Transcontinental migratory connectivity predicts parasite prevalence in breeding populations of the European barn swallow

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Abstract

Parasites exert a major impact on the eco-evolutionary dynamics of their hosts and the associated biotic environment. Migration constitutes an effective means for long-distance invasions of vector-borne parasites and promotes their rapid spread. Yet, ecological and spatial information on population-specific host–parasite connectivity is essentially lacking. Here, we address this question in a system consisting of a transcontinental migrant species, the European barn swallow (*Hirundo rustica*) which serves as a vector for avian endoparasites in the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon*. Using feather stable isotope ratios as geographically informative markers, we first assessed migratory connectivity in the host: Northern European breeding populations predominantly overwintered in dry, savannah-like habitats in Southern Africa, whereas Southern European populations were associated with wetland habitats in Western Central Africa. Wintering areas of swallows breeding in Central Europe indicated a migratory divide with both migratory programmes occurring within the same breeding population. Subsequent genetic screens of parasites in the breeding populations revealed a link between the host's migratory programme and its parasitic repertoire: controlling for effects of local breeding location, prevalence of Africa-transmitted *Plasmodium* lineages was significantly higher in individuals overwintering in the moist habitats of Western Central Africa, even among sympatrically breeding individuals with different overwintering locations. For the rarer *Haemoproteus* parasites, prevalence was best explained by breeding location alone, whereas no clear pattern emerged for the least abundant parasite *Leucocytozoon*. These results have implications for our understanding of spatio-temporal host–parasite dynamics in migratory species and the spread of avian borne diseases.

Introduction

The study of host–parasite systems has a long tradition in the evolutionary sciences (Van Valen, 1973) and continues to be an active research field today (Altizer *et al.*, 2011). Examples are manifold including effects on

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inbreeding dynamics (Acevedo-Whitehouse *et al.*, 2003), sexual selection (Moore & Wilson, 2002), speciation (Filchak *et al.*, 2000; Eizaguirre *et al.*, 2009) on the host side and rapid adaptation on the side of the parasite (Weeks *et al.*, 2007). Of central importance to the dynamics and evolutionary significance of host–parasite interactions is their spatio-temporal distribution. Yet, our knowledge on host–parasite connectivity in a spatially explicit population context is highly limited (Altizer *et al.*, 2003) but see Dodge *et al.*, and Hellgren *et al.* (2013).

Transcontinental migration constitutes an extreme form of spatio-temporal heterogeneity where individuals cycle

between disjunct geographical areas and heterogeneous habitats. Every year, billions of long-distance migratory birds are potential vectors for exchanging pathogens and parasites between tropical areas and their temperate breeding grounds. Migration is a main natural cause for the rapid spread of infectious diseases across the globe (Newton, 2008; Altizer *et al.*, 2011) affecting not only the host, but also its associated biotic environment with the potential to significantly alter local evolutionary population dynamics (Fuller *et al.*, 2012). To predict the expected geographical spread and possible consequences of parasite infections, a better understanding of the connectivity between host and parasite populations is needed, both for overwintering areas and breeding grounds (Webster *et al.*, 2002).

To investigate this question, we here consider a host–parasite system consisting of a transcontinental migrant host species, the barn swallow *Hirundo rustica*, and arthropod-borne parasites of the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon* that during a part of the life cycle inhabit erythrocytes of the vertebrate host (Valkiunas, 2004). All three genera show a high degree of genetic lineage diversity and commonly infect bird species around the globe (Bensch *et al.*, 2009b; Loiseau *et al.*, 2012) exerting significant selection pressures (Marzal *et al.*, 2008; Knowles *et al.*, 2010; Asghar *et al.*, 2011).

Barn swallows from European populations are obligatory migrants and winter almost exclusively south of the Saharan desert in Africa (Turner, 2006). Although

explicit wintering areas of individuals from specific breeding populations are rarely known, extensive band recovery data suggest different wintering locations for Northern and Southern European populations (Ambrosini *et al.*, 2009). British, Scandinavian and north-eastern European breeding birds (hereafter referred to as Northern) presumably overwinter in savannah-like habitats in Southern Africa (Evans *et al.*, 2003; Ambrosini *et al.*, 2009), whereas breeding birds from Switzerland, Italy and Spain (hereafter Southern) overwinter in Central and Western Africa (Fig. 1a,b) (Saino *et al.*, 2004; Maumary *et al.*, 2007; Ambrosini *et al.*, 2009). In addition to the clear separation of Northern from Southern populations, previous data indicate that Central European populations show heterogeneous migration patterns (Ambrosini *et al.*, 2009) (Fig. 1c). This suggests the existence of a migratory divide in swallows (Hobson *et al.*, 2012a), a zone of secondary contact and admixture between neighbouring populations with divergent migratory behaviour that arose as a possible consequence of pleistocene glaciations (Newton, 2008; Bensch *et al.*, 2009a). Analogous to hybrid zones identified on the basis of other characters [e.g. steep morphological contrasts (Hewitt, 1988; Poelstra *et al.*, 2014)], migratory divides constitute a natural evolutionary experiment and are well suited to study interactions of host and parasite populations in a geographical context (Møller *et al.*, 2011; Santiago-Alarcon *et al.*, 2011).
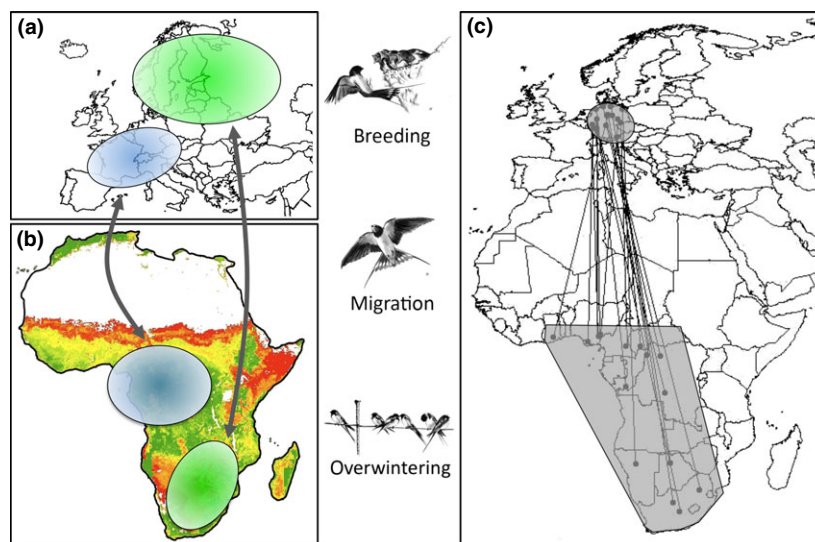
Fig. 1 Geographical context of barn swallow breeding and wintering ranges. (a) Breeding ranges of Southern (blue) and Northern (green) breeding populations. The allopatric samples for this study were collected from locations in Switzerland and Sweden (Fig. S1). (b) Expected wintering range of Southern (blue) and Northern European (green) breeding populations in Africa on the basis of banding data (Ambrosini *et al.*, 2009). Colour coding indicates modelled distribution of plant δ^{13}C stable isotope ratios, that is warmer colours illustrate higher δ^{13}C values (Still & Powell, 2010). These are used to predict population-specific isotope ratios from feathers grown in Africa during the winter (Figs S2 and S3). (c) Breeding range of Central European populations. Capture and recovery locations of banded birds from Germany and Austria (dots) are connected with a line. The banding data suggests a broader, wintering range overlapping with wintering ranges from both Northern and Southern populations (Table S1).

In this study, we examined host–parasite connectivity across a range of barn swallow breeding populations including allopatric Southern and Northern populations and populations from within the migratory divide. In a first step, we quantified migratory connectivity of these populations with their African overwintering locations using a combination of banding information and stable isotope data from feathers grown in Africa. Stable isotopes are powerful biomarkers for linking breeding and wintering areas of migratory species worldwide (Hobson & Wassenaar, 2008). They have successfully been applied in birds [including swallows; (Hobson *et al.*, 2012a)] to pinpoint overwintering location (Chamberlain *et al.*, 2000; Rubenstein *et al.*, 2002; Bearhop *et al.*, 2005), habitat (Evans *et al.*, 2003; Yohannes *et al.*, 2008) and predict the position of migratory divides (Chamberlain *et al.*, 2000). In the swallow system, expectations for carbon and nitrogen stable isotope ratios (δ^{13}C and δ^{15}N) can be derived by mapping ring recovery data from overwintering locations on isotope maps. Northern European individuals overwintering in savannah-like habitat characterized by ^{13}C-enriched C4 plants of Southern Africa are expected to show relatively high δ^{13}C values, whereas Central African overwintering grounds used by Southern European breeding populations should be depleted in ^{13}C. For δ^{15}N, a positive correlation has been predicted for Southern Africa (Van der Merwe *et al.*, 1990; Szép *et al.*, 2009), whereas a negative correlation has been observed for Central Africa (Bensch *et al.*, 2006; Veen *et al.*, 2007).

Subsequently, we screened for prevalence and diversity of haemosporidian parasite lineages and investigated the poorly established link between breeding location, overwintering habitat and intercontinental pathogen transfer. In that we made use of the system's property of population divergence in migration programmes and their admixture in a Central European migratory allowing to separate breeding-population-specific effects on pathogen prevalence from effects specific to the conditions in Africa. Given the large ecological differences between Southern and Western Central Africa, differences in parasite communities between the two overwintering areas ought to be expected. Notably, large differences in humidity should affect incidence and prevalence of infection with mosquito-transmitted endoparasites like avian malaria and other haemosporidian parasites (Valkiunas, 2004). We hypothesized that populations with different wintering areas would differ in prevalences of Africa-transmitted parasites. For populations and individuals wintering in humid areas of Africa, we predicted higher individual infection risk than for those wintering in dryer areas. No difference should be expected for parasitic lineages where arthropod vectors and/or parasite survival capacity are not restricted to the overwintering location.

Materials and methods

Study sites and sampling

Breeding birds were captured between May and August in colonies at five different sites corresponding to Northern (Sweden, $n_{colonies} = 2$), Central (Northern Germany: Itzehoe and Greifswalder Oie $n_{colonies} = 50$) and Southern (Southern Germany, $n_{colonies} = 6$; Switzerland, $n_{colonies} = 10$) European populations in years 2006–2009 (Fig. S1). Central colonies were chosen in close proximity to the location of a central European suture zone common to many species (Remington, 1968). In the absence of knowledge on the precise geographical location of the migratory divide, this constitutes a good approximation consistent with the assumption of a Pleistocene glacial origin (Møller *et al.*, 2011). Every colony essentially reflects a single farm, with one exception being the small (0.54 km^2) island Greifswalder Oie (Fig. S1) located 10 km off the German coast in the Southern Baltic Sea, which is considered a single colony. We here report the results for joint analyses of populations (Northern: Sweden, Central: Itzehoe, Greifswalder Oie, Southern: Switzerland, Southern Germany, Fig. S1); the results remain qualitatively the same if populations are treated separately. Every bird was individually marked and before release, feather (adult birds: $n_{Northern} = 50$, $n_{Central} = 695$, $n_{Southern} = 89$) and blood samples were collected (adult birds: $n_{Northern} = 50$, $n_{Central} = 435$, $n_{Southern} = 90$; 1st-year birds: $n_{Central/Greifswalder\ Oie} = 17$). Sample size information by site is given in Table S2.

Stable isotope analysis

For stable isotope analysis, 5–20 small body feathers were picked from the upper back, stored in paper envelopes in the field and later kept in a freezer at −20 °C. European barn swallows of the study populations undergo a complete moult in their African wintering areas (Jenni & Winkler, 1994). Feathers from the upper back are predominantly moulted between December and February in Africa (Broekhuysen & Brown, 1963; Ginn & Melville, 1983) when migratory movements are supposedly minimal and probably do not exceed an average daily foraging radius of 50 km around the night roost (Oatley, 2000). Moreover, δ^{13}C and δ^{15}N are inert in feather keratin once feather growth has finished (Hobson & Clark, 1992). Feather stable isotope ratios are thus expected to represent local habitat features of the overwintering habitat with high confidence (Evans *et al.*, 2003; Møller & Hobson, 2004). Prior to analysis, following the procedure described by Evans *et al.* (2003), single feathers were washed in 0.5 M sodium hydroxide solution, rinsed in distilled water and dried. Single clean feathers were weighed (~0.5 mg) into tin cups and analysed for δ^{13}C and δ^{15}N. Measurements of δ^{13}C and δ^{15}N were reported

relative to the international standards Vienna PeeDee Belemnite (VDPB) and AIR, respectively.

We measured four feathers from individual birds ($n = 6$) and calculated individual isotopic repeatability (Lessells & Boag, 1987). Because standard errors of the mean $\delta^{13}C$ and $\delta^{15}N$ ratios of repeated measures were very close (feather $\delta^{13}C$: 0.2 ‰; internal laboratory standard $\delta^{13}C$: 0.1 ‰) or equal (feather $\delta^{15}N$: 0.3 ‰; internal laboratory standard $\delta^{15}N$: 0.3 ‰) to the standard errors of the mean $\delta^{13}C$ and $\delta^{15}N$ ratios calculated in the same way from repeated measures of the internal laboratory standards (fish muscle tissue (*Rutilus rutilus*), mean $\delta^{13}C$: −27.6 ‰, mean $\delta^{15}N$: 7.2 ‰), we suggest that the $\delta^{13}C$ and $\delta^{15}N$ ratios of a single feather adequately reflect the diet over the moulting season (Evans *et al.*, 2003).

Expected distribution of stable carbon isotope values

Based on climate data and vegetation cover of C3 and C4 plants, Still & Powell (2010) modelled the distribution of $\delta^{13}C$ values for plants across Africa. Using Arc-GIS 7.2, we derived spatially explicit distributions of expected plant $\delta^{13}C$ values for the wintering areas predicted for our study populations based on an extensive analysis of band recovery data (Ambrosini *et al.*, 2009). More specifically, we superimposed the geographical positions of ellipses numbers 1.2 and 2.1 from Figure 5b in Ambrosini *et al.* (2009) on the modelled isoscape (as in Fig. 1b) from Still & Powell (2010) to extract the expected $\delta^{13}C$ values from the selected grid cells ($n_{grid\ cells\ ellipsis1.2} = 3\ 924\ 396$, $n_{grid\ cells\ ellipsis2.1} = 2\ 895\ 727$). The European sampling sites of our Northern and Southern study populations lay well within the corresponding ellipses superimposed on Europe in the same figure [numbers 1.2 and 2.1 in Figure 5b in Ambrosini *et al.* (2009)]. We repeated the procedure for band recovery information from Germany and Austria and the depicted polygon in Fig. 1c (Table S1) to extract the corresponding information for our Central European populations ($n_{grid\ cells\ polygon\ Fig.\ 1c} = 8\ 774\ 722$). For the calculation of the expected $\delta^{13}C$ distributions, we truncated the extreme ends of the raw data by the mean carbon isotope values for C3 and C4 plants (−27 and −12 ‰, respectively), because these values greatly dominated the data and were hardly represented in any of the feather isotope samples.

Assignment of wintering grounds using stable isotope data

We used discriminant function analysis to assign Central European barn swallows into Northern-like, Intermediate and Southern-like individuals. The $\delta^{13}C$ and $\delta^{15}N$ ratios of Northern and Southern barn swallows served hereby as learning data sets. Group-specific posterior probabilities of assignment of larger than 90% were used as a cut-off to assign individuals into Northern-like or Southern-like groups, whereas if individual group-specific posterior probabilities were below 90%, individuals were assigned as intermediates, that is individuals which cannot be assigned with high confidence to either Northern-like or Southern-like groups. The 90% cut-off was chosen arbitrarily and constitutes a good compromise for the trade-off between sample size and assignment precision. For the large-scale geographical assignment of individual moulting and wintering areas, we further used a recently developed clustering method (Hobson *et al.*, 2012c). As an *ad hoc* approach, we calculated mean population $\delta^{13}C$ and $\delta^{15}N$ ratios for the Northern (Swedish) and Southern (Swiss) populations and used these as input data for the clustering, separately. Although some geographical clusters show a dispersed pattern across sub-Saharan Africa (Hobson *et al.*, 2012c), information from band recovery data still allows a geographical placement; Northern barn swallows largely winter south of 17°S latitude and Southern barn swallows winter predominantly north of 17°S latitude (Ambrosini *et al.*, 2009).

Haemosporidian parasites – classification and geographical origin

Total DNA from avian blood was extracted using a standard chloroform/isoamyl alcohol extraction method (Sambrook & Russell, 2001). For parasite detection, we used a nested PCR protocol (Hellgren *et al.*, 2004). Amplification success was evaluated by running 2.5 µL of the final PCR product on a 2% Agarose gel. PCR products of positive samples were sequenced using Big-Dye end terminator sequencing (ABI 3730). Editing and alignment of sequences was performed using the software BIOEDIT (Hall, 1999). The resulting cytochrome *b* sequences were compared to data from the *MalAvi* database (Bensch *et al.*, 2009b) and were assigned to any of the three avian malaria genera mentioned above (Table S2 and Fig. S4). All lineages are reported in Table S2, and sequences from new lineages were deposited in GenBank accession numbers KP696485-98.

To examine how overwintering habitat influences rates of avian malaria transmission, considerations need to be restricted to parasite lineages known to be transmitted exclusively during the wintering season in Africa. Information about transmission areas for most of the *Haemoproteus* and *Leucocytozoon* lineages found is scarce or absent (Bensch *et al.*, 2009b) due to the fact that they were previously unknown or only occasionally observed. However, most *Plasmodium* lineages detected in this study are already known from different bird species and knowledge about their transmission area has accumulated (Bensch *et al.*, 2009b). Strict sub-Saharan transmission was established as follows: we classified a lineage as being exclusively transmitted in

Africa (i) when it has never been recorded in juvenile individuals of any bird species in Europe, (ii) when no record has ever been made in any resident or migratory bird species which do not winter in sub-Saharan Africa and (iii) when none of the lineages was found in juvenile barn swallows aged up to 2 months that had never been in Africa ($n = 17$). For the analysis of infection risk with *Plasmodium* sp. in different wintering areas, we restricted our analysis to lineages found in adult birds in at least two of the three study areas (Northern, Central, Southern) or in at least three of five study sites (Table S2) to avoid any spurious effects of parasite spillover (Hellgren *et al.*, 2009). The approach rests on the reasonable assumption that birds are mostly infected on the overwintering grounds rather than during the much shorter migration period (Liechti *et al.*, 2014). To test for differences in parasite prevalence according to wintering location, we used generalized linear models with a binomial error structure and logit link function. All statistical analyses were conducted in R (Ihaka & Gentleman, 1996).

Results

Migratory connectivity of the host species

Estimates of stable isotope ratios from feathers grown in Africa were used to predict overwintering habitat and location (see Materials and methods). High repeatability estimates (sensu Lessells & Boag, 1987) of both δ^{13}C and δ^{15}N ratios for feathers sampled from the same individual and year suggested that δ^{13}C and δ^{15}N ratios of a single feather adequately reflect the diet over the moulting season (carbon: repeatability $R = 0.99$, $F_{5,18} = 397.65$, $P < 0.001$; nitrogen: repeatability $R = 0.77$, $F_{5,18} = 14.68$, $P < 0.001$). Significant autocorrelation across multiple years further indicated that individuals returned to locations in similar isoscapes ($n_{individuals} = 80$, carbon: $R = 0.85$, $F_{79, 112} = 14.97$, $P < 0.001$; nitrogen: $R = 0.48$, $F_{79, 111} = 3.18$, $P < 0.001$).

Observed δ^{13}C values of individuals from Northern breeding population were significantly higher (enriched in ^{13}C) than those from Southern European populations (Fig. 2, Welch's *t*-test: $t = -9.01$, d.f. = 77.14, $P < 0.001$) which suggests migration to more savannah-like overwintering areas for Northern European birds and more humid locations for Southern European birds. We also found significant correlations between expected and observed distributions (see Materials and methods) of δ^{13}C from both samples from Northern ($n = 50$, $r_{Pearson} = 0.65$, $P = 0.019$) and Southern breeding areas ($n = 89$, $r_{Pearson} = 0.92$, $P < 0.001$, Fig. S3b). Interarea correlations serving as a control negative were nonsignificant (Southern$_{exp}$ vs. Northern$_{obs}$: $r_{Pearson} = -0.52$, $P = 0.083$, Northern$_{exp}$ vs. Southern$_{obs}$: $r_{Pearson} = -0.28$, $P = 0.404$) suggesting that δ^{13}C ratios
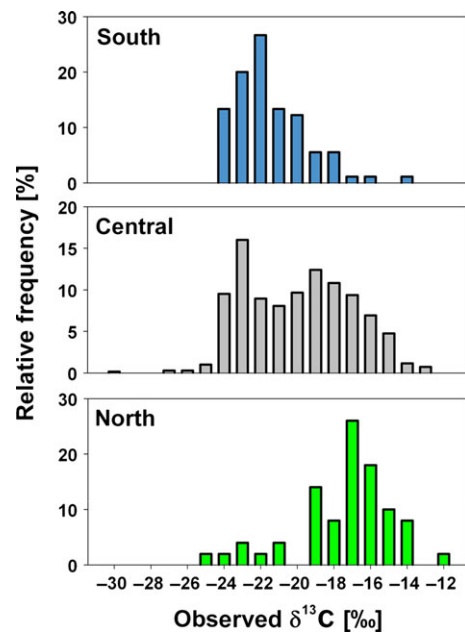

Fig. 2 Observed distributions of δ^{13}C stable isotope ratios from feathers moulted in the wintering area of individuals from Southern (top), Central (middle) and Northern (bottom) breeding locations in Europe. For expected distributions, see Fig. S3.

serve as a reliable indicator for habitat use and for geographically where in Africa an individual bird can be found in the winter. This observation falls square within the expectations derived from ring recovery data (Fig. 1a,b).

It has further been suggested that not only single stable isotope ratios, but correlations between δ^{13}C and δ^{15}N ratios show discrete geographical patterning across Africa: positive correlations being common in Southern African regions and negative correlations further North (see Discussion). The swallow isotopic data recaptured this pattern (Fig. 3). Northern European birds that overwinter in Southern Africa showed a positive correlation between carbon and nitrogen ($n = 50$, $r_{Pearson} = 0.45$, $P < 0.001$). Birds from Southern breeding grounds that overwinter in Central Africa showed a negative correlation ($n = 89$, $r_{Pearson} = -0.47$, $P < 0.001$). Using the method of Hobson *et al.* (2012c) with population level mean δ^{13}C and δ^{15}N ratios, breeding birds from Northern Europe were placed with high probabilities into Southern Africa (cluster 2-2, Table S3) and Southern European breeding birds into 'Western Central Africa' (cluster 2-3, see Table S3), respectively. These results, together with high repeatability estimates of both δ^{13}C and δ^{15}N led us to conclude that δ^{13}C and δ^{15}N ratios provide a solid proxy for in which habitat and geographical area an individual can be found during the course of several years. Barn swallows from Northern European breeding areas
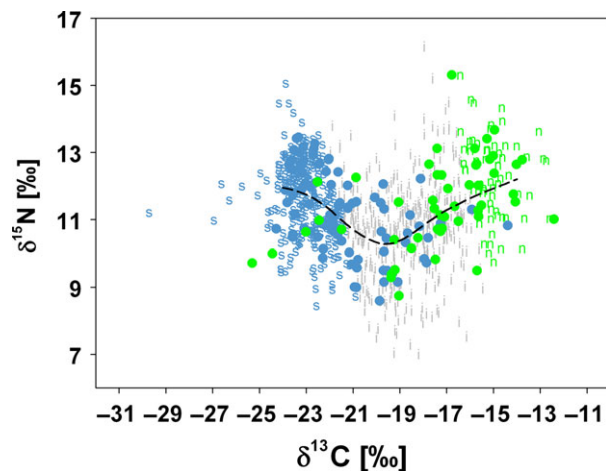
Fig. 3 : Relationship between carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotope ratios from feathers grown in the African overwintering habitat. Northern European populations (green dots) migrating to Southern Africa show a positive correlation, and Southern European populations (blue dots) show a negative correlation. Feather samples from within the migratory divide in Central European populations span the entire range and can be classified into Northern-like (green 'n'), Southern-like (blue 's') and Intermediate (grey 'i').

appear to spend the winter predominantly in dry areas in Southern Africa, Southern European birds in wet Central Africa.

In contrast to Northern and Southern European breeding populations, band recovery data from geographically intermediate populations in Central Europe suggest that individuals use the whole range of Central-Western to Southern African wintering locations (Fig. 1c). This scattered overwintering area was reflected in δ^{13}C and δ^{15}N ratios spanning the whole range of values observed in Southern and Northern populations combined (Fig. 1a–c). We observed an intriguing bimodal distribution of δ^{13}C values with peaks corresponding to δ^{13}C values recorded from Northern and Southern breeding populations (Fig. 2). Again, expected distributions inferred on the basis of banding data (see Fig. 1c) and observed δ^{13}C values were strongly correlated ($n = 695$, $r_{Pearson} = 0.70$, $P = 0.004$, Fig. S3b). Notably, the correlation was almost identical for two relatively distant (290 km) German populations located at similar latitude (Fig. S3b).

Breeding birds from the Central European populations further showed an expected composite pattern for the covariance of δ^{13}C and δ^{15}N ratios indicated by the U-shaped curve in Fig. 3. On either side of the (local) minimum, the respective correlations observed for Southern and Northern breeding populations were found (Fig. 3; positive correlation on the right side of the minima: $r_{Pearson} = 0.42$, $t = 8.55$, d.f. = 348, $P < 0.001$; and nega-

tive correlation on the left side of the minima: $r_{Pearson} = -0.43$, $t = -8.76$, d.f. = 339, $P < 0.001$). Using the discrete signatures of the allopatric Southern and Northern breeding populations as the learning data sets in a discriminant function analysis, we subsequently classified breeding birds from the Central breeding grounds in Northern Germany into 'Southern-like (s)', 'Northern-like (n)' and 'Intermediates (i)' (Fig. 3). This division was supported by the clustering approach developed by Hobson *et al.* (2012c) (Table S3).

Avian malaria and related parasites in the barn swallow

Screening blood samples of 575 adult swallows for avian malarial parasites, we detected 27 mitochondrial cytochrome b lineages from the three genera *Plasmodium* ($n = 9$), *Haemoproteus* ($n = 9$) and *Leucocytozoon* ($n = 9$) (Table S2 and Fig. S4). Most *Plasmodium* lineages could be attributed to already known isolates from different host species (7/9 known), whereas most lineages belonging to *Haemoproteus* (4/9 known) and *Leucocytozoon* (2/9 known) were previously unknown (Bensch *et al.*, 2009b). The most commonly observed lineages belong to *Plasmodium* sp. (overall prevalence 12.2%), whereas *Haemoproteus* sp. (3.8%) and *Leucocytozoon* sp. (2.4%) were far less prevalent. For 5 *Plasmodium* lineages, exclusive African transmission could be established with high confidence (Table S2, see Materials and methods). We did not find any infections with avian malaria or related parasites in 1st-year birds ($n = 17$).

Influence of overwintering range on parasite prevalences

To investigate potential differences in parasite connectivity, we first used the Northern and Southern breeding populations to test for differences in prevalence of avian malaria parasites. Prevalence of *Plasmodium* parasites exclusively transmitted in Africa was significantly higher in birds of Southern European origin that overwinter in moist areas than in Northern European birds that overwintered in dryer Southern Africa over winter (Fig. 4a, Table 1). The pattern observed in the Northern and Southern populations was confirmed in the populations from Central Europe where local effects in the breeding populations can be excluded: individuals from Central German populations with lower δ^{13}C values had a significantly higher probability of being infected than individuals that showed higher δ^{13}C values (Fig. 4b, Plasmodium: GLM: $\chi^2 = 13.76$, d.f. = 1, $p_{carbon} < 0.001$). No such pattern was detected for neither Haemoproteus nor Leucocytozoon parasites (GLM: $\chi^2 = 0.21/0$, d.f. = 1/1, $p_{carbon} = 0.647/0.993$, respectively, Table 1 and Fig. S5). Prevalence of Africa-transmitted parasites was highest in Southern-like individuals, intermediate in those individuals that could
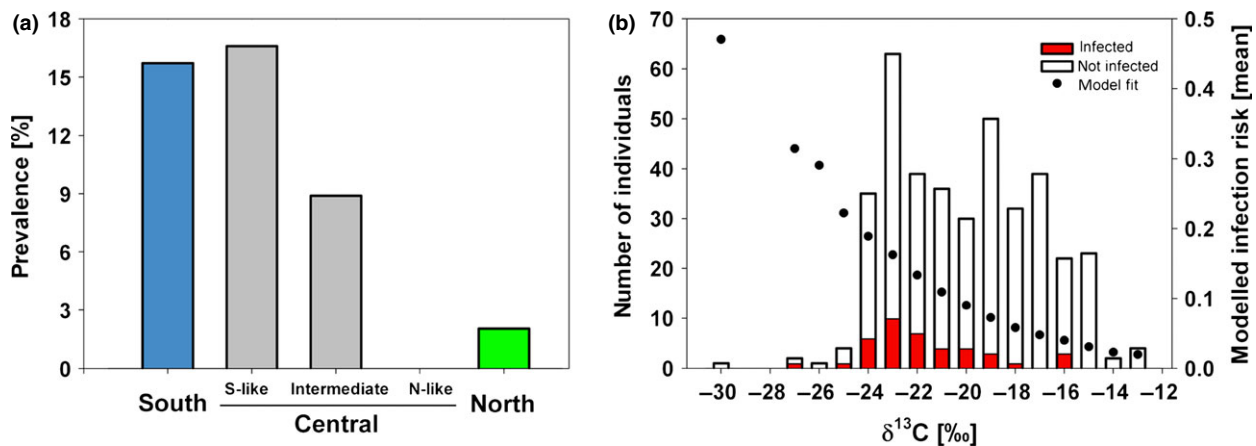
Fig. 4 Prevalence of Africa-transmitted *Plasmodium* sp. lineages shown (a) in relation to the European breeding area and (b) as a function of individual carbon isotope ratio of breeding birds from northern Germany. Individuals from Southern (S, n = 89) breeding populations overwinter in moist Central Africa, of Northern (N, n = 50) populations in dry Southern Africa (compare Figs 1–3). Individuals from intermediate Central German populations are classified by their carbon and nitrogen stable isotope ratios into Northern-like (N-like, n = 144), Southern-like (S-like, n = 38) or intermediate (n = 201). Lower δ^{13}C ratios are indicative of moister, C3-plant dominated wintering areas, whereas higher δ^{13}C ratios are hint towards a dryer, savannah-like wintering area.

Table 1 Comparison of endoparasite prevalence between allopatric Northern (N) and Southern (S) European breeding populations and between individuals of the sympatric Central European breeding population that are classified by their putative overwintering habitat into Northern-like (N-like), intermediate (Int) and Southern-like (S-like) (cf. Figs 1 and 3). The direction of effect sizes is indicated by symbols (<, =, >). Statistical values of the logistic regression (χ^2-statistic, degrees of freedom and type I error probabilities) are given below.

	Parasite genera	Allopatric Northern vs. Southern Europe	Sympatric N-like, intermediate, S-like
Europe or Africa transmitted	*Haemoproteus* spp.	N > S χ^2 = 5.3818, d.f. = 1, P = 0.020	N-like = Int = S-like χ^2=0.450, d.f.=2, P = 0.798
	Leucocytozoon spp.	N = S χ^2 = 0.2374, d.f. = 1, P = 0.633	N-like < Int > S-like χ^2 = 8.373, d.f. = 2, P = 0.015
Exclusively Africa transmitted	*Plasmodium* spp.	N < S χ^2= 5.3284, d.f. = 1, P = 0.021	N-like < Int < S-like χ^2 =13.406, d.f. = 2, P = 0.001

not be classified ('i' in Fig. 3) and lowest in Northern-like individuals (Fig. 4a, Table 1).

Transmission patterns of *Haemoproteus* and *Leucocytozoon* parasites showed no relationship with the overwintering habitat (Table 1, Fig. S5). For *Haemoproteus*, prevalence was significantly higher in swallows of Northern origin (Table 1, Fig. S5) than in Southern European populations. Prevalence in individuals from Central German populations differing in overwintering location was undistinguishable (Table 1). Prevalence of *Leucocytozoon* within Central breeding birds from Germany was highest in individuals with intermediate migratory behaviour (Table 1, Fig. S5).

Discussion

In this study, we examined migratory connectivity in the barn swallow and its implications for host–parasite association. We provide independent evidence for high migratory connectivity between European breeding populations and their African overwintering grounds and support the existence of a migratory divide in Central Europe (Ambrosini *et al.*, 2009; Hobson *et al.*, 2012a). We further characterized the endo-parasite haemosporidian fauna, and for avian malaria parasites disclosed the relationship between migration and host–parasite connectivity.

Isotopes as a proxy for bird migration in a Palearctic–Afrotropical migration system

As opportunistic aerial foragers, barn swallow feather stable isotope values integrate general habitat characteristics (Evans *et al.*, 2003). In contrast to species with small-scale micro habitat choice (Chamberlain *et al.*, 2000; Veen *et al.*, 2007; Yohannes *et al.*, 2008), isotopic values reflect broad scale geographical patterns well. Calibration of stable isotope ratios with a large number

of band recoveries (Northern and Southern Europe: Ambrosini *et al.* (2009), Central Europe: our own ring recovery data, Appendix: Table A1) places this study in a unique position to predict wintering location with good accuracy. Several lines of evidence support this conclusion: (i) repeatabilities within and among years were high and (ii) carbon isotope profiles of disjunct Northern and Southern breeding populations were highly correlated with the prediction from ringing data. Disjunct wintering areas were further reflected in a marked bimodal distribution of birds from the migratory divide with co-occurring migratory programmes (Fig. 2). (iii) $\delta^{13}C$ and $\delta^{15}N$ ratios showed a negative correlation for Southern breeding populations and a positive correlation for Northern breeding populations. Importantly, birds from the migratory divide showed a U-shaped carbon-to-nitrogen relationship including both negative and positive correlations consistent with a population consisting of birds using two different overwintering areas. Such decoupling of N and C has been observed elsewhere: positive correlations have been reported by other wide ranging animals from Southern Africa, for example elephants (Van der Merwe *et al.*, 1990; Szép *et al.*, 2009), whereas negative correlations seem to reflect areas in West and Central Africa (Bensch *et al.*, 2006; Veen *et al.*, 2007), but see Evans *et al.* (2003). These differences reflect the existence of contrasting isoscapes across the wider geographical area of Africa. Explanations for the spatial variation in baseline $\delta^{13}C$ and $\delta^{15}N$ values that drive African isoscapes are diverse and include variation in precipitation, plant community composition and coverage (e.g. C3 vs. C4, relative amount of agricultural crops), the extent of nitrogen fixation and proximity to saline environments (Heaton, 1987; Still & Powell, 2010; Wang *et al.*, 2010). The positive correlation between $\delta^{15}N$ and $\delta^{13}C$ found in Southern Africa (and northern swallows here) likely reflects a combination of the shift in $\delta^{13}C$ values between ^{13}C-depleted C3 plants (e.g. broad leaved plants of the wooded savannah) and ^{13}C-enriched C4 plants (e.g. grasses and sedges), with the negative correlation between both foliar $\delta^{13}C$ and $\delta^{15}N$ and water availability (Heaton, 1987; Swap *et al.*, 2004; Still & Powell, 2010; Wang *et al.*, 2010). The negative relationship between $\delta^{15}N$ and $\delta^{13}C$ within the southern birds is less easy to explain. As the southern birds have feather $\delta^{13}C$ values < −21 ‰, the factors driving the pattern are likely largely restricted to those influencing C3 plants (Swap *et al.*, 2004). As noted above, at large spatial scales, C3 plants typically show a negative relationship in both $\delta^{13}C$ and $\delta^{15}N$ with precipitation (Heaton, 1987; Swap *et al.*, 2004; Still & Powell, 2010) indicating that the variation is not driven by a large-scale gradient of water availability (see left-hand side of Fig. 3, where $\delta^{13}C$ increases, whereas $\delta^{15}N$ decreases). However, the sign of the relationship between water availability and foliar

$\delta^{15}N$ can switch under certain localized conditions, such as in mesic areas surrounded by xeric habitats (Handley *et al.*, 1999). Flanagan *et al.* (1997) also reported on ^{13}C depletion in plants from well-shaded mesic habitats found in xeric zones. The fact that other studies have reported a similar negative correlation between feather $\delta^{13}C$ and $\delta^{15}N$ in West and East Africa indicates that the region is worthy of further study in terms of understanding the factors driving isoscapes in the region.

Overall, the above-mentioned evidence support that carbon and nitrogen stable isotope ratios can be used as reliable indicators of the large-scale geographical wintering area of swallows in this Palearctic–Afrotropical migration system (Evans *et al.*, 2003; Møller & Hobson, 2004).

Although the current study was interested in broad patterns of habitat use, and how this affected transmission patterns for key parasites, future work may consider the use of alternative markers of overwintering habitat such as δ^2H (Hobson *et al.*, 2012a,b) and newly developed spatially explicit models using a Bayesian approach (Hobson *et al.*, 2014; Veen *et al.*, 2014) to further refine overwintering location and parasite transmission risk.

Avian migration, disease ecology and evolutionary implications

The set-up of breeding populations with divergent and mixed migratory behaviour enabled us to investigate overwintering location effects on host population–parasite association while excluding local breeding ground effects. Using this approach, the study provides evidence for an effect of winter location on the prevalence of *Plasmodium* parasites transmitted in the wintering area and transported into the breeding grounds. Transmission of *Haemoproteus* and *Leucocytozoon* lineages was not necessarily restricted to the overwintering habitat, and we consequently did not find similar associations. For *Plasmodium*, however, Southern breeding populations wintering in humid Central African areas were significantly more likely to be infected and carry parasites into Europe than northern populations wintering in dryer Southern African regions. Individuals from central European populations showed a mixed signature being best explained by overwintering habitat.

Host–parasite systems spanning across migratory divides can have interesting evolutionary implications (see also (Dodge *et al.*, 2013). As long as parasite transmission is limited to the overwintering habitat as was the case for the most common *Plasmodium* lineages here, the divide can serve as a barrier to parasite dispersal and impose divergent selection regimes. These in turn are expected to facilitate habitat specific adaptations and might eventually drive ecological speciation even in the face of gene flow (Rundle & Nosil, 2005). Rolshausen *et al.* (2009), for example, suggested that

differential migratory orientation in blackcaps *Sylvia atricapilla* rapidly generated higher genetic and phenotypic divergence between sympatric migratory 'eco-'types than between allopatric populations in blackcaps. Selection pressures, however, remain uncertain: Santiago-Alarcon *et al.* (2011) found no association between migration route and endoparasite prevalence, but instead suggested intraparasitic interactions patterns. Future studies on relative fitness of migratory types in particular with respect to parasite fauna (Møller *et al.*, 2004) provide interesting research avenues to follow.

Broader considerations

Information on host–parasite association and the ecological conditions for interpopulation spread are important not only from an evolutionary perspective. Parasites such as malaria, influenza or West-Nile viruses also have a large impact on natural ecosystem function (Hochachka & Dhondt, 2000; LaDeau *et al.*, 2007; Atkinson & LaPointe, 2009), livestock farming and human health (Altizer *et al.*, 2011). Our findings illustrate the need to understand both migratory connectivity (Webster *et al.*, 2002) and infection risk in the wintering habitat to be able to predict parasite prevalence and future infection risks in the breeding areas. Most of the detected African-transmitted *Plasmodium* lineages are not host specific (Bensch *et al.*, 2009b), as was also the case here, and will be carried into Europe by other long-distance migrants (Hellgren *et al.*, 2007). The spatial distribution of parasite influx will thus depend on the migratory connectivity and overwintering habitat of many bird species and clearly motivates further research in this direction. In particular, under a scenario of increasing future temperatures across Europe (Alcamo *et al.*, 2007), we expect a simultaneous increase in both vectors and transmission success of parasites (Garamszegi, 2011). Changing environmental conditions can expand the distribution of vectors or generate more benign conditions for the development in already ubiquitous vectors (Loiseau *et al.*, 2012) in the breeding ranges. This can have far-reaching eco-evolutionary consequences, as parasites significantly contribute to host population dynamics including species extinctions (Daszak *et al.*, 2000). Integrating the information from models on environmental change, migratory connectivity and eco-evolutionary dynamics of diseases and their vectors will be instrumental in identifying zones of elevated risk and will be a necessary first step to take early action.

Acknowledgments

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Geographic locations of the breeding populations studied corresponding to Northern, Central and Southern breeding populations.

Figure S2 Map of expected plant δ^{13}C stable isotope values for Africa (Still & Powell, 2010; A) and two-isotope isoscape for Africa, derived from cluster analysis of the δ^{13}C and δ^{15}N isoscapes (Hobson *et al.*, 2012c; B).

Figure S3 Distributions (a) and correlations (b) of observed and expected δ^{13}C ratios (‰).

Figure S4 Neighbour joining tree of cytochrome b sequences representing the parasite lineages.

Figure S5 Prevalence of all (a) *Haemoproteus* sp. and (b) *Leucocytozoon* sp. found in allopatric Northern (N,

green), Southern (S, blue) and Central (N-like, S-like, intermediate, grey) European breeding populations.

Table S1 Ring-recovery data of barn swallows shown in Fig. 1c.

Table S2 Summary of blood parasite lineages (cyt *b*) found in European barn swallows breeding in the study areas (N – north, C – central, S – south).

Table S3 Assignment of barn swallows from different breeding populations according to the approach described in Hobson *et al.* (2012c).