Materials and methods**

Blood samples were collected from Great Reed Warblers at four non-breeding localities in Africa: Nigeria (n = 41), Botswana (n = 6), Zambia (n = 41) and Kenya (n = 13; Table 1). Blood samples were stored either in a standard salt buffer or in ethanol. All birds were released immediately after blood sampling.

**Table 1**: Sampling localities, sampling period, number of sequences (n), number of haplotypes (nH), haplotype diversity (H) and nucleotide diversity (π). Data from the breeding sites are from Hansson et al. (2008).

| Country | Site | Coordinates | Sampling year | n  | nH | H    | π   |
|---------|------|-------------|---------------|----|----|------|-----|
| **Non-breeding sites** | | | | | | | |
| Nigeria | Lake Chad, Malamfatori | 13°33′ N, 13°23′ E | 2000 | 41 | 22 | 0.918 | 0.00792 |
| Botswana | Phakalane, Gabarone | 24°32′ S, 25°58′ E | 1998–1999 | 6 | 4 | 0.867 | 0.00630 |
| Kenya | Ngulia, Tsavo West NP | 3°00′ S, 38°08′ E | 1990, 1991 | 13 | 6 | 0.821 | 0.00342 |
| Zambia | Choma | 16°39′ S, 27°00′ E | 2011, 2012 | 41 | 14 | 0.871 | 0.00636 |
| **Breeding sites** | | | | | | | |
| Spain | Hondón Natural Park, Alicante | 38°12′ N, 0°42′ W | 1996 | 11 | 5 | 0.709 | 0.00202 |
| Netherlands | Zwarte Meer, Weerribben | 52°37′ N, 5°55′ E | 1995 | 10 | 4 | 0.533 | 0.00205 |
| Sweden | Kvismaren, Närke | 59°10′ N, 15°25′ E | 1987–1990 | 22 | 7 | 0.649 | 0.00480 |
| Latvia | Engure/Kanieris, Tukums | 57°07′ N, 23°20′ E | 1992 | 20 | 11 | 0.916 | 0.00654 |
| Germany | Müggelsee, Berlin | 52°26′ N, 13°39′ E | 1992, 1993 | 19 | 12 | 0.918 | 0.00652 |
| Czech Republic | Hodonín fishponds, Moravia | 48°54′ N, 17°02′ E | 2006 | 17 | 11 | 0.934 | 0.00912 |
| Hungary | Apaj Channels, Kiskunlachaza | 47°07′ N, 19°05′ E | 2000 | 20 | 14 | 0.947 | 0.00565 |
| Bulgaria | Kalimok, Trutakan | 44°01′ N, 26°26′ E | 2005–2006 | 20 | 12 | 0.900 | 0.00836 |
| Greece | Limni Mikri Prespa, Florina | 40°50′ N, 21°05′ E | 1990 | 20 | 13 | 0.932 | 0.00906 |
| Belarus | Mitríkou Lake, Rodopi | 40°58′ N, 25°17′ E | 2005 | 17 | 9 | 0.890 | 0.00737 |
| Ukraine | Turov, Zhiltovichi | 52°01′ N, 27°49′ E | 2000 | 18 | 11 | 0.941 | 0.00799 |
| Russia | Usovka, Poltava | 50°19′ N, 32°32′ E | 2000 | 13 | 9 | 0.900 | 0.00737 |
| Denmark | Denisyka, Poltava | 49°53′ N, 32°36′ E | 2000 | 13 | 9 | 0.900 | 0.00737 |
| Vilkovo, Odessa | 45°28′ N, 29°35′ E | 2006 | 28 | 12 | 0.857 | 0.00527 |
| Russia | Stepepilman, Saratov | 50°43′ N, 46°27′ E | 2006 | 28 | 12 | 0.857 | 0.00527 |
| Solynka, Saratov | 50°49′ N, 47°05′ E | 2006 | 28 | 12 | 0.857 | 0.00527 |
| Furmanovo, Saratov | 51°38′ N, 49°07′ E | 2006 | 28 | 12 | 0.857 | 0.00527 |
| Kazakhstan | Stone Lake, Zhambyl | 42°49′ N, 70°56′ E | 2001 | 35 | 9 | 0.793 | 0.00275 |
| Lake Balkhash, Almaty | 45°12′ N, 73°59′ E | 2001 | 35 | 9 | 0.793 | 0.00275 |
| Turkey | Mogan Lake, Ankara | 39°46′ N, 32°48′ E | 2005 | 17 | 7 | 0.824 | 0.00374 |
| Iran | Zarin Kola, Mazandaran | 36°44′ N, 53°00′ E | 2004 | 7 | 2 | 0.286 | 0.00117 |
| **Pooled** | | | | 382 | 72 | 0.921 | 0.00765 |
after being examined. Samples were collected from February to April 2000 in Nigeria, November 1998 to February 1999 in Botswana, January to March 2011 and 2012 in Zambia and November to December 1990 and 1991 in Kenya. Since Nigerian birds captured late in the spring were likely on migration (stop-over), we conducted additional analyses including only birds captured in early spring (before March), which were at or close to their wintering sites (presented in Supplementary Table S1).

Birds from Kenya were captured during the short mid-winter movement, i.e. close to their wintering sites (Backhurst and Pearson 1984), and birds from Botswana and Zambia were at or close to their wintering sites.

Ethics statement: the protocols for handling and examining the birds were approved by the County Administrative Board and the Lund/Malmö Animal Review Board in Sweden. Ringing, examination and sampling were done in collaboration with experienced ringers at the respective study sites.

DNA was extracted from blood (5–50 µL stored in SET-buffer) by a standard phenol–chloroform extraction protocol. The mitochondrial control region II in Great Reed Warblers (GenBank accession no. AF111791) was amplified and sequenced with the primers BCML4 (5′-TTCACAGATAAATGCTTGGG-3′) and FTPH3 (5′-AAGCTGGAGAGTGTGTA-3′) (Bensch and Hasselquist 1999). The polymerase chain reactions (PCRs) were performed in volumes of 25 µL and included 10–50 ng of total genomic DNA, 0.125 mM of each nucleotide, 1.5 mM MgCl₂, 0.6 µM of each primer (BCML4 and FTPH3) and 0.5 U AmpliTaq polymerase. The PCRs were run using the following conditions: 30 s at 94 °C, 30 s at 50 °C, 30 s at 72 °C (35 cycles). The PCR product was precipitated (NH₄Ac and ethanol) and then dissolved in 20 µL of water. For sequencing, we used 2–4 µL of the PCR product. Sequencing was done from both directions using the BigDye Terminator sequencing kit (Applied Biosystems) in an ABI Prism 3100 capillary sequencer (Applied Biosystems). The primers give a product of 577 bp and previous analyses have confirmed that 494 bp of this fragment are from the control region II and the 81 bp flanking the control region (3′) from the rRNA Phe gene (Bensch and Hasselquist 1999). Maternal inheritance of haplotypes in families and absence of double base calling (Bensch and Hasselquist 1999) confirm the mitochondrial (and not nuclear mitochondrial DNA) origin of this fragment. In total, 101 mitochondrial sequences from four wintering populations were generated specifically for the present study (Table 1). In addition, we also used mitochondrial control region II sequences from 281 Great Reed Warblers from 15 populations throughout the species’ breeding range (from Spain in the west to Kazakhstan in the east, and from Iran in the south to Sweden in the north; Table 1) that were available from a previous phylogeographic study (Hansson et al. 2008). The consensus alignment of all 382 mitochondrial control region II sequences was 489 bp (GenBank accession nos. KR055071–KR055452).

Population statistics were calculated using the program DnaSP 5.10.01 (Rozas et al. 2003) and haplotype diversity (H) and nucleotide diversity (π) were calculated according to Nei (1987). Genetic differentiation measured as FST (following Equation 3 in Hudson et al. 1992) between pairs of populations was also calculated in DnaSP 5.10.01 (sites with alignment gaps were considered as a fifth state). Bootstrap statistics for pair-wise genetic differentiation came from 506 permutations and were conducted in Arlequin 3.5 (Excoffier and Lischer 2010). We also used Arlequin for AMOVA analysis to partition the genetic variance to populations and regions (breeding vs non-breeding populations).

In addition, we calculated a haplotype sharing index (HSI) for each pairwise combination of wintering and breeding populations as the multiplied haplotype frequencies of each haplotype summed over all haplotypes (i.e. P[H1]Wintering × P[H2]Breeding + P[H2]Wintering × P[H2]Breeding + P[H3]Wintering × P[H3]Breeding + ...). The HSI-values were standardised for each wintering site by dividing each value with the maximum value for that wintering site (this resulted in HSI-values ranging between 0 and 1).

Results

The mitochondrial control region II contained 45 variable sites, which defined 72 different haplotypes. The haplotype diversity for all samples was 0.921 (range within populations: 0.286–0.947) and the nucleotide diversity 0.00765 (range within populations: 0.00117–0.00912) (Table 1). The four African wintering sites were intermediate in both haplotype and nucleotide diversity (Table 1).

The FST-based analyses showed that the Nigerian population was most similar, i.e. had lowest FST-values, to populations in Central Europe (FST < 0.01 compared to seven populations; Table 2). Analyses including all Nigerian birds or only those individuals that were caught in early spring produced very similar results (Supplementary Table S1). The populations from Botswana and Zambia were most similar to populations in Central and East Europe (Botswana: FST < 0.01 to nine populations; Zambia: FST < 0.05 to six populations), and the Kenyan population to the Middle East (FST < 0.05 to Turkey and Iran) (Figure 1, Table 2). Populations from Nigeria, Botswana and Zambia were most differentiated from Spain and the Netherlands in the west and to Kazakhstan, Turkey and Iran in the east (Table 2). However, the Kenyan population was highly differentiated (FST ≥ 0.29) from all populations except Turkey and Iran (Table 2).

P-values for the FST-values are given in Supplementary Table S2. An AMOVA showed that 0.8% of the variation was partitioned between regions (breeding vs non-breeding populations), 8.4% between populations and the remaining 90.8% within populations.

The haplotype sharing analyses showed a somewhat different pattern (Figure 2, Table 2). Nigeria had the best match (HSI > 0.6) to populations in West Europe (Spain, the Netherlands and Sweden), whereas Botswana showed similarities to West, Central and East Europe (Spain, the Netherlands, Sweden, Latvia, Belarus and Russia) and Zambia shared the highest proportion of haplotypes with populations from East Europe and the Middle East (Latvia, Bulgaria, Belarus and Iran). Kenya had the highest haplotype sharing to Bulgaria, Turkey and Iran (Figure 2, Table 2).
Table 2: Pair-wise genetic differentiation ($F_{ST}$) and haplotype sharing index (HSI) between African non-breeding populations and Eurasian breeding populations. The significance levels for the $F_{ST}$-values are given in Supplementary Table S2

|          | Nigeria | Botswana | Zambia | Kenya |
|----------|---------|----------|--------|-------|
|          | $F_{ST}$ | HSI      | $F_{ST}$ | HSI   | $F_{ST}$ | HSI | $F_{ST}$ | HSI |
| Spain    | 0.128   | 0.885    | 0.105   | 0.844 | 0.428    | 0.246 | 0.759    | 0.000 |
| Netherlands | 0.147   | 1.000    | 0.129   | 1.000 | 0.456    | 0.000 | 0.780    | 0.000 |
| Sweden   | 0.005   | 0.933    | −0.028  | 0.909 | 0.241    | 0.298 | 0.617    | 0.053 |
| Latvia   | −0.006  | 0.422    | −0.068  | 0.643 | 0.050    | 0.835 | 0.487    | 0.350 |
| Germany  | 0.007   | 0.444    | −0.043  | 0.564 | 0.171    | 0.345 | 0.555    | 0.246 |
| Czech Republic | −0.006 | 0.428    | −0.057 | 0.588 | −0.024   | 0.345 | 0.327    | 0.229 |
| Hungary  | 0.043   | 0.403    | 0.013   | 0.429 | 0.200    | 0.598 | 0.599    | 0.058 |
| Bulgaria | 0.041   | 0.227    | −0.015  | 0.571 | 0.001    | 0.802 | 0.291    | 0.642 |
| Greece   | −0.001  | 0.422    | −0.020  | 0.357 | 0.049    | 0.147 | 0.363    | 0.156 |
| Belarus  | −0.020  | 0.535    | −0.082  | 0.756 | 0.006    | 0.664 | 0.414    | 0.435 |
| Ukraine  | 0.005   | 0.354    | −0.055  | 0.476 | −0.029   | 0.326 | 0.364    | 0.389 |
| Russia   | 0.035   | 0.575    | −0.022  | 0.689 | 0.259    | 0.266 | 0.616    | 0.208 |
| Kazakhstan | 0.290  | 0.130    | 0.316   | 0.122 | 0.529    | 0.000 | 0.785    | 0.000 |
| Turkey   | 0.460   | 0.260    | 0.489   | 0.168 | 0.345    | 0.598 | 0.047    | 0.663 |
| Iran     | 0.550   | 0.111    | 0.580   | 0.000 | 0.426    | 1.000 | 0.045    | 1.000 |

Discussion

Our results support a clear but relatively weak migratory connectivity in Great Reed Warblers. The emerging pattern when combining the results from the analyses of $F_{ST}$-based differentiation and the haplotype sharing is that the population in (1) Nigeria (West Africa) had the best genetic match to breeding populations in West and Central Europe, whereas (2) Botswana (southern Africa) showed most similarities with West, Central and East Europe, (3) Zambia (southern Africa) with Central and East Europe and the Middle East, and (4) Kenya with East Europe and in particular the Middle East. The relatively weak connectivity in the present study corroborates previous indications from ringing recoveries from several European localities, which also suggest weak genetic connectivity and wide wintering areas for European birds (Yohannes et al. 2008). Moreover, a recent geolocator analysis found that Great Reed Warbler individuals from a single population in Sweden were distributed over a wide wintering range in West Africa, from Senegal in the west to south-western Chad in the north, and north-western Congo in the east (Lemke et al. 2013).

It is important to point out that the resolution of any marker-based connectivity analysis is set by the resolution of the population genetic differentiation over the breeding range: the stronger the population differentiation, the higher the ability to assign wintering individuals to distinct breeding populations. The strength of the present study lies in the species’ documented high mitochondrial population differentiation over the breeding range (Bensch and Hasselquist 1999; Hansson et al. 2008). This population structure has been largely influenced by postglacial expansions over the current breeding range from two glacial refugia, presumably located in western Europe and the Middle East, respectively (Bensch and Hasselquist 1999; Hansson et al. 2008). Thus, mtDNA-based methods are highly suitable for determining the origins of populations of wintering Great Reed Warblers. This is not the case for microsatellites, for which populations are much less differentiated (B Hansson unpublished data). However, the postglacial expansion also implies that populations share a common ancestry, which lower population structures due to ancestral haplotype sharing. Therefore, both ancestral and current demographic processes can influence the degree of population genetic structure and thus the power to use molecular markers to dissect the migratory links between breeding and wintering grounds.

Of the four wintering populations analysed in the present study, Kenya showed the most unique connectivity pattern, as it was highly associated with breeding populations in the Middle East, both when considering differentiation and haplotype sharing. In contrast, the other three populations showed more diffuse connectivity mainly to populations from West to East Europe. This strongly suggests that different Great Reed Warbler populations use different flyways and that there are restricted movements between at least some parts of the species’ wide winter range. For example, it seems as if birds from Central Europe take a south-westerly migration direction, and birds from the Middle East a southerly direction, a pattern that would facilitate the crossing of two challenging barriers, the Mediterranean Sea and the Sahara Desert. The possible separation in eastern and western flyways during migration and weak parallel connectivity, with at least some degree of restricted east–west movement, is consistent with results from studies of Nearctic–Neotropical passerines (Yellow Warbler Setophaga petechia [Boulet et al. 2006]; Wilson’s Warbler Wilsonia pusilla [Kimura et al. 2002]). It should be noted that none of the four wintering populations matched the breeding population in Central Asia (Kazakhstan), which indicates that the breeding populations in that region winter in parts of Africa that we did not sample, possibly in south-eastern Africa. Thus, it is possible that somewhat stronger connectivity in Great Reed Warblers would be supported if additional sites were included. Nevertheless, because the included African wintering sites were positioned over such a wide geographic area (West, South and East Africa), our present results offer strong support for weak migratory connections between breeding and wintering grounds.

Weak migratory connectivity in Great Reed Warblers
Figure 1: Degree of genetic similarity between non-breeding and breeding populations of Great Reed Warbler. Solid lines connect pairs of populations with $F_{ST} < 0.01$, and dashed lines pairs with $F_{ST} < 0.05$. (a) Nigeria, (b) Botswana, (c) Zambia and (d) Kenya.
may be explained by their colonisation history. After the last glaciation, less than 10,000 years ago, Great Reed Warblers colonised the breeding range from two refugia source populations – possibly located in western Europe and the Middle East (Hansson et al. 2008). It is possible that newly established populations have kept the same migration routes as their ancestral populations resulting in many breeding populations sharing the wintering range (cf. Ruegg and Smith 2002). Weak connectivity can also partly be explained by the Great Reed Warbler’s two-stage over-wintering pattern. Most Great Reed Warblers utilise two main wintering sites: first, stopping to undertake a complete moult before conducting a mid-winter movement to a second location for the remaining winter months (Hedenström et al. 1993; Lemke et al. 2013). Recently, geolocators have illustrated multi-stage migration in a number of Palearctic–African migrant passerines (Stach et al. 2012; Tøttrup et al. 2012), including the Great Reed Warbler (Lemke et al. 2013), suggesting that this migratory strategy may be widespread. This strategy could result

Figure 2: Bar charts showing pair-wise haplotype sharing index (HSI) between African wintering populations and Eurasian breeding populations for each wintering site. HSI-values > 0.6 are indicated. Analyses for Nigeria include data from all birds captured (February–April).
in connectivity patterns changing throughout the winter months and should be considered when interpreting results from migratory connectivity analyses.

In conclusion, we found clear but weak migratory connectivity in the Great Reed Warbler, which may reflect the demographic history of the species and its life-history strategies (e.g. natal dispersal and mid-winter movement). Weak connectivity suggests that the effects of local habitat deteriorations on the wintering grounds will be diffuse rather than concentrated (Taylor and Norris 2010) and that Great Reed Warbler breeding populations may be buffered against local extinctions.

Acknowledgements — Samples were kindly provided by J Waldenström (Nigeria), D Hasselquist, S Bensch (Kenya) and S Tyler (Botswana). We thank S Bensch and D Hasselquist for long-term support and M Tarka for laboratory work. This study was supported by grants from the Swedish Research Council (621-2007-5381; 621-2009-4945; 621-2014-5222), the Crafoord Foundation, the Oscar and Lili Lamm Foundation, the Carl Trygger Foundation, Lunds Djurskyddsfond and the Centre for Animal Movement Research (CAnMove; a Linnaeus excellence centre supported by the Swedish Research Council [349-2007-8690] and Lund University).

References

Backhurst GC, Pearson DJ. 1984. The timing of the southward right migration of Palearctic birds over Ngulia, southeast Kenya. In: Ledger J (ed.), Proceedings of the Fifth Pan African Ornithological Congress, held at Lilongwe, Malawi in 1980. Johannesburg: Southern African Ornithological Society. pp 361–369.

Bensch S, Bengtsson G, Åkesson S. 2006. Patterns of stable isotope signatures in willow warbler Phylloscopus trochilus feathers collected in Africa. Journal of Avian Biology 37: 323–330.

Bensch S, Hasselquist D. 1999. Phylogeographic population structure of great reed warblers: an analysis of mtDNA control region sequences. Biological Journal of the Linnean Society 66: 171–185.

Bensch S, Hasselquist D, Nielsen, Hansson B. 1998. Higher fitness for philopatric than for immigrant males in a semi-isolated population of great reed warblers. Evolution 52: 877–883.

Boulet M, Gibbs HL, Hobson KA. 2006. Integrated analysis of genetic, stable isotope, and banding data reveal migratory connectivity and flyways in the northern yellow warbler (Dendroica petechia; aestiva group). Ornithological Monographs 61: 29–78.

Chamberlain CP, Bensch S, Feng X, Åkesson S, Andersson T. 2000. Stable isotopes examined across a migratory divide in migratory connectivity in the western sandpiper Calidris mauri. Journal of Avian Biology 43: 155–167.

Gratto-Trevor C, Amirault-Langlais D, Catlin D, Cuthbert F, Fraser J, Maddock J, Roche E, Shaffer F. 2012. Connectivity in piping plovers: do breeding populations have distinct winter distributions? Journal of Wildlife Management 76: 348–355.

Hansson B, Bensch S, Hasselquist D, Nielsen B. 2002. Restricted dispersal in a long-distance migrant bird with patchy distribution, the great reed warbler Oecologia 130: 536–542.

Hansson B, Hasselquist D, Tarka M, Zetladnij P, Bensch S. 2008. Postglacial colonisation patterns and the role of isolation and expansion in driving diversification in a passerine bird. PLoS ONE 3: e2794.

Hasselquist D. 1998. Polygyny in the great reed warbler: a long-term study of factors contributing to male fitness. Ecology 79: 2376–2390.

Hedenström A, Bensch S, Hasselquist D, Lockwood M, Ottoison U. 1993. Migration, stopover and moult of the great reed warbler Acrocephalus arundinaceus in Ghana, West Africa. Ibis 135: 177–180.

Hedenström, A. & Pettersson, J. 1987. Migration routes and wintering areas of Willow Warblers Phylloscopus trochilus (L.) ringed in Fennoscandia. Ornis Fennica 64: 137–143.

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405: 907–913.

Hobson KA, van Wilgenburg SL, Faaborg J, Toms JD, Rengifo C, Llanes Sosa A, Aubry Y, Brito Aguilar R. 2014. Connecting breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopes: a call for an isotopic atlas of migratory connectivity. Journal of Field Ornithology 85: 237–257.

Hudson RR, Slatkin M, Maddison WP. 1992. Estimation of levels of gene flow from DNA-sequence data. Genetics 132: 583–589.

Iwamura T, Possingham HP, Chades I, Minton C, Murray NJ, Rogers DI, Tremli EA, Fuller RA. 2013. Migratory connectivity magnifies the consequences of habitat loss from sea-level rise for shorebird populations. Proceedings of the Royal Society B: Biological Sciences 280: 20130325. DOI: 10.1098/rspb.2013.0325.

Kennerley P, Pearson D. 2010. Reed and bush warblers. London: Christopher Helm.

Kimura M, Clegg S, Lovette I, Holder K, Girman D, Wade M. 2002. Phylogeographical approaches to assessing demographic connectivity between breeding and overwintering regions in a Nearctic–Neotropical warbler (Wilsonia pusilla). Molecular Ecology 11: 1605–1616.

Klaassen RHG, Hake M, Strandberg R, Koks B, Trierweiler C, Exo KM, Bairlein F, Alerstam T. 2014. When and where does mortality occur in migratory birds? Direct evidence from long-term satellite tracking of raptors. Journal of Animal Ecology 83: 176–184.

Kraus RH, Zeddeman A, Van HP, Sartakov D, Soloviev SA, Ydenberg RC, Prins HH. 2011. Evolution and connectivity in the world-wide migration system of the mallard: inferences from mitochondrion DNA. BMC Genetics 12: 99.

Lemke HW, Tarka M, Klaassen RHG, Åkesson M, Bensch S, Hasselquist D, Hansson B. 2013. Annual cycle and migration strategies of a trans-Saharan migratory songbird; a geolocator study in the great reed warbler. PLoS ONE 8: e79209.

Marra PP, Hobson KA, Holmes RT. 1998. Linking winter and summer events in a migratory bird by using stable-carbon isotopes. Science 282: 1884–1886.

Martin TG, Chades L, Arcese P, Marra PP, Possingham HP, Norris DR. 2007. Optimal conservation of migratory species. PLoS ONE 2: e751.
Received 25 January 2015, accepted 20 May 2015
Associate Editor: Victor Rambau