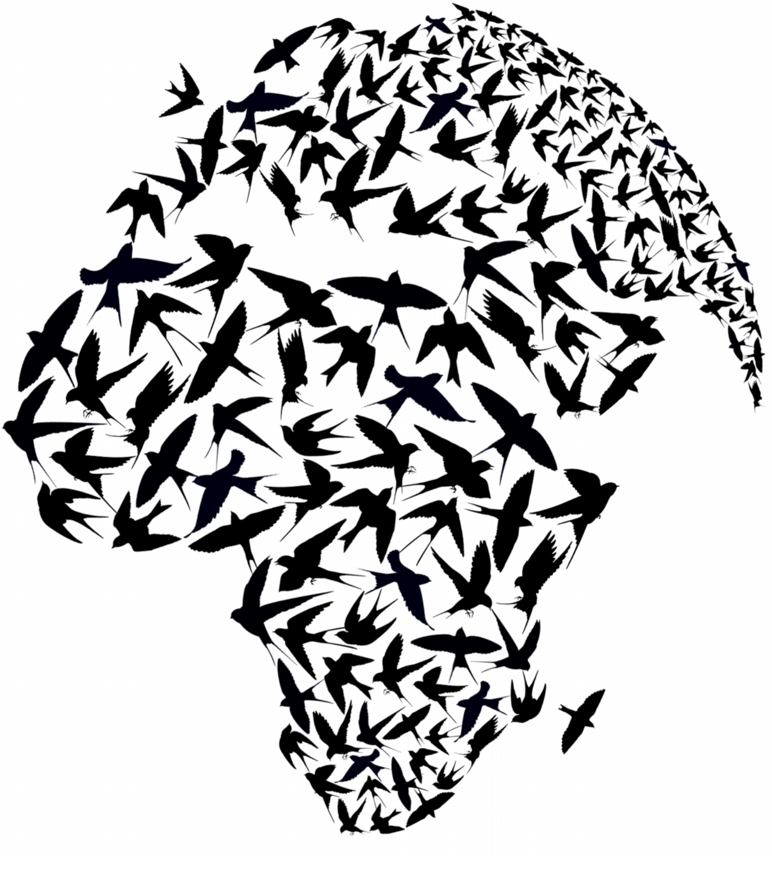
Linking different worlds: migration ecology in two species of hirundines



PhD Thesis Cosme López Calderón 2019

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Conectando mundos distintos: ecología de migración en dos especies de hirundínidos

Tesis doctoral Cosme López Calderón 2019

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Agradecimientos

No podría empezar de otra forma que agradeciendo a mi madre, Isabel Calderón, pues ella fomentó que desarrollara desde niño mi pasión por la zoología y la investigación.

Agradezco a mi familia y amigos por soportar reiteradamente conversaciones sobre pájaros y por aguantarme en periodos de estrés. Especialmente doy gracias a Álvaro Merino y Aleksander Kubicki, que sin duda han contribuido en mi formación como científico.

Tengo muy buen recuerdo de mi etapa en la Universidad de Extremadura, donde además de formarme en estudios de medio ambiente, conocí a buenos amigos y aprendí a valorar nuestra tierra. En especial, quiero agradecer a Juan M. Sánchez su labor de mentor más allá de un simple trabajo fin de grado. Agradezco también la influencia de Juan M. Pleguezuelos, con quien di mis primeros pasos en la investigación.

Gracias a Blas Torres y José Manuel Guerra, supe del proyecto de investigación “Migración, conectividad y malaria en aves”. Así conocí al director de mi tesis, Javier Balbontín, que sin duda es la persona que más tengo que agradecer. Gracias a su proyecto, financiado por la Junta de Andalucía, he contado con el apoyo económico necesario para dedicarme por completo a esta tesis doctoral. Además de los innumerables conocimientos científicos que

Javier me ha trasmitido, le agradezco los buenos momentos que hemos pasado juntos. Continúo dando gracias a mi co-director Alfonso Marzal, a quien debo mucho también. Gracias a Alfonso, hoy en día trabajo en algunas temáticas que no hubiera podido imaginar años atrás. También agradezco a Florentino de Lope, que compagina una extensa labor científica con un buen sentido del humor. Sigo agradeciendo al resto de componentes de nuestro grupo de investigación: Sergio, Luz, Maribel, Yolanda, Manu y Jaime. A ellos les debo el buen ambiente de trabajo y que siempre respetaron mis hábitos de alimentación. En especial doy gracias a Sergio, que fue la primera persona que me enseñó a manejar un pájaro, momento que nunca se olvida.

Muchas gracias también a nuestros colaboradores externos Keith Kobson y Anders Møller, que he podido conocer durante mis estancias en Canadá y Francia. Siempre os consideraré influencias fundamentales en mi desarrollo científico. Además os agradezco la buena comida servida. Tengo mucho que agradecer también a Xiomara, que hizo todo lo posible para facilitar mi estancia en Canadá. Gracias también a Steven Van Wilgenburg y Kevin Kardynal, que me permitieron avanzar en programación.

Aunque en la Universidad de Sevilla no tenemos aún grupo de investigación consolidado, he de agradecer la compañía ofrecida por los miembros del Laboratorio de Biología Marina. Gracias a Guerrita, Free, Carlos, Maca, Pablo, Sedano, Altai, Íñigo, Gema, Ale, Marta

y Juanmi entre otros, que siempre tuvieron conversación para comer y tomar un café. A Francisco Sedano© además tengo que agradecer las buenas fotografías.

Por último, agradezco a las personas que han hecho posible nuestro seguimiento de la golondrina común. Mil gracias a las familias Sangrán, Lora y Marzal, así como a Plácido, que nos han permitido trabajar en sus propiedades. Juan Sangrán merece un agradecimiento especial ya que nos ayudó a buscar nuevas zonas de estudio. También doy gracias a todos los trabajadores de las fincas, que tuvieron una paciencia ilimitada para recibirme a cualquier hora del día: Fernando, Antonio, Martín, Manuel, Yosi, Javier, Gabriel, Antonio Jesús y Yolanda. Pido disculpas ante cualquier molestia que os hemos podido causar y agradezco de corazón vuestra buena voluntad. También tengo que dar gracias a Marina Bollo y Sergio Reinoso por acompañarme en el trabajo de campo, su ayuda se hizo esencial en la etapa final de mi doctorado. Finalmente, agradezco también a todos los voluntarios que participaron en el trabajo de campo: Valentín, Carlos, Julia, Luis, Alejandro y Victoria.

Resumen

El comportamiento migratorio constituye una de las adaptaciones más espectaculares de los seres vivos frente a los cambios ambientales del planeta. Este comportamiento ha evolucionado en diversos grupos taxonómicos, desde mariposas a mamíferos, pero sin duda es en las aves donde ha encontrado su mayor desarrollo. Conocer dónde migran las aves siempre ha sido un misterio y no fue hasta finales del siglo XIX cuando comenzó el anillamiento científico. Desde los años ochenta, el desarrollo de nuevas técnicas de marcaje ha permitido obtener datos de gran precisión sobre el comportamiento migratorio. Esta información es necesaria para poder conservar adecuadamente poblaciones de aves migratorias en declive. En esta tesis doctoral, hemos utilizado análisis de isótopos estables y geolocalizadores para estudiar la ecología de migración en la golondrina común (*Hirundo rustica*) y el avión común (*Delichon urbicum*). En primer lugar, hemos descrito en detalle el comportamiento migratorio de la golondrina común reproductora en el sur de España. Además hemos identificado que la migración prenupcial es el principal nexo de unión entre lo que ocurre en África durante el invierno y lo que ocurre en Europa durante la temporada de reproducción. A continuación, hemos detectado que las condiciones ambientales experimentadas durante el invierno pueden tener consecuencias sobre la reproducción, lo que resulta sorprendente teniendo en cuenta que ambos eventos se encuentran separados varios meses en el tiempo y miles de kilómetros

en distancia. Nuestros resultados revelan que el efecto del hábitat de invernada sobre el posterior éxito reproductor es muy complejo, pues depende de la edad, el sexo y de diferentes variables mediadoras que pueden interactuar entre sí. Aparte del propio valor científico de esta tesis doctoral, nuestros resultados tienen implicaciones en la conservación de especies migratorias y de aves insectívoras. De hecho, muchas especies propias de estos grupos se encuentran amenazadas de extinción o están sufriendo declives poblacionales.

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General introduction

Migratory behaviour constitutes one of the most amazing adaptations of life to face the changing conditions on Earth (Newton 2008; Hobson & Wassenaar 2008). This behavior has evolved in a wide variety of animals, from zooplankton to mammals (e.g. Hays 2003; Bolger et al. 2008). Every time we are impressed by new studies describing extraordinary movements, such as butterflies travelling about 4,000 km in several generations (Wassenaar & Hobson 1998), juvenile marine turtles crossing entire oceans (Luschi et al. 2003) or songbirds moving from Alaska to eastern Africa (Bairlein et al. 2012). Undoubtedly, migratory behaviour has developed to the greatest extent in birds, thanks to the domination of flying (Newton 2008). Bird migration has been always a mystery and people of the past thought very curious ways to explain why some species disappear after spring. For instance, it was supposed that breeding species transformed themselves into wintering species, or that swallows hibernated within the mud of lakes. Interestingly, these unbelievable explanations came first than thinking of birds travelling to other continents (Hobson & Wassenaar 2008).

The first evidence of bird migration was discovered in 1822 by chance, when a white stork was found in Germany carrying an African spear (Kinzelbach 2005). During subsequent years, more than twenty storks were found in the same way, confirming the discovery.

Later in 1899, the first bird was ringed with an aluminium ring (Preuss 2001), and since then this has been the most extended method to unravel the whereabouts of migratory birds. Over a century of bird banding we have gained many information so far (du Feu et al. 2009), but recapture rates of ringed birds is very low (e.g. Hill et al. 1997) and so the information given by ringing recoveries is very limited.

Since the 1980s, advances in satellite telemetry have allowed scientists to obtain high precision data on migratory behaviour (Perras & Nebel 2012; Kays et al. 2015). Once a bird is capture and attached with a satellite transmitter, researchers can monitor movements for long periods without having to recapture it. However, these devices are very expensive, which in turn restrict the sample size in most of studies. And even more importantly, due to their high size and weight, satellite transmitters cannot be deployed on small migratory birds (Kays et al. 2015; but see Hallworth & Marra 2015).

An alternative solution to study migratory movements emerged after 1988 from stable isotope analyses (Hobson & Wassenaar 2008). Under this approach, the measurement of stable isotope ratios of a given tissue (e.g. feather) is matched to predicted values in maps, because the tissue incorporates the isotopic signature from the place where it was grown. The main advantages of the isotope method are that only one capture is needed, their lower cost compared to other methods, and that there is not restriction on body size (Hobson 1999). Nonetheless, stable isotope analyses can only provide broad geographical information for certain periods of the migration cycle (e.g. moult areas), so other geographic information such as migratory routes or stopover sites are missing. This strong limitation encouraged the development of solar geolocation as the only alternative to obtain precise information for migratory songbirds (McKinnon et al. 2013).

The first migratory songbird was tracked with light-level geolocators on 2007 (Stutchbury et al. 2009), and since then, thousands of birds have been tagged with these devices all over the world (e.g. Finch et al. 2017). Geolocators record light levels, which are then transferred into position estimates on the basis that photoperiod is associated with latitude and the time of solar noon or midnight with longitude (Ekstrom 2004; 2007). Within the short life of solar geolocation, this method has provided more information than banding recoveries over a century (McKinnon et al. 2013). Nevertheless, this technology is still far more expensive than stable isotope analyses, and furthermore ethical considerations must be taken into account (Costantini & Møller 2013; Brlík et al. *in press*).

Therefore, both stable isotope analyses and light-level geolocators are entirely complementary (Hobson & Kardynal 2016) and they represent the best options to study migration in passerines. For instance, if the researcher is just interested in generalizing geographical origins (e.g. winter areas), then stable isotope analyses should provide the most cost-effective choice. By contrast, if the purposes of the study require to know full migratory routes and schedules, then solar geolocation is the best option. Moreover, the combination of both tracking techniques has already provided refined results and so they are prone to feedback from each other (Contina et al. 2013; Hobson & Kardynal 2016; Imlay et al. 2018). To date, few studies have tested if moult origins inferred by stable isotopes agree with position estimates given by geolocators (González-Solís et al. 2011; Hallworth et al. 2013; Seifert et al. 2018). The degree of consistency between these methods depends on the structure of underlying isoscapes, accuracy of geolocators, and also to biological traits of the study species such as habitat or foraging behavior (Oppel et al. 2011; Hobson & Kardynal 2016; Seifert et al. 2018). Consequently, it is hard to generalize at this point, and so previous studies have found mixed results (González-Solís et al. 2011; Hallworth et al. 2013; Seifert et al. 2018). The recent development of solar geolocation has made possible to ground-truth geographical assignations based on stable isotopes, which was necessary to identify the flaws of that approach. In this line, we expect that future studies will introduce new statistical methods to increase the match between both tracking techniques.

Independently of the geographical assignment of origin, stable isotopes analyses have been proved to be very powerful in the study of migration ecology (Hobson 1999). Precisely, the forensic isotope approach has contributed profoundly to establish links between different worlds (Webster et al. 2002; Norris & Marra 2007; Harrison et al. 2011). Since the late 1990s, it has become widely accepted that events occurring in one part of the migration cycle may severely affect subsequent performance in another, i.e. “carry-over effect” or “seasonal interaction” (Hobson & Wassenaar 2008). The first clear example of a carry-over effect in a migratory songbird was described by Marra et al. (1998) using stable isotopes analyses. These authors demonstrated for the American redstart (*Setophaga ruticilla*), that winter habitat quality in Jamaica was related to spring arrival to the breeding grounds in New Hampshire. Since then, many other ground-breaking studies have described carry-over effects for passerines using stable isotope analyses (e.g. Norris et al. 2004a, 2004b; Bearhop et al. 2005; Gunnarson et al. 2005). More recently, the development of solar geolocation has inaugurated a new golden age for the study of carry-over effects (e.g. Gils et al. 2016; Saino et al. 2017; Kramer et al. 2017; Patchett et al. 2018).

Traditional ornithology has been mainly focused on the breeding period, but the development of new tracking technologies has stimulated a shift of paradigm (Faaborg et al. 2010). Before the 1980s, it was assumed that migratory birds are temperate breeders that overwinter in warmer latitudes feeding on "excess" available resources. Currently, our model of migration includes birds with an evolutionary tropical origin that started to breed northwards as a seasonal reproductive strategy. In fact, migratory birds usually spend more time travelling and wintering than actually breeding (Faaborg et al. 2010; Cornelius et al. 2013), therefore knowing the whereabouts of migrants and the extent of carry-over effects is essential for conservation and management (Norris & Marra 2007; Harrison et al. 2011; McKinnon et al. 2013). First, it is necessary to know where do migratory birds stopover and winter; second, it is important to determine the most suitable non-breeding habitats that may drive subsequent fitness advantages; and finally, we should prioritize the protection of these key non-breeding habitats. This is because the protection of certain suitable habitats along the migratory route should have a profound impact on widespread population trends (Sillett & Holmes 2002; Norris et al. 2004; Gunnarson et al. 2005; Delmore et al 2012; Laughlin et al. 2013).

In addition, any disturbance at these specific non-breeding habitats will have different consequences depending on the pattern of connections with the breeding areas, i.e. “migratory connectivity” (Webster et al. 2002; Webster & Marra 2005; Finch et al. 2017). Thus, if individuals of the same breeding population congregate together at localized non-breeding habitats (strong connectivity), that breeding population should be more vulnerable to environmental changes that another with widespread non-breeding distributions (weak connectivity). This is because gene flow among wintering populations must be higher in a situation of weak migratory connectivity, what leads to lower rates of local adaptation and thus provides more resilience against rapid environmental changes (Webster & Marra 2005).

Recently, it has been shown an outstanding example that integrates different aspects of migration ecology to provide guidelines for conservation (Kramer et al. 2018). By using light level geolocators, these authors identified the wintering areas of golden-winged warblers (*Vermivora chrysoptera*) and blue-winged warblers (*Vermivora cyanoptera*). They found a strong migratory connectivity for the golden-winged warbler, which was associated with historical trends in breeding populations. Interestingly, the steepest population decline at the breeding grounds was found for birds overwintering in habitats that had undergone severe deforestation. As predicted by Webster et al. (2002), the lower migratory connectivity was found for the blue-winged warbler, who has already replaced golden-winged warblers in many regions (Gill 1980).

It is well known that migratory behaviour differs across sex and age classes (Cristol et al. 1999; Newton 2008). Females usually select partners that are established in suitable nesting habitats, and thus competition for earlier arrival to breeding areas is higher among males (Møller 1992; Lozano et al. 1996; Hasselquist 1998). As a consequence, males have developed different strategies for this purpose, such as wintering in high quality habitats to improve physical condition and depart earlier from winter areas (Marra et al. 1998). In addition, young birds breeding for the first time usually arrive later to the breeding areas and have lower reproductive success than more experienced birds. This lower performance of younger individuals could be either because they are less competitive than socially dominant older individuals, because of their lower experience, or due to the selective disappearance of poor quality individuals (Forslund & Pärt 1995; Newton 2008). Consequently, carry-over effects along different stages of the life cycle also change according to age and sex (Saino et al. 2004; Norris et al. 2004; Balbontín et al. 2009; Drake et al. 2013; Sergio et al. 2014). Indeed this is another source of variance to take into account in migration ecology, and also in management decisions regarding conservation efforts.

In addition to intrinsic factors such as age and sex, environmental factors play an important role in shaping migration strategies. For instance, weather conditions strongly affects migrants, determining when they can travel, their energy requirements, potential risks encountered during the journey, and the visibility of any celestial or geographical cue used for navigation (Newton 2008). Less obvious are the effects of other extrinsic factors such as parasitism pressure. Because diversity and prevalence of parasites is usually higher in the tropical wintering grounds than in the temperate breeding areas (Bensch et al. 2009; Sehgal 2015), migratory behaviour may have evolved as a mean to escape infections (Møller & Szép 2010). Alternatively, the exposure to more parasites in the tropics could be seen as a cost to migration (Figuerola & Green 2000; Waldenström *et* *al*. 2002). Along this line, winter habitat choice may affect probability of infection, which in turn may have carry-over fitness effects back in the breeding areas. A deep understanding of the relationships between parasitism and migration is necessary under the current scenario of global change (Altizer et al. 2011). To cope with global warming, many different species have already shifted distribution ranges (Walther et al. 2002; Parmesan 2006). Specifically, migratory birds have modified their wintering grounds or are predicted to do so (Austin & Rehfisch 2005; Barbet-Massin et al. 2009; Ambrosini et al. 2011). On the other hand, it has been found that prevalence of avian malaria has increased during the last decades in correlation with temperature (Garamszegi et al. 2011). Animal migration can potentially enhance global spread of pathogens, but also it may allow animals to escape from infected habitats, it may reduce disease levels when the infected individual dies along the migratory route and it may lead to the evolution of less-virulent pathogens (Altizer et al. 2011).

Because they are relatively easy to monitor compared to other animals and because they hold the most developed migratory behaviour (Newton 2008), avian species provide unique study models to describe biological links between different worlds. Besides this important scientific value, studying carry-over effects and migratory connectivity it is necessary for the effective conservation of migratory birds under the current scenario of global change (Webster & Marra 2005; Gilroy et al. 2016; Patchett et al. 2018).

In this thesis, we used two species of hirundines as our study models to disentangle the extent of biological connection between Europe and Africa. In Chapter 1, we used light-level geolocators to describe in detail the migration system of barn swallows (*Hirundo rustica*) breeding in southwestern Spain. In that chapter, we identified non-breeding areas across Africa, we inferred migration schedules, we quantified migratory connectivity, we determined factors influencing migratory behaviour and also we tested carry-over effects along migration schedules. In Chapters 2-4, we used stable isotope analyses to identify carry-over effects operating in house martins (*Delichon urbicum*) breeding in southwestern Spain. Specifically, in Chapter 2, first we inferred the most probable African wintering areas, second we tested the importance of age and sex on winter area choice, and third we determined the association between winter area choice and subsequent reproduction. Chapter 3 was focused on the mechanisms underlying carry-over effects from winter habitat to following reproductive success, taking into account causal relationships among mediator variables and different responses across age and sex classes. In Chapter 4, we were interested in the role that may play winter area choice and age class on the probability of infection by haemosporidian parasites. Finally, in Chapter 5, we used stable isotope analyses to identify carry-over effects occurring over the last three decades in barn swallows breeding in northern Denmark. In that chapter, first we depicted the most probable wintering areas in Africa, second we tested if environmental conditions at these winter areas have changed during a 30-year period, and third then we ascertained if these environmental conditions affected subsequent reproductive success. In addition, we were interested in the causal relationships involved and different responses according to age and sex.

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Chapter 1

The migration system of barn swallows (*Hirundo rustica*) breeding in southwestern Spain

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Abstract

Animal migration has always fascinated biologists. Beyond the inherent scientific knowledge, it is necessary to know the whereabouts of migrants as a prior requisite for their conservation. The recent development of solar geolocation has allowed ornithologists to track extensively small sized birds, which make up the majority of bird abundance. Here we used light-level geolocators to describe in detail the migration system of barn swallows breeding in southwestern Spain. We used the most up to date statistical framework to re-construct migratory routes from light data in order to determine migratory schedules, stopover and wintering areas, migratory connectivity, factors determining variability in migration and carry-over effects. We compare our results with previous literature and provide ecological interpretations. Solar geolocation has already filled traditional gaps of knowledge within an unprecedented time.

Introduction

Migratory behavior has evolved in a variety of taxonomic groups, such as those constituting zooplankton, insects, fish, amphibians, reptiles and mammals (e.g. Wassenaar & Hobson 1998; Hays 2003; Luschi et al. 2003; Quinn 2005; Bolger et al. 2008; Kovar et al. 2008). However, with no doubts, migratory behavior has developed to the greatest extent in birds, yet they can fly and move with relatively low energy requirements (Newton 2008). Thus, more than 50 billion birds perform regular annual movements between breeding and wintering areas, often involving thousands of kilometers (Berthold 1993). Specifically, an estimate of two billion birds migrate each autumn from Europe to sub-Saharan Africa (Hahn et al. 2009). This bulk of individuals constitutes a strong ecological link between different continents across the world, demanding resources along migratory routes and potentially transporting associated species such as pollen, seeds, parasites and invasive species (Kays et al. 2015).

Beyond the inherent scientific knowledge that has always fascinated ornithologists, it is necessary to know where migratory birds stay during the non-breeding period as a prior requisite for their conservation (Faaborg et al. 2010). Indeed, many migratory bird species are declining in population numbers, especially long-distance migrants (Sanderson et al. 2006), aerial insectivores (Nebel et al. 2010) and species with low variability in migratory performance

(Gilroy et al. 2016). Mortality rates are usually much higher during migration than during the stationary stages (Sillett & Holmes 2002; Newton 2008; Drake et al. 2014; Klaassen et al. 2014), and conditions experienced along the journey and during the wintering stage may affect subsequent reproductive performance (i.e. “carry-over effects”; Norris & Marra 2007; Harrison et al. 2011; O’Connor et al. 2014). Thus, in order to implement effective management decisions, it is needed to ascertain migratory pathways, the distribution and quality of habitats visited, migratory schedules, as well as the factors leading to differences in migratory performance among individuals (Faaborg et al. 2010). In addition, it is important to understand how breeding and wintering areas are geographically linked (i.e. “migratory connectivity”; Webster et al. 2002), because it determines the vulnerability of a given species to face environmental changes (Webster & Marra 2005; Gilroy et al. 2016).

Passerine species make up the majority of long-distance migratory birds (e.g. more than 73% of European breeding individuals; Hahn et al. 2009). However, most of the detailed information on bird migration is only available for raptors and waterfowls. This gap of knowledge is obviously led by their small body size, which has traditionally hampered the attachment of satellite transmitters onto migratory passerines (Kays et al. 2015). For this reason, GPS tags have been continuously miniaturized until a ~1 g archival GPS tag was for the first time attached on to a small migratory bird (Hallworth & Marra 2015). However, the temporal resolution of these GPS tags is still very limited (e.g. only 28 days for the latter study), and consequently it is expected during the coming years a continued miniaturization of this technology together with an increase in their temporal resolution (Kays et al. 2015).

In the meantime, archival light-level geolocators (or geologgers) represent the most extended tracking technique to obtain position estimates during the non-breeding period of passerines (Bridge et al. 2013; McKinnon et al. 2013; Finch et al. 2017). These devices record light levels at fixed time intervals during the whole migration cycle and once the marked individuals are recaptured, the stored light data can be downloaded for analysis. Then, following astronomical equations (Ekstrom 2004; 2007), position estimates are calculated according to the duration of day light (i.e. latitude) and time of solar noon/midnight (i.e. longitude). Light data recorded by geolocators is noisy due to different physical (e.g. proximity to equator, equinoxes, cloud cover) and biological factors (shading caused by dense vegetation or by the use of cavities). Consequently, current efforts in this research area focus in statistical methods for quantifying uncertainty of estimated positions taking into account all sources of error (Rakhimberdiev et al. 2015; 2017). Although geologgers presents many limitations and relatively low precision in position estimates, their simplicity make them able to collect data during full migration cycles at a reasonable economic expense. That is why since the first published migratory track of a passerine species (Stutchbury et al. 2009), more than 7,800 small birds have been tagged across the world (Brlík et al. *in press*). Nonetheless, the attachment of geolocators onto small birds (body mass <100 g) slightly reduces survival rates, and thus the miniaturization of these devices is still mandatory due to ethical reasons (Brlík et al. *in press*). After all, geologgers have provided so far invaluable scientific information on the whereabouts of migratory passerines, such as the distribution and quality of non-breeding habitats, migration phenology, migratory connectivity, factors influencing migration and carry-over effects throughout different stages of the life cycle (McKinnon et al. 2013; Patchett et al. 2018).

The barn swallow (*Hirundo rustica*) is a small passerine (15-20 g) that breeds semi-colonially and feeds on insects while flying. The available dataset on ringing recoveries for this species is one of the largest (Ambrosini et al. 2009), but it is still strongly biased to African countries with high ringing effort (e.g. South Africa). Thanks to ringing recoveries, we know that populations breeding in southwestern Europe overwinter from West to Central Africa, whereas northern breeding populations winter from Central to South Africa (Ambrosini et al. 2009). Specifically, little is known about the stopover and wintering areas used by barn swallows breeding in the Iberian Peninsula, because ringing recoveries for these populations are scarce (de Lope 1980; Ambrosini et al. 2009). In addition, distribution maps developed by BirdLife International (2016) are wrong for the southern Iberian Peninsula. Across North America, the barn swallow has been tagged with light-level geolocators in several breeding populations (Hobson et al. 2015). However, in Europe, this species has been extensively tagged at a single breeding area (Liechti et al. 2014). Taking into account the overwhelming scientific literature available for the barn swallow, filling gaps of knowledge in its migratory behavior should benefit many other research areas that used this species as study model.

In this study, we used light-level geolocators to elaborate a detailed description for the migration system of barn swallows breeding in the southwestern Iberian Peninsula. First, we re-constructed migratory tracks for our study individuals. Second, we determined migration schedules for our study individuals. Third, we identified stopover areas in autumn, wintering areas and stopover areas in spring used by our study population. Fourth, we quantified migratory connectivity at a local geographical scale. Fifth, we tested if barn swallows performed migration differently according to breeding colony, year or sex. Sixth, we tested if any advance or delay across migration timetables were accumulated during the migration cycle.

Materials and methods

Field procedures

During 2016-2018, we monitored four breeding populations of barn swallows in southwestern Spain (provinces of Badajoz and Seville): “Asesera” (38°39'N, 7°13'W), “La Alegría” (37°29'N, 6°11'W), “Las Coladas” (37°36'N, 6°14'W) and “La Calera” (37°34'N, 6°13'W). In our study sites barn swallows breed in traditional farms surrounded by Mediterranean *Dehesa,* an extensive agrosilvopastoral management system derived from the Mediterranean forest ecosystem (Olea and San Miguel 2006). Dominant vegetation was sclerophyllous oak-trees (*Quercus ilex* and *Q. suber*), accompanied by a wide range of bushes (e.g. *Cistus, Genista*, *Retama*, *Pistacia* and *Ulex*) and scattered pine plantations. The study sites had different economic activities such as livestock (ovine, bovine and porcine), wildlife gaming, cork production, rural tourism and agriculture (cereals, sunflower and olives). The number of breeding pairs differed across the study sites, ranging from eight to thirty.

From February to July, we captured adult barn swallows with mist nets and identified each individual with both metallic and color rings. Every year, we trapped birds until 90-100% of the population was captured in each breeding site. Individuals were sexed from the presence or absence of brood patch, by the length of outermost tail feathers, and also by observation of breeding behavior (Møller 1994).

In 2016 and 2017, we attached respectively 64 and 59 geolocator devices to adult barn swallows as leg-loop position (Rappole & Tipton 1991). Tag model was ML6540 (Biotrack, Wareham, UK) in both years. In 2016, every tag was built with a light stalk of 5 mm, and all harnesses were made of elastic material (Street Magic cord, Pepperell Braiding Company). The stalk of geologgers places their light sensor outside from body feathers, thus reducing shading. In this year, the weight of both tag and harness was on average 0.59 g (3.31% of body weight), and loop diameter of the harnesses were 26-31 mm depending on body size. In 2017, tags were built without light stalk (so called "flat geolocator"), 33 harnesses were made of elastic material and 27 of non-elastic material (cotton thread obtained from broken mist nests). In this year, the weight of both tag and harness was on average 0.56 g (3.22% of body weight), loop diameter of elastic harnesses was 30-33 mm and that of non-elastic harnesses was 34-40 mm. To test if return rates of tagged birds were lower than natural ones, 48 and 40 adult swallows were considered to be controls (i.e. colour banded adults within our study sites) in 2016 and 2017 respectively.

Light data analysis

Geolocators measured light levels each minute and recorded the maximum light value every two minutes. Raw light data were corrected for clock drift with *Decompressor* (British Antarctic Survey). Twilight transitions were individually inspected with library *BAStag* (Wotherspoon et al. 2016) of *R* (R Core Team 2016). Transitions with light peaks or non-linear transitions that substantially disrupted twilight time were manually excluded from further analysis. This process resulted in the rejection of 4-29% of twilight events in each tag record. We never edited non-natural twilights, we delete them, but we did not fill these gaps with an expected twilight time. This criterion should lead to higher uncertainty in the estimated positions during periods of low quality light data, but we were confident that this is the most conservative approach. A minimum dark period of five hours was considered, and the arbitrary threshold value defining twilight was set at 16.

Migratory routes were reconstructed from light data with library *FLightR* (Rakhimberdiev et al. 2017), which provides refined accuracy and precision of calculated positions by using a hidden Markov chain model and a template fit observation model (Rakhimberdiev et al. 2015). We used two different calibration periods of approximately one week each, and we accounted for the loss of transparency in the surface of the light sensor. The use of cavities for nesting had a strong impact in the light profiles (Liechti et al. 2014; Hobson et al. 2015), and thus the first calibration period was taken just after reproduction but when the bird was still at a breeding colony before departure. The second calibration period was taken after recapture in the following year, exposing the tags to the sun light on a rooftop. We used a spatial-mask, constraining our model to consider states of residence only over land, with a buffer distance of 30 km into the sea. Following Rakhimberdiev et al. (2015), we incorporated the priors of 0.1 for probability of migratory behavior and 300 ± 150 km for distance covered between consecutive twilights.

Key dates of the migration cycle (i.e. departure from breeding area, arrival to winter area, departure from winter area and arrival to breeding area) were calculated using the function `find.times.distribution` from the library *FLightR* (Rakhimberdiev et al. 2017). This function derives the time at which an animal crossed a specified latitude, together with the measure of its uncertainty (we always used Q.50 value). We considered departure/arrival from/to breeding areas when a given swallow crossed the boundary latitude of 36º (i.e. Gibraltar Strait). Regarding arrival/departure to/from wintering areas, we considered specific boundary latitudes for each individual. We defined boundary latitude of arrival to winter area as the latitude when the tracked bird stopped its southward journey, and boundary latitude of departure from winter area as the latitude when the given bird started its northward journey.

We defined the wintering areas for each tracked swallow as polygons generated by the function `plot_util_distr` from the library *FLightR* (Rakhimberdiev et al. 2017). This function calculates the accumulated probabilities of occurrence during a specified period (i.e. between arrival and departure date from wintering areas). For example, the polygons obtained with this function setting `percentiles=0.5` indicated the grid points which accumulated more than 50% of probability of occurrence during winter. These polygons were circles of 85 km diameter because *FLightR* discretizes the space to reduce computation time (Rakhimberdiev et al. 2015).

In order to identify the stopover locations for each tracked swallow, we generated our own function in *R* (R Core Team 2016). We considered a stopover as any location during autumn (between departure date from breeding area and arrival date to winter area) or spring migration (between departure date from winter area and arrival date to breeding area) in which the given bird stayed for at least two days. If the coordinates (being latitude and longitude rounded to one decimal) of twilight "i" matched the coordinates of twilight "i + n" but there were missing twilights among them due to poor quality light data, we considered this location as a stopover only if the bird remained here for at least two consecutive days, and we obtained the stopover duration from the first and last day at this stopover (even if during this period some twilights were missing).

To depict the wintering and stopover areas used by our whole population under study we rasterized respectively, polygons with accumulated probabilities of occurrence during winter and error squares of stopover locations (as defined by upper and lower 95% Credible Intervals for latitude and longitude). Pixels within these polygons were coded as one and all others as zero, resulting in one binary map per individual. Finally, for each stage of the migration, we summed over each individual raster layer. All GIS operations were conducted in R version 3.3.1 (R Core Team 2016) with packages *raster* (Hijmans 2016) and *sp* (Pebesma & Bivand 2005).

Statistical analysis

First, as complementary descriptive information, we quantified migratory connectivity following Finch et al. (2017). This was accomplished by calculating two new variables: population spread and inter-population mixing. The former was simply the mean pairwise distance between individual wintering areas (i.e. centroids) for each breeding colony, and the latter was obtained by Mantel correlation coefficient between pairwise distance matrices of individual wintering and breeding locations (Ambrosini et al. 2009). This coefficient corresponds to a Pearson product moment correlation coefficient between two matrices, whose significance is assessed with randomized permutation. The mantel coefficient ranges from -1 to +1, and it quantifies whether distances between individual breeding colonies are maintained during the winter. Strong positive Mantel coefficients indicate that individuals which breed close together also spend the winter season relatively close together (i.e. low inter-population mixing), whereas weak or negative coefficients indicate that individuals which breed close together spend the winter season apart from each other (i.e. high inter-population mixing).

Second, we tested if barn swallows performed migration differently according to year, breeding colony and sex. For this purpose, we fit five Generalized Linear Models for each stage of the migratory cycle. During autumn migration, our dependent variables of interest were: departure date from breeding areas, number and mean duration of stopovers (days), arrival date to wintering areas and migration period (days). During winter, our dependent variables of interest were: latitude and longitude of the wintering area centroid, size of the wintering area (km^2), great circle migratory distance (kilometres between winter area centroids and breeding colonies) and wintering period (days). All these dependent variables were obtained for wintering areas defined as grid points which accumulated >50% probability of occurrence during winter. During spring migration, our dependent variables of interest were: departure date from wintering areas, number and mean duration of stopovers (days), arrival date to breeding areas and migration period (days). For every dependent variable, we fit our models including year of tag detachment, breeding colony and sex as fixed effects. We did not account for any interaction term because of our low sample size on migratory tracks. We always used Gaussian distribution of errors and the identity link function.

Residuals of each GLM were visually inspected for deviation from normality using normal “qqplots”.

Third, we tested if differences in departure date from breeding areas were consistently accumulated through the migration cycle to finally affect arrival date to breeding areas. Because key dates during migration flow sequentially in a time series fashion, we used a Structural Equation Model (hereafter: SEM). This is a probabilistic model that hypothesizes a causal network with multiple variables that can appear as both predictor and response variables (Lefcheck 2016). We used “confirmatory path analysis” or piecewise SEM, based on applications from graph theory (Shipley 2016). In piecewise SEM, the causal network is translated to a set of linear equations (e.g. linear mixed models), which are then evaluated individually thus allowing a wide range of distributions and sampling designs. Our causal network was made up of three linear mixed models: the first model tested the effect of departure date from breeding areas on arrival date to wintering areas, the second model tested the effect of arrival date to wintering areas on departure date from wintering areas and the third model tested the effect of departure date from wintering areas on arrival date to breeding areas. In every linear mixed model, we included detachment year and breeding colony as cross-random intercepts, thus controlling for inter-annual effects and among breeding site variation. We always used Gaussian distribution of errors and the identity link function. To qualitatively evaluate each individual model fit, we visually inspected residuals for deviation from normality using normal “qqplots”. Finally, we quantified the goodness of fit of our entire causal network with directed separation test (“d-separation test”; Shipley 2016), which tests the assumption that all variables are conditionally independent (i.e. that there are no missing relationships among unconnected variables).

Results

Description of migratory behavior

In 2017, we recaptured 21 out of 64 tagged swallows. However, in 2018 we recaptured only 7 out of 59 tagged swallows. Four of the tags recovered in 2017 did not provide any useful light data. Thus we count for this study with a total sample size of 24 migratory tracks. In 2017, the apparent survival rate of tagged birds (32.81%) was significantly lower than that of control birds (56.25%) ($\chi^2 = 6.152$; df = 1; $p = 0.013$). In 2018, the apparent survival rate of tagged birds (11.86%) was also lower than that of control birds (20%), but this difference was not statistically significant ($\chi^2 = 1.227$; df = 1; $p = 0.268$). Four swallows were equipped with geolocators in both attachment years of our study, but only one of them survived the second migration (ring: Y65702). Thus, we only could obtain longitudinal data on migration for a single individual. To avoid pseudo-replication, we excluded the first recapture of this individual from subsequent analyses (we retained the second recapture to increase sample size in our second migration year).

Individual migratory routes are presented in Figures 1-24 of Supplementary Materials. Generally, barn swallows used the Strait of Gibraltar to cross the sea. Considering the limitations of our light data (i.e. precision and missing twilights), few individuals crossed open sea at the Gulf of Cádiz or the Alboran Sea. Depending on each track, individuals used either similar or different routes for autumn and spring migration. Roughly, half of our tracked individuals conducted a loop migration. In many cases, migratory routes followed in autumn were closer to the Atlantic coast than routes followed in spring. Nevertheless, it seems that our study individuals did not show consistent soaring behavior and rather they usually crossed large regions of Sahara desert.

Our studied barn swallows left their breeding areas generally in August (Fig. 1 and 2). One third of them stayed to refuel in northwestern Morocco (Fig. 3), and they took between 4 - 60 days to conduct the autumn migration (mean ± SD = 20.79 ± 18.01 days; n = 24). Around 1st of September (Fig. 1 and 2), barn swallows arrived to their wintering areas (Fig. 4), where they spent approximately five months (mean ± SD = 152.88 ± 23.53 days; n = 24). Wintering areas were largely spread across West Africa, roughly from 5º to 25º in latitude and from -15º to 5º in longitude. The main core of habitats used for wintering (i.e. grid points which accumulated more than 50% of probability of occurrence) was concentrated along Ivory Coast, Guinea, Republic of Sierra Leone, Mali, Burkina Faso and Ghana. These areas were mainly dominated by savannah and cropland mixed with natural vegetation (Allen 2016). Considering also the areas used occasionally during winter (i.e. grid points which accumulated more than 95% of probability of occurrence), our study populations spread out through other different African biomes, such as desert, grassland and broadleaf forest. From January to March, barn swallows left their wintering areas (Fig. 1 and 2). Two thirds of them stayed to refuel from western Algeria to northern Morocco (Fig. 5), and they took between 20 - 103 days to conduct the spring migration (mean ± SD = 48.92 ± 25.73 days; n = 24). Finally, our studied individuals arrived back to their breeding colonies from February to May (Fig. 1 and 2).

Interestingly, a single individual did not migrate to Africa, but instead it travelled northwards during autumn migration (ring: 2L12590; Fig. 23 Supplementary Materials). This swallow took two months to reach Manchester (UK) and then only one week to come back to Spain. Then, this individual spent about three months wintering in the northwestern Iberian Peninsula (from Central Portugal to Galicia). After this stage, it travelled northwards again, crossing 1,000 km of open sea and reaching Ireland only in two days. Then, after a few days, this bird started the southward journey back to Spain and it arrived to its breeding colony in about 20 days. This time, it made a detour through Cornwall and Brittany, but still crossing open sea at the Bay of Biscay. Because this was a very especial case in our study population, we excluded this single individual from our subsequent statistical analyses.

Migratory connectivity

Population spread during winter was 672.69 km, 653.26 km, 521.19 km and 733.41 km for “Las Coladas”, “La Calera”, “La Alegría” and “Asesera”, respectively. Inter-population mixing on the wintering grounds was high (Mantel $r = 0.022$; $p = 0.343$). Therefore, the distance between two individuals during the breeding season did not correspond with the distance between the same individuals during the winter (i.e. weak migratory connectivity; Fig. 6).

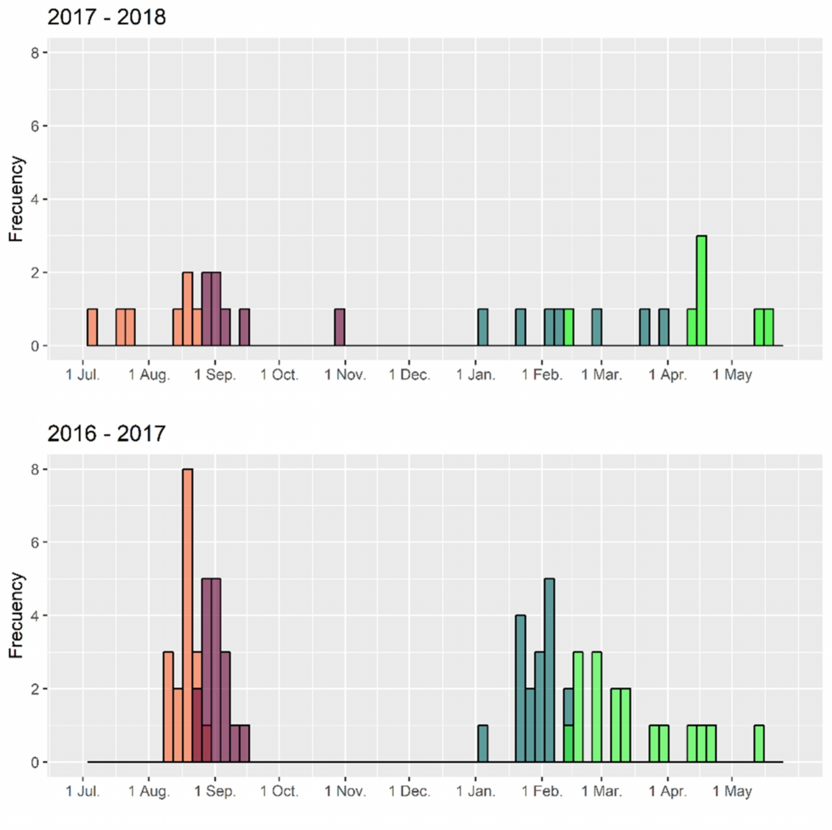

Figure 1. Distribution of migration schedules for barn swallows breeding in southwestern Spain. Orange bars indicate departure date from breeding areas, purple bars indicate arrival date to wintering areas, blue bars indicate departure date from wintering areas and green bars indicate arrival date to breeding areas. Sample size was 24 migratory tracks.

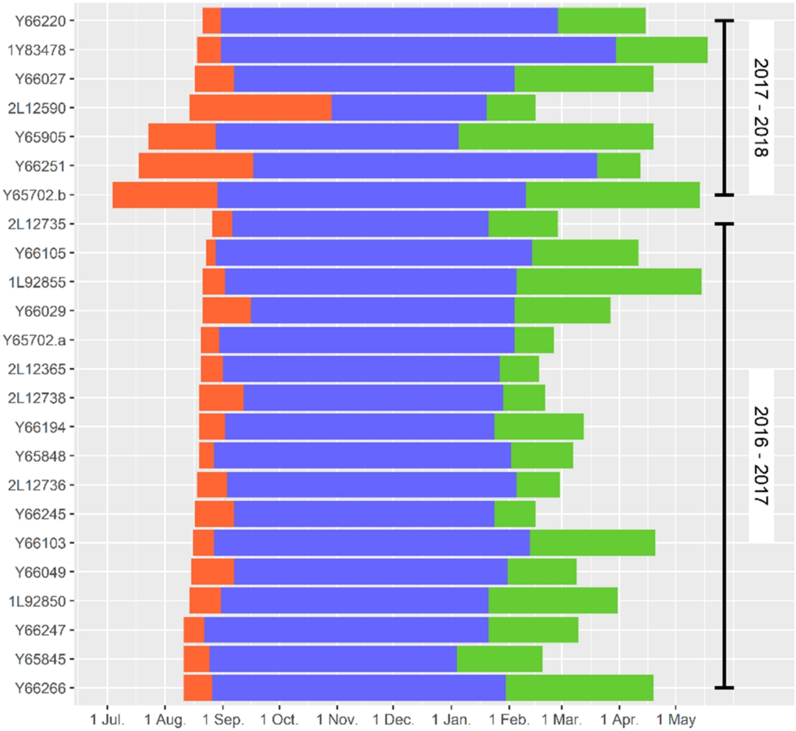

Figure 2. Timelines with migration schedules of barn swallows breeding in southwestern Spain. Autumn migration period is indicated in orange, wintering period in blue and spring migration period in green. Sample size was 24 migratory tracks.

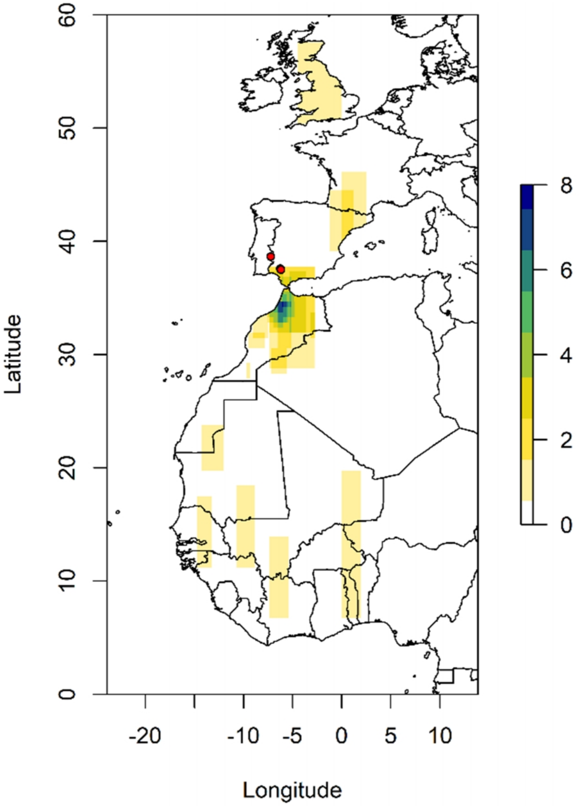

Figure 3. Stopover areas used during autumn migration by our study populations. Colors indicate the number of times that a specific pixel was used by any of the tracked individuals to stop over for at least two days. Stopover areas for each individual were defined as error squares of stopover locations (see Methods for details). Breeding colonies are denoted by red points. Sample size was 24 migratory tracks.

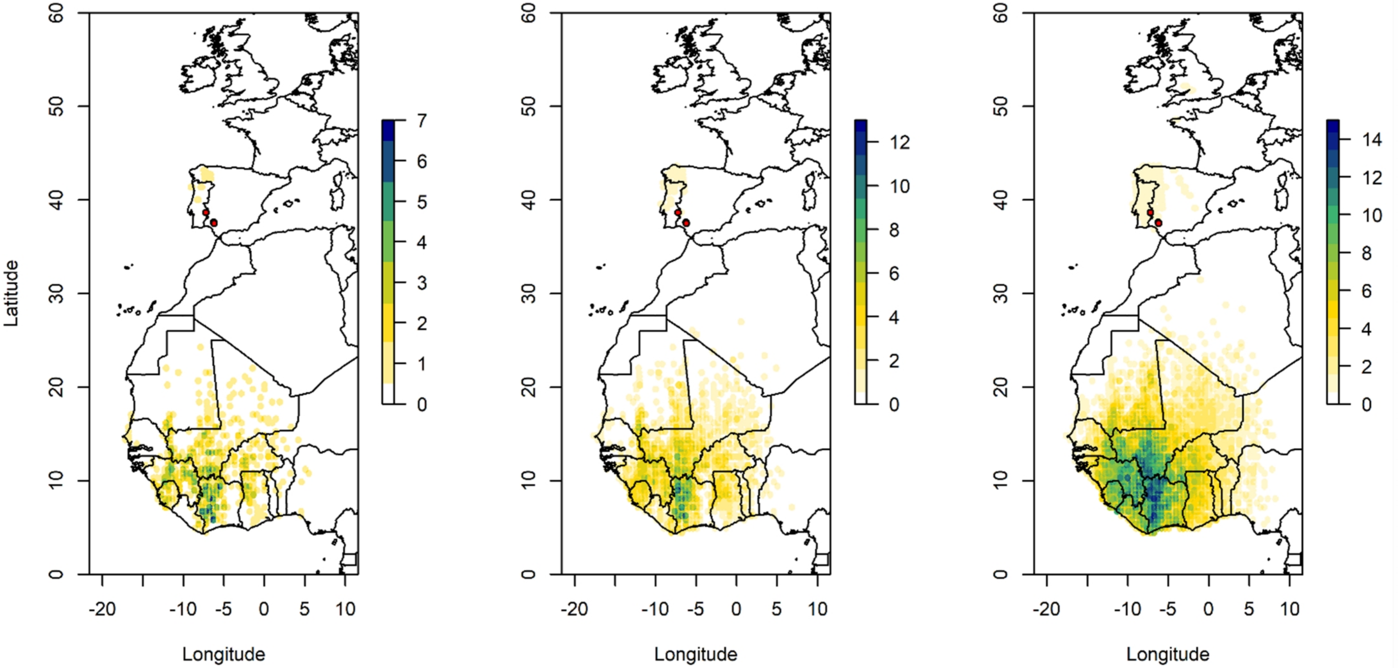

Figure 4. Wintering areas used by our study populations. Colors indicate the number of tracked birds that were located at the specific pixel during the wintering period. From left to right different spatial scales are shown (i.e., grid points that accumulated more than 50%, 75% and 95% of probability of occurrence, respectively). Breeding colonies are denoted by red points. Sample size was 24 migratory tracks.

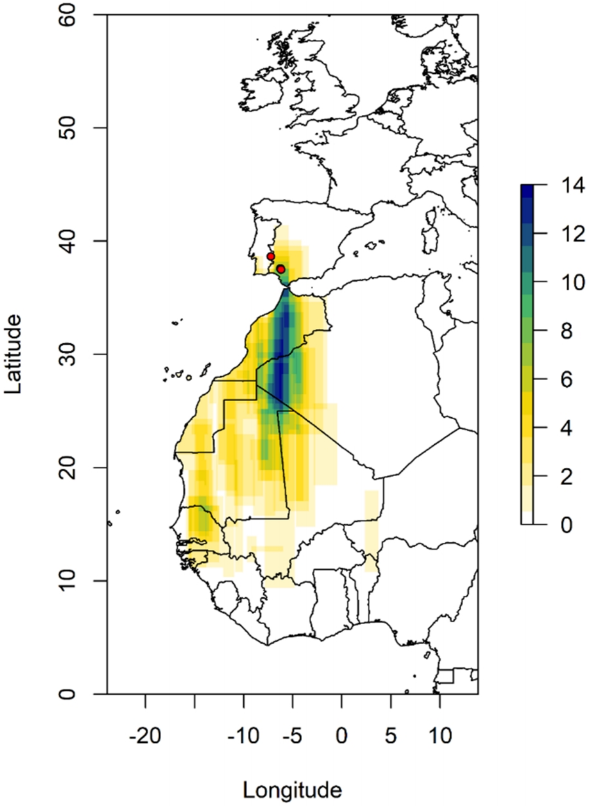

Figure 5. Stopover areas used during spring migration by our study populations. Colors indicate the number of times that a specific pixel was used by any of the tracked individuals to stop over for at least two days. Stopover areas for each individual were defined as error squares of stopover locations (see Methods for details). Breeding colonies are denoted by red points. Sample size was 24 migratory tracks.

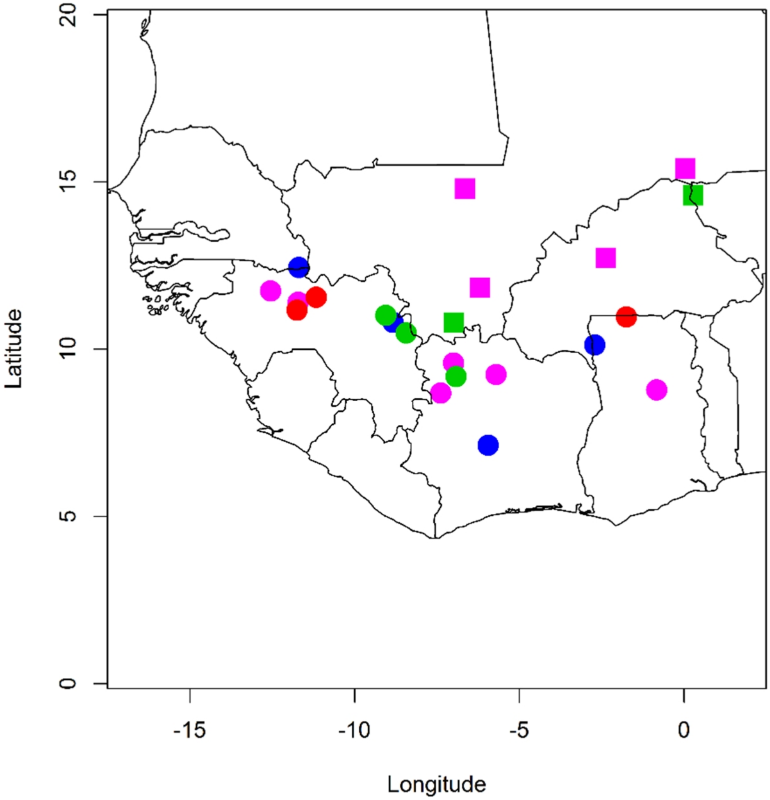

Figure 6. Centroids of each individual wintering area, defined as grid points that accumulated more than 50% of probability of occurrence. Colors indicate individuals breeding at the same colony (magenta: "Las Coladas"; blue: "La Calera"; green: "La Alegría"; red: "Asesera"). Shapes indicate different migration years (circles: 2016-2017; squares: 2017-2018). Sample size was 22 migratory tracks.

Factors influencing migratory behavior

Regarding the factors that could influence our migratory variables of interest, we found widespread inter-annual effects and some differences across breeding colonies during spring migration. Sex was never a significant predictor of migratory performance.

During autumn migration, individuals left their breeding areas earlier, stopped to refuel more times and thus took more days to complete the journey in the second migration year (Table 1). Mean duration of stopovers in autumn was higher in the second migration year, although these differences were not statistically significant. On the other hand, arrival date to winter areas was very similar across years.

Individual wintering areas showed consistent differences across years (Table 2). Specifically, individuals spread out through a larger surface, wintered northerly in the African winter quarter and consequently the migratory distance was lower in the second migration year. In addition, individuals wintered further to the east and during a longer period in the second migration year, although these differences were not statistically significant.

During spring migration, we found widespread differences according to breeding colony (Table 3). Individuals breeding at “Las Coladas” stopped to refuel more times (mean ± SD = 5.2 ± 2.3 days) than individuals breeding at other sites (mean ± SD = 1.75 ± 1.87 days), stopped over for longer periods (mean ± SD = 5.83 ± 2.81 days) than swallows from other populations (mean ± SD = 2.75 ± 0.71 days), arrived later to the breeding colony (mean ± SD = 23th April ± 18.26 days) than the other swallows (mean ± SD = 8th March ± 19.5 days), and therefore took more days to accomplish the journey (mean ± SD = 73 ± 19.5) than the other individuals (mean ± SD = 33 ± 11.28). Besides, barn swallows arrived later to their breeding colonies during the second migration year. Additionally, individuals left their wintering areas later in the second migration year, but these differences were not statistically significant (Table 3).

Table 1. F statistic and associated *p* value of GLM developed for each migration variable in autumn. Dependent variables are underlined. Independent variables with significant effects are highlighted in bold. Averages and standard deviations are given for dependent variables by year. Sample size was 22 migratory tracks.

Model	**F (*p*)**	**Mean (SD): 2016**	**Mean (SD): 2017**
Departure date:			
Year	**7.05 (0.017)**	**17/08 (4.38)**	**01/08 (20.03)**
Breeding colony	0.12 (0.947)		
Sex	0.04 (0.849)		
Nº stopovers:			
Year	**4.76 (0.044)**	**0.88 (1.09)**	**2.67 (2.5)**
Breeding colony	0.29 (0.835)		
Sex	1.03 (0.325)		
Duration stopovers:			
Year	1.57 (0.258)	3.85 (1.77)	5.69 (3.64)
Breeding colony	0.83 (0.523)		
Sex	0.66 (0.447)		
Arrival date:			
Year	0.41 (0.530)	31/08 (6.81)	02/09 (7.63)
Breeding colony	0.55 (0.658)		
Sex	1.44 (0.248)		
Migration period:			
Year	**7.44 (0.015)**	**14.00 (5.91)**	**31.83 (21.89)**
Breeding colony	0.02 (0.996)		
Sex	0.66 (0.430)		

Table 2. F statistic and associated *p* value of GLM developed for each migration variable in winter. Dependent variables are underlined. Independent variables with significant effects are highlighted in bold. Averages and standard deviations are given for dependent variables by detachment year. Sample size was 22 migratory tracks.

Model	F (*p*)	Mean (SD): 2017	Mean (SD): 2018
Lat. of winter area:			
Year	**16.14 (0.001)**	**10.27 (1.40)**	**13.36 (1.85)**
Breeding colony	0.32 (0.812)		
Sex	0.56 (0.465)		
Long. of winter area:			
Year	3.28 (0.089)	-7.72 (3.68)	-3.65 (3.38)
Breeding colony	0.03 (0.994)		
Sex	0.18 (0.676)		
Winter area size:			
Year	**7.58 (0.014)**	**102,921 (61,128)**	**212,410 (77,319)**
Breeding colony	0.05 (0.984)		
Sex	0.020 (0.887)		
Migratory distance:			
Year	**18.74 (0.001)**	**3,086 (134)**	**2,725 (181)**
Breeding colony	0.03 (0.992)		
Sex	0.60 (0.448)		
Wintering period:			
Year	2.75 (0.117)	150.06 (11.10)	170.83 (28.44)
Breeding colony	0.12 (0.947)		
Sex	0.26 (0.619)		

Table 3. F statistic and associated *p* value of GLM developed for each migration variable in spring. Dependent variables are underlined. Independent variables with significant effects are highlighted in bold. Averages and standard deviations are given for dependent variables by year. Sample size was 22 migratory tracks.

Model	**F (*p*)**	**Mean (SD): 2017**	**Mean (SD): 2018**
<u>Departure date:</u>			
Year	3.68 (0.073)	28/01 (9.85)	20/02 (30.93)
Breeding colony	0.15 (0.929)		
Sex	< 0.01 (0.955)		
<u>Nº stopovers:</u>			
Year	1.10 (0.311)	3.13 (2.96)	3.83 (1.83)
Breeding colony	**5.34 (0.009)**		
Sex	0.20 (0.658)		
<u>Duration stopovers:</u>			
Year	0.003 (0.960)	4.13 (2.93)	4.67 (1.42)
Breeding colony	**4.12 (0.027)**		
Sex	2.16 (0.164)		
<u>Arrival date:</u>			
Year	**11.86 (0.003)**	**16/03 (26.42)**	**25/04 (15.64)**
Breeding colony	**12.98 (0.001)**		
Sex	0.87 (0.366)		
<u>Migration period:</u>			
Year	0.56 (0.464)	46.50 (22.47)	64.17 (30.82)
Breeding colony	**9.62 (0.001)**		
Sex	0.78 (0.389)		

Carry-over effects across migratory schedules

We also found that differences in departure date from breeding areas did not accumulate throughout the migration cycle (Fig. 7). In other words, carry-over effects of migratory schedules did not propagate during the whole migration cycle. Nevertheless, we found that departure date from wintering areas affected arrival date to breeding areas (Fig. 7). This latter result highlights that the wintering period is linked to the subsequent breeding season through mediation of spring migration. Finally, our path analysis provided robust fit to data, as revealed by d-separation test (Fisher's C = 9.05; df = 6; $p = 0.171$). Thus, we concluded that the hypothesized causal relationships we examined were consistent with the data. By further inspection of "qqplots", we determined that every single linear mixed model was adequately fitted.

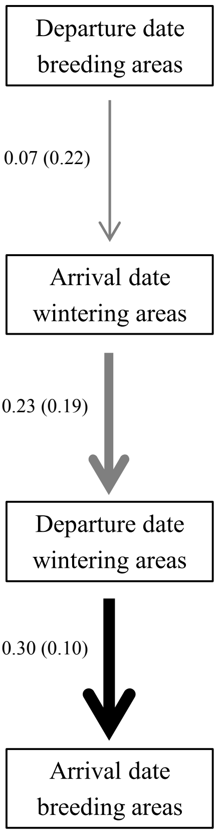

Figure 7. Path diagram of the causal model explaining migratory schedules. One-headed arrows represent a causal effect of one variable on another (i.e. a path). The width of arrows reflects the magnitude of standardized path coefficients, which are presented above each arrow (with standard errors in brackets). Black arrows indicate significant effects and grey arrows non-significant effects.

Discussion

In this study, we have unveiled the migration system of barn swallows breeding in southwestern Spain. We utilized the most updated statistical framework to estimate positions from light data. We have determined that our studied individuals started autumn migration generally in August, and arrived to their African wintering areas around the beginning of September. From January to March, barn swallows departed from their wintering sites and arrived back to their breeding colonies from February to May. The main stopover area used during autumn migration was located in northwestern Morocco. The highest overlap of individual wintering areas was found in Ivory Coast and surroundings countries, places dominated by savannahs and crops. Regarding spring migration, most of individuals stopped over to refuel from western Algeria to northern Morocco. Surprisingly, one swallow migrated in autumn to the British Islands, then it stayed for winter in the northwestern Iberian Peninsula and afterwards it migrated again to the north. We also found a weak migratory connectivity for our breeding populations. In addition, our results highlight strong inter-annual effects on migratory performance. We only detected differences across breeding colonies for the spring migration. By contrast, we did not find differences between sexes in migratory performance. Finally, we showed that migration schedules did not accumulate during the full migration cycle, but only departure date from wintering areas significantly predicted arrival date to breeding sites.

In the first migration year of our study, we found a significant negative effect of tag attachment on apparent survival rates, which agrees with previous studies on aerial insectivores (Scandolara et al. 2014; Szép et al. 2017; Morganti et al. 2018). In order to reduce this negative effect, in the second migration year we attached geologgers without light stalk. This had been recommended in previous studies highlighting that light stalk should considerably increase air drag, with especial detrimental effects for aerial insectivores (Bowlin et al. 2010; Costantini & Møller 2013; Morganti et al. 2018). In agreement, during our second migration year, the negative effect of tag deployment on return rates was not significant.

We successfully reduced the impact of geologgers on apparent survival rates, but this came at the cost of increased noisy light data (Bowlin et al. 2010). Consequently, it can be appreciated that uncertainty in estimated position is usually wider for migratory tracks during the second migration year (see Suppl. Mat.). As it is the basis in solar geolocation (Ekstrom 2007; Bridge et al. 2013; Rakhimberdiev et al. 2015; 2017), we found larger uncertainties for latitude than for longitude, especially when getting closer to equinoxes (e.g. two weeks after and before). In addition, we acknowledge that our position errors should be higher than others found for species moving at northern latitudes, because day length at the equator is constant through the year. We noticed that barn swallows spent nights within rural buildings some time before they actually arrived to their breeding colonies. Therefore, strong shading events started to appear usually in February or March, corresponding to estimated positions across northern Africa. Following previous studies (Liechti et al. 2014; Hobson et al. 2015), we excluded these periods from our raw light data. This latter source of error together with the spring equinox provided in some cases extremely large uncertainties for our estimated positions (e.g. Figures 9, 10, 12 from Supp. Mat.).

Most of our studied barn swallows started autumn migration in August, which is about a month earlier than previous findings on hirundines (Liechti et al. 2014; Hobson et al. 2015; Szép et al. 2017). This difference should be explained by latitude of breeding colony, which was lower in our study compared to others. In agreement, Hobson et al. (2015) sampled barn swallow breeding populations across a wide geographical range, and they found the earliest departure date for the southernmost breeding area. Actually, July and August in southwestern Spain can be extremely warm and dry, so it is expected that barn swallows leave their breeding areas by the time when resources decline. During autumn migration, roughly half of our study individuals stopped over to refuel for at least two days, up to six times and during a maximum period of 12 days. In a previous study, five out of six common swifts (*Apus apus*) made stopovers in autumn migration, up to five times and staying between 10 and 56 days

(Åkesson et al. 2012). Although migratory distance in the latter study was much larger than in our study, and the body size of common swift is rather bigger than of the barn swallow, to our concern this is the closest example for comparison. Further, we found in this study that duration of autumn migration varied substantially among individuals, being on average twenty days, which perfectly matches previous findings (Liechti et al. 2014; Szép et al. 2017). Our studied barn swallows arrived to their wintering areas from mid-August to mid-September (excluding bird 2L12590), which is about a month earlier than barn swallows breeding in Italy and Switzerland (Liechti et al. 2014). Then, Spanish barn swallows stayed on average five months wintering in Africa, which matches very well the previous results on hirundines (Liechti et al. 2014; Szép et al. 2017).

From mid-January to mid-February, most of our tagged swallows departed from the winter quarters, which was about one month earlier than barn swallows breeding in Italy and Switzerland (Liechti et al. 2014). In spring migration, most of our studied individuals stopped to refuel, with the number of stopovers and the mean duration of stay showing substantially variability. On average, our study barn swallows stopped over to refuel three times during spring migration and stayed for four days at these stopover sites. By contrast, sand martins breeding in Hungary stopped over 5-6 times with an average duration of 1.5 day, whereas house martins breeding in Hungary stopped over 3-7 times with an average duration of one day (Szép et al. 2017). The larger migratory distances of sand martins and house martins may require stopping over more times and for less time than our studied barn swallows. This is because covering a large distance on flight must demand many energy resources, but the time to travel a large distance may be more constrained compared to a short distance. Consequently, our studied barn swallows took on average 49 days to conduct spring migration, which is a considerably longer period than previous findings for martins (10-14 days on average; Szép et al. 2017) and barn swallows (one month on average; Liechti et al. 2014). Finally, our studied barn swallows arrived back to their breeding areas from mid-February to mid-May, with most of individuals arriving before mid-March (still earlier than above studies).

Previous studies on hirundines showed that migratory schedules in autumn (i.e. departure date from breeding areas and arrival date to winter areas) were less variable among individuals than migratory schedules in spring (i.e. departure date from winter areas and arrival date to breeding areas) (Liechti et al. 2014; Szép et al. 2017). Here we have found the same pattern, especially for our first migration year. This may help to explain why we already know so many carry-over effects from the winter to the breeding stage (Norris & Marra 2007; Harrison et al. 2011). Because some individuals arrive back to the breeding colonies much earlier than others do, they should experience a relatively large shift in subsequent fitness, since it is generally accepted that earlier arrival is associated with higher reproductive success (Winkler & Allen 1996; Smith & Moore 2004; Marzal et al. 2013).

With respect to the stopover areas used during autumn migration, most of our studied individuals refueled in northwestern Morocco. Only a few birds used other sites to stop over along West Africa, but they never overlapped. We believe that barn swallows use these habitats in northern Morocco to gain fat reserves before crossing the Sahara desert (Schaub & Jenni 2000; Rubolini et al. 2002). Indeed, these areas belong to the most humid region of Morocco, and they constitute some of the last patches of Mediterranean influence before continuing to harsher conditions in the desert (Hama et al. 2013). The great importance of Morocco for Palearctic migrants is well known and thus, the stopover areas we have identified in this study should be shared by many other species (e.g. Bächler et al. 2010).

The wintering areas we have identified spread out along most of West Africa, with the main core situated across Ivory Coast, Guinea, Republic of Sierra Leone, Mali, Burkina Faso and Ghana. These wintering areas fall within the expectations for barn swallows breeding in Spain (de Lope 1980; Ambrosini et al. 2009), but also they are indeed similar to wintering areas identified for other species (e.g. Bächler et al. 2010 ; Eraud et al. 2013; Lemke et al. 2013; Ouwehand et al. 2016). This outcome suggest that management decision related to conservation efforts should affect many of the Palearctic migrants, and not just single species. At a landscape-level, our studied barn swallows used normally savannahs and croplands to overwinter, and more occasionally open dry areas and broadleaf forest. These are the main biomes that we can infer my matching our geolocator data to available maps of land cover (e.g. Allen, 2016). Nevertheless, we think that our studied individuals forage and roost specifically in wetlands and reedbeds, as it is generally accepted for barn swallows (Seifert et al. 2017). Interestingly, the main core of wintering areas was located at a very similar longitude to our breeding colonies (approximately 6-7ºW), what reveals the importance of orientation along the N-S axis during migration.

During the spring migration, our study barn swallows usually stopped over to refuel from western Algeria to northern Morocco. The second-most important stopover area was located in Senegal, and there were also many scattered stopover areas from Western Sahara to Mali. These stopover areas we identified for the spring migration were similar to that found for other species (Eraud et al. 2013; Arizaga et al. 2013). Given that our main stopover area spread along a S-N gradient from western Algeria to northern Morocco, we believe that barn swallows used these habitats as temporary refuges to recover from fatigue after crossing the desert, and not because they needed to increase fat loads to continue migration (Maggini & Bairlein 2011; Arizaga et al. 2013).

Our results suggest that flexibility in spring is higher than in autumn migration, yet we found for the former a much wider range of staying locations and a larger variance in the number of stopovers within individuals. This outcome also contributes to explain carry-over effects from the winter to the breeding stage (Norris & Marra 2007; Harrison et al. 2011), since individuals may experience very different environmental conditions as they can stop to refuel across many scattered locations along the Sahara.

Surprisingly, a single individual did not migrate to Africa (ring: 2L12590), but it travelled up north in autumn migration until reaching England. Then, this individual came back and stayed for two months wintering at the northwestern Iberian Peninsula. After that stage, this swallow traveled northwards again, this time crossing 1,000 km of open sea in two days and reaching Ireland. To the best of our knowledge, this reverse migration has never been found for a Palearctic barn swallow. There was not any technical issue to discard light data recorded by this tag (Sean Walls, personal communication), and besides we found other biological reasons to accept the track. (1) Two barn swallows were sighted along the coast of Dorset during December 2017 (http://dorsetbirds.blogspot.com/2017/12/; accessed 21 January 2018), which is close in space and time to our findings (Fig. 23 Suppl. Mat.). (2) The wintering areas used by this swallow matched perfectly with a known winter roost of barn swallows in Europe (van Nus & Neto 2017). (3) A previous study showed that tree swallows, *Tachycineta bicolor*, were able to cross more than 1,000 km of open sea within 12-36 hours (Bradley et al. 2014). This extraordinary behavior could be related to inclement weather in August 2017 or just to an orientation mistake. In fact, we found that barn swallows departed earlier from their breeding areas, stopped over more times and required more days to accomplish autumn migration in 2017 compared to 2016. In addition, apparent survival rates during the second year of our study were much lower than that of the first one, what may be driven by mortality rate in autumn migration. Further studies are needed to identify if any environmental variable showed anomalies in August 2017 and how it affected migrants. In the meantime, we have tagged again the bird ringed 2L12590. If we are lucky to recapture that bird again, we will be able to test if this strange migration is repeated. If this rare behavior is not random, it would be very interesting to develop future genetic studies for this individual and its offspring.

Finch et al. (2017) analyzed virtually all published geolocator tracks and they found an overall weak migratory connectivity, i.e. that different breeding populations of the same species spread across large areas and mix themselves during winter. However, for the barn swallow in particular these authors found a significant migratory connectivity (Mantel $r = 0.25$), consistent with previous results from ringing recoveries (Ambrosini et al. 2009). Nevertheless, we have found a very weak migratory connectivity for our study populations.

These encountered results must be driven by different geographical scales (Mckinnon et al. 2013). Because our study breeding colonies were located close to each other, it is easy for these birds to spread and mix together at the wintering grounds. However, when considering breeding populations sampled across all Europe, barn swallows ringed in southwestern countries were usually recovered nearby the Gulf of Guinea, but individuals ringed up to the north were mainly recaptured in South Africa (Ambrosini et al. 2009). The same pattern was found for the American barn swallows, yet migratory connectivity was strong at a broad geographical scale (Hobson et al. 2015; Finch et al. 2017), but it was weak at a local scale (Imlay et al. 2018). Some previous studies have advocated quantifying migratory connectivity only when using multiple sampling locations throughout the distribution range (e.g. Hallworth et al. 2015). Nevertheless, we think that quantifying migratory connectivity at a local scale would be useful depending on the purposes of the study or given economic and logistic limitations.

We have found in this study generalized inter-annual effects in migratory performance, which is already known for barn swallows breeding in Italy (Liechti et al. 2014). As we did in our study, these authors detected significant variability among the years for departure date from breeding colonies, duration of autumn migration, locations of wintering areas and arrival date to breeding colonies. Such strong inter-annual effects were hypothesized to be weather related, and for example, departure date from the Italian breeding colonies coincided with heavy rains (whose timing differed among years). Food availability for aerial foragers should decrease with heavy rain and therefore barn swallows may use rainfall as an environmental index to start migration, in addition to other triggers of circannual rhythms such as photoperiod (Coppack et al. 2008). Moreover, our results showed differences in spring migration across breeding colonies. Interestingly, barn swallows breeding in the largest colony under study stopped over to refuel more times and for longer periods, took more days to finish the journey and arrived later to the breeding colony than barn swallows from smaller colonies. Future studies may address the relationship between colony size and spring migration, because a higher intra-specific competition could select certain migratory behaviors (e.g. Studds & Marra 2005). In agreement with Hobson et al. (2015), we did not find any difference in migratory performance between sexes, what should be driven by small sample size on tracked individuals (Liecthi et al. 2014). This is because protandry, i.e. the earlier arrival to breeding colonies of males compared to females, has been proved for the barn swallow using much larger datasets (e.g. Møller 2004).

Previous studies have found either that duration of both migrations is similar or that autumn migration last longer than spring migration (Stutchbury et al. 2009; Åkesson et al. 2012; McKinon et al. 2013; Liechti et al. 2014; Szép et al. 2017; Cooper et al. 2017).

Shorter duration of spring migration could be expected taking into account the competition among individuals to arrive earlier at the breeding grounds, given the benefits associated with earlier arrival (Marra et al. 1998; Winkler & Allen 1996; Smith & Moore 2004; Newton 2008; Marzal et al. 2013). In contrast, we found a longer duration for the spring migration, but also a higher variability in migratory schedules or the range of stopover locations. In addition, migratory timetables did not accumulate during the whole migration cycle (Senner et al. 2014), but only departure date from wintering areas significantly predicted arrival date to breeding grounds. This finding may also reflect competition for earlier arrival to the breeding grounds; but more importantly, it highlights the tight link between the winter and the breeding stage and provides a mechanism for carry-over effects to occur in spring (McKinon et al. 2013; Lemke et al. 2013; Szép et al. 2017; Cooper et al. 2017).

Summarizing, we have extensively deployed light-level geolocators to describe important details of the migration system of barn swallows breeding in southwestern Europe. Such information relates to distribution of non-breeding habitats, phenology of migration, migratory connectivity, factors determining migration and carry-over effects. These have been traditional gaps of knowledge in ornithology, and we have started to fill them within an unprecedented time thanks to solar geolocation.

Acknowledgements

This study was funded by research projects of the *Junta de Andalucía* (P12-RNM-2144), *Ministerio de Economía y Competitividad* (CGL2015-646509) and *Junta de Extremadura* (IB16121). The first author was supported by an operating grant from *Junta de Andalucía* (P12-RNM-2144). We thank Juan Sangran and his family, Borja Lora and his family, Plácido and Marzal family for allowing us to work on their properties. Thanks also to Martin, Manuel, Antonio, Fernando, Javier, Gabriel and other staff that work in the farms. We also thank Marina Bollo and Sergio Reinoso for helping in fieldwork and data base management. Finally, we thank Álvaro Merino Alejandre for helping to elaborate R scripts.

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Chapter 2

Wintering areas predict age-related breeding phenology in a migratory passerine bird

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López-Calderón et al. *Journal of Avian Biology* (2017),
doi: 10.1111/jav.01070

Abstract

Understanding connections between breeding, stopover and wintering grounds for long-distance migratory birds can provide important insight into factors influencing demography and the strength of carry-over effects among various periods of the annual cycle. Using previously described, multi-isotope (δ^{13}C, δ^{15}N, δ^{2}H) feather isoscapes for Africa, we identified the most probable wintering areas for house martins *Delichon urbica* breeding at Badajoz in southwestern Spain. We identified two most-probable wintering areas differing in isotopic signature in West Africa. We found that the probability to winter in the isotopic cluster two was related to age and sex of individuals. Specifically, experienced males (i.e. two years or older) winter in the isotopic cluster two with a greater probability than experienced females, whereas first-year females winter in the isotopic cluster two with a greater probability than first-year males. In addition, wintering area was correlated with breeding phenology, with individuals wintering in the isotopic cluster two initiating their clutches earlier than those wintering in the isotopic cluster one. For birds wintering in the isotopic cluster two, there was no relationship between age and clutch initiation date. In contrast, young birds wintering in the isotopic cluster one initiated their clutches earlier than experienced birds wintering in this area. There was no significant correlation between wintering area and clutch size or the number of fledglings produced. We hypothesize that the relationship among social status, population

density and winter habitat quality should be the most important driver of the carry-over effect we found for this population.

Introduction

Migratory connectivity, or the degree of connection among individuals between breeding and wintering sites and along migratory routes, has important consequences for the ecology, evolution and conservation of migratory species and populations (Webster et al. 2002). Populations of migratory birds with strong connectivity may be more vulnerable to habitat disturbance occurring at their wintering areas compared to populations consisting of individuals wintering across several wintering locations (Webster et al. 2002; Fraser et al. 2012). Examination of such connected ecosystems can provide important insights into how environmental changes may affect migrants, since different habitats along the migratory route may experience different perturbations or environmental changes (Both et al. 2006). Many migratory birds, especially aerial insectivores and long-distance migrants, have shown general declining population trends (Sanderson et al. 2006; Nebel et al. 2010; but see Michel et al. 2015). Specifically, the Palearctic population of the house martin *Delichon urbica* has declined during 1990–2000 and the global population is also declining (Sanderson et al. 2006; Birdlife International 2012). However, causes for these declines and the degree of migratory connectivity in this species are poorly known (Hobson et al. 2012a).

In spite of the huge amount of information provided by ring recoveries, this traditional approach to studying migration has been insufficient to identify the wintering sites of the Palearctic house martin. The overall trans-Sahara recovery ratio for house martins ringed in the European breeding quarters is 1:51,000. Unfortunately, for the 23,948 individuals ringed in Spain, none have been recaptured in their African winter areas (Hill 1997). Probably, this was because this area is greatly impoverished and it is involved in multiple political conflicts which prevent ornithological research in the area. Fortunately, the analysis of endogenous markers like stable isotope ratios in feathers, allow estimation of the wintering areas of birds that are otherwise not easily studied by other methods (Chamberlain et al. 1997, 2000; Hobson & Wassenaar 1997, 2001, 2008; Rubenstein et al. 2002; Rubenstein & Hobson 2004; Robinson et al. 2009).For instance, Hobson et al. (2012a) assigned a house martin breeding population from the Netherlands to wintering sites in West Africa based on stable isotope ratios in winter-grown feathers and constrained their assignment longitudinally based on ring recoveries. These authors proposed a method of combining isotope data with ring recoveries to estimate wintering ranges among breeding and wintering geographical locations in Europe and Africa, respectively. Other European breeding populations of house martins were also assigned with highest probability to winter in West Africa, by examining concordance between the normalized difference vegetation index (NDVI) in Africa and local breeding population trends (Ambrosini et al. 2011), and by linking winter rainfall values with survival rate (Robinson et al. 2008).

Establishing migratory connectivity using isotopic and other methods has also provided information on how individuals differing in their age or sex also differ in their migration strategies or their use of winter habitat. For instance, male American redstarts *Setophaga ruticilla* wintering in high-quality winter habitats (inferred from stable-carbon isotope analyses) arrived earlier to their breeding areas and in better physical condition than males wintering in low quality winter habitats (Marra et al. 1998). Furthermore, Norris et al. (2004) predicted for redstarts that individuals wintering in higher-quality habitats also reared more fledglings at the breeding grounds; moreover the strength of this carry-over effect was higher for females than for males. Another example is found in yellow warblers *Setophaga petechial*, where only yearling females wintering in high-quality habitats (inferred from carbon and nitrogen stable-isotope analyses), initiated reproduction earlier and thus raised more fledglings than yearling females wintering in drier habitats (Drake et al. 2013). Comparable results were obtained utilizing NDVI, a large-scale measure of plant productivity on the wintering grounds. For example, in the Palearctic barn swallow *Hirundo rustica*, Saino et al. (2004) detected earlier spring arrival dates in Italy for older individuals after winters with more favorable conditions in Africa. Similarly, Balbontin et al. (2009) found an advance in arrival dates of barn swallows with improved ecological conditions at stopover areas in North Africa. The extent to which winter habitats cause carry-over effects on subsequent breeding performance may depend on age and sex (Marra et al. 1998; Norris et al. 2004; Saino et al. 2004; Balbontin et al. 2009; Drake et al. 2013), but also on the specific migratory species. For instance, it has been shown that delayed arrivals or departures at a given stage of the migratory route, do not accumulate and therefore do not affect subsequent breeding success in Hudsonian godwit *Limosa haemastica* or wood thrush *Hylocichla mustelina* (Senner et al. 2014; McKinnon et al. 2015). In addition, for 19 migratory bird species breeding in the United Kingdom, climatic conditions on the breeding grounds were more important drivers of breeding phenology than carry-over effects from the winter areas (Ockendon et al. 2013). Thus, it is necessary to be cautious when generalizing carry-over effects across different age and sex categories, or across different taxa.

Here we used stable isotope methods to examine migratory connectivity with the aim to 1) identify general wintering areas in Africa of two house martin colonies breeding in southwest Spain, 2) to determine if age or sex were correlated with winter habitat use, and 3) to determine whether African winter habitat use had any carry-over effect during the subsequent breeding season in Europe by investigating breeding phenology (i.e. laying date) and breeding success (i.e. clutch size and number of fledglings). Based on the previous studies, we expected our house martins to winter in West Africa. We predicted that experienced birds (i.e. two years old or older), which have already accomplished the migratory journey at least on one occasion, would winter in habitats of higher quality than young birds that had never accomplished the migratory journey before. Also, we predicted that males would be more competitive than females with respect to winter habitat selection since they are prone to arrive earlier than females to the breeding areas to increase mating and reproductive success. We also predicted that those individuals wintering in high quality habitats could begin to reproduce earlier and could have a greater reproductive success than birds wintering in low quality habitats.

Materials and methods

Field procedures

Our study took place in two breeding colonies of house martins at Badajoz (38°52’N, 7°05’W), southern Spain, during an 8-yr period (2005–2013, excluding 2006). Adult house martins were captured at dawn in nests or using mist nets placed at the colony. Birds captured for the first time were marked with numbered metal rings, and recaptured birds were identified by their ring. From February to July, we followed reproductive events inspecting nests every second day to determine the start of laying and hatching. When nestlings were nine days old, we captured adult house martins at dawn in their nests and recorded tarsus length with a digital caliper to the nearest 0.01 mm, and body mass with a Pesola spring balance to the nearest 0.5 g. From each adult we took the outermost rectrix for isotopic analysis (original sample size of 300 birds). Each year we performed more than one hundred capture sessions from March to late June. When recapture rate of individuals captured in a given session was >90%, capture sessions were stopped. We aged house martins confidently because this species shows high breeding site philopatry, and recruitment occurs at the age of one year (de Lope and da Silva 1988). Briefly, breeding dispersal is negligible in our study sites with less than 0.1% of breeding individuals (i.e. ≥ first year of age) among the more than 10 000 adults ringed in the study population during more than 20 yr ever being recorded at another colony in the same or subsequent years (Marzal et al. 2016). Moreover, age at first reproduction occurred at the age of one year for the majority of individuals since all but one of more than 500 individuals marked as nestlings were recruited as first-year breeding adults. For the analyses of winter habitat use and carry-over effects (respectively second and third objectives), we included 197 adult house martins. We categorized as young birds individuals ringed as nestlings/fledglings and recaptured in the next year as yearlings (n = 51; 22 males and 29 females; Online Supplementary Material Appendix 1, Table A1). We categorized as experienced birds house martins ringed as nestlings/fledglings and recaptured two years or more after their first capture (n = 28; 13 males and 15 females). In addition we also included as experienced birds individuals ringed for the first time as adults and recaptured in subsequent years (n = 118; 46 males and 71 females). Hence, the total sample size for experienced birds was 146 individuals (59 males and 86 females; Online Supplementary Material Appendix 1, Table A1).

Stable isotope analysis

All feathers were cleaned of surface oils in 2:1 chloroform:methanol solvent rinse and prepared for δ^2H, δ^{13}C and δ^{15}N analysis at the Stable Isotope Laboratory of Environment Canada, Saskatoon, Canada. Deuterium in the non-exchangeable hydrogen of feathers was determined using the method described by Wassenaar & Hobson (2003) and using three calibrated keratin hydrogen-isotope reference materials (CBS: -197 ‰ SPK: -121.6 ‰, KHS: -54.1 ‰). Hydrogen isotopic measurements were performed on H_2 gas derived from high-temperature (1350°C) flash pyrolysis of 350±10 µg feather subsamples and keratin standards using continuous-flow isotope-ratio mass spectrometry CFIRMS with a Eurovector 3000 (Milan, Italy - www.eurovector.it) elemental analyser interfaced with an Isoprime (Manchester, UK) mass spectrometer. Measurement of the three keratin laboratory reference materials corrected for linear instrumental drift were both accurate and precise with typical within-run (n=5) SD values of < 2 ‰. All results are reported for non-exchangeable H expressed in the typical delta (δ) notation, in units of per mil (*‰*), and normalized on the Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale.

For δ^{13}C and δ^{15}N analyses, between 0.5 and 1.0 mg of feather material was combusted online using a Eurovector 3000 elemental analyzer. The resulting CO_2 and N_2 was separated by Gas Chromatograph (GC) and introduced into a Nu Horizon (Nu Instruments, Wrexham, UK - www.nu-ins.com) triple-collector isotope-ratio mass-spectrometer via an open split and compared to CO_2 or N_2 reference gas. Stable nitrogen (^{15}N/^{14}N) and carbon (^{13}C/^{12}C) isotope ratios were expressed in delta (δ) notation, as parts per thousand (*‰*) deviation from the primary standards: atmospheric nitrogen and VPDB (Vienna Pee Dee Belemnite carbonate) standards, respectively. Using previously calibrated internal laboratory C and N standards, powdered keratin (BWBIII, δ^{13}C: -20‰, δ^{15}N: 14.4‰) and gelatin (PRGEL, δ^{13}C: -13.6‰, δ^{15}N: 4.73‰), within run (n=5), precisions for δ^{15}N and δ^{13}C measurements were ~ ± 0.15 *‰.*

Defining wintering areas

Hobson et al. (2012b) identified four isotopically distinct feather clusters in Africa based on combined $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values (i.e. isoscapes). Briefly, this approach assigns each sample feather to its most-probable cluster origin in Africa, given the regional mean expected values of the three isotopes and the covariance among them. Here, the $\delta^{13}C$ and $\delta^{15}N$ isoscapes were based on plant physiology and soil models and the $\delta^{2}H$ isoscape was based on the measured long-term Global Network for Isotopes in Precipitation (GNIP). These isoscapes were converted to feather isoscape equivalents using assumed isotopic discrimination factors ($\delta^{13}C$ and $\delta^{15}N$) or transfer functions ($\delta^{2}H$) based on measured data for known-origin birds. Feather isoscapes were then grouped in different multivariate spaces (i.e. isotopic clusters). This was accomplished using the “clara” algorithm, which is based on the cluster analysis using the partitioning around the medoids (PAM clustering algorithm; see details Hobson et al. 2012b). The isotopic clusters separated well in multivariate space, with relatively low overlap between 90% confidence ellipsoids between clusters (a crossvalidated DFA suggested that 94% of GIS cells within the African continent could be accurately placed into a given isotopic cluster). The optimal number of clusters was determined using a cross-validation criterion known as “prediction strength”: the highest mean prediction strength was found by using four different clusters for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$. Across 50 randomized clustering experiments, sample pairs were placed in the same cluster 88% of the time (Hobson et al. 2012b). By utilizing linear discriminant function analysis (DFA), we predicted the posterior probability that a feather sample with a given multi-isotope signature could have originated from any given developed cluster within Africa. Finally, each sample feather was associated within the cluster for which the highest likelihood was found. This method has already been successfully used to identify the most-probable wintering origins of house martins breeding at a single site in The Netherlands (Hobson et al. 2012a).

Every isotopic cluster is spread across a great portion of the African continent, so in order to narrow down potential wintering regions corresponding to assigned clusters, we utilized a regression analysis across known breeding and wintering geographical locations of house martins based on recoveries of ringed individuals (Hobson et al. 2012a). As shown by this linear regression, house martins breeding in Western Europe tend to winter in Western Africa and house martins breeding in Eastern Europe tend to winter in Eastern Africa. Although this regression analysis is based on a small sample of trans-Sahara recapture data of birds ringed in their Europeans breeding areas, cues on winter areas provided by regression analysis were consistent with previous studies that have assigned house martins breeding in Europe to wintering sites in West Africa (Robinson et al. 2008; Ambrosini et al. 2011; Hobson 2012a).

Statistical analysis

To investigate how age or sex may influence the probability of winter site use, we used a Generalized Linear Mixed Model with wintering area (i.e. isotopic cluster one or two) as the response variable. We used a binomial distribution of errors and used the *logit* as the link function. As explanatory variables, we included sex and age ('young' and 'experienced') as fixed effects, and body condition index as a covariate. To measure body condition we used the scaled body mass index, which standardizes body mass at a fixed value of a linear body measurement based on the scaling relationship between mass and length (Peig & Green 2010). To determine if birds hatched within our study colonies (i.e., recruits) winter in different sites than birds hatched outside from the study area, we included as well the 'origin of bird' as another fixed effect with two levels ('hatched at the study colonies' and 'hatched outside study colonies'). We included individual identity, colony and year as three cross-random effects (intercepts), thus controlling for inter-annual and between-breeding site variation in isotopic values and for individuals sampled on more than one occasion. This global model included the two breeding colonies with a sample size of 163 observations after exclusion of missing data for any of the explanatory variables.

We tested whether winter area may have influenced subsequent breeding performance. We used Generalized Linear Mixed

Models in which the date females laid their first eggs (i.e., laying date), the number of eggs laid (i.e., clutch size) and the number of fledglings produced were the response variables while winter area (isotopic cluster one or two), sex, and body condition were explanatory variables. We included all possible two-way interactions between these factors. Two cross-random effects (intercepts) in our model were nest identification (i.e., the nest attended by the breeding pair) and year, thus controlling for inter-annual and within-nest variation in the response variables. For this analysis we used data from just one of our breeding sites (i.e., Rucab) because we did not monitor the breeding pairs of the other breeding site (i.e., Botoa). Given the low number of recaptures in Rucab (N = 5), we utilized only one observation per individual, and finally sample size was 196 observations for this analysis. We used a Poisson distribution of errors and used *log* as the link function. In addition, we ran a similar Generalized Linear Mixed Model with the same response variables indicating fitness, but including age (young, experienced) and winter area and their interaction as fixed factors leaving the random part of the model unchanged. Sample size for this last analysis was reduced to 112 observations, after inclusion of age. For the models in which clutch size and the number of fledglings were the response variables, we also included laying date as an additional covariate because it has been shown that this variable affected breeding success in this species (Marzal et al. 2013).

We evaluated all possible candidate models that could be built with our initial global models following the procedures described by Grueber et al. (2011). We first standardized the input variables entering the global model, scaling them by dividing means by two standard deviations, which allowed comparison on the same scale of coefficients of binary factors (e.g., sex), and covariates. Therefore, parameter estimates were standardized effect sizes and were on a comparable scale (Gelman 2008; Grueber et al. 2011). The most parsimonious of all possible candidate models was determined using Akaike Information Criterion corrected for small sample size (AICc). We calculated Akaike weight (w) for each candidate model (i) that can be interpreted as the probability that (i) is the best model, given the data and set of candidate models (Burnham & Anderson 2002). The final model was obtained by averaging the parameter estimates from top models at a cut-off criterion of ΔAICc < 6.0 (Richards 2008).

We employed the libraries *MuMIn* (Bartón 2015), *lme4* (Bates et al. 2015) and *AICcmodavg* (Mazerolle 2015) using R version 3.0.2 (R Core Team 2016).

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5sg24 (Lopez-Calderon et al. 2016). Online Supplementary Materials can be found in the published version: http://avianbiology.org/appendix/jav-01070.

Results

First objective: identification of most-probable wintering areas

We matched a previously described isoscape proposed for Africa based on three isotopes (δ^{13}C, δ^{15}N, δ^{2}H) with the isotopic proportions measured in the feathers of house martins captured in our breeding sites in Spain (Fig. 1).

Within our original sample size (n = 300; Online Supplementary Material Appendix 1, Table A1), a total of 101 sampled house martins were assigned to ‘cluster one’ and 198 to ‘cluster two’. Only one individual was assigned to 'cluster three’, and this individual was excluded from subsequent analyses. Summary results from the probability assignment procedure are shown in Online Supplementary Material Appendix 1, Table B2). Both clusters (one and two) were present in several main regions of Africa: North, West, Central, East and South Africa. Using the regression equation based on ring-recoveries we estimated a wintering longitude of 0.50°E [95% CI: (–7.51, 8.52)] given a breeding geographical longitude of 7.015°W (Badajoz geographical longitude). This analysis allowed us to apply prior knowledge to discard Central, East and South Africa as possible wintering areas for the studied house martins. Therefore, two pockets of potential wintering areas were possible: in North Africa and in West Africa. House martins are classified as wintering across Africa south of 20°N, which excludes North Africa (Turner 2004). Therefore, we identified the most probable wintering area for our studied populations as West Africa (Fig. 1). The West Africa wintering area is characterized by two different biomes corresponding respectively to isotopic cluster one and two: moist broadleaf forest and savannah mixed with tropical seasonal forest (SEDAC 2012). The average, standard deviation and range of the isotopic values for each winter area are shown in Table 1.

Table 1. Average, standard deviation and range of stable isotopic values obtained from sample feathers assigned to either isotopic cluster. Sample size is 101 birds assigned to isotopic cluster one and 198 to isotopic cluster two.

	Mean (‰)	**S.d. (‰)**	**Range (‰)**
δ^{13}C			
Cluster two	-16.72	1.44	(-19.6, -12.4)
Cluster one	-19.83	1.11	(-23.5, -16.7)
δ^{15}N			
Cluster two	9.06	0.99	(6.9, 12.6)
Cluster one	9.03	1.09	(7.0, 12.4)
δ^{2}H			
Cluster two	-42.39	8.82	(-66.7, -21.2)
Cluster one	-37.37	11.05	(-76.8, -13.5)

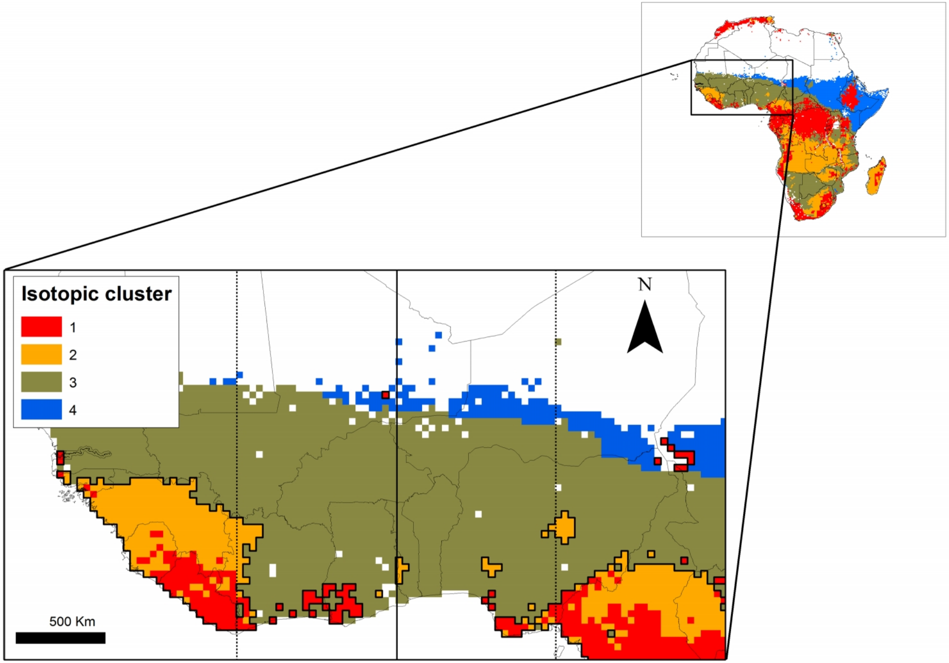

Figure 1. Map depicting assigned wintering areas for house martin *Delichon urbica* breeding at Badajoz (southwestern Spain). The continuous and dotted vertical lines indicate the mean and 95% confidence intervals wintering longitude estimated for the studied population using linear regression analysis (Hobson et al. 2012a). Predicted wintering areas correspond to isotopic clusters 1 and 2 throughout West Africa. Sample size is 300 feathers collected from our two study populations.

Second objective: relationships of age and sex on winter habitat use

The final model of the analysis of probability to winter either in the isotopic cluster two or the isotopic cluster one showed a significant effect of the interaction term between age and sex (Table 2). The confidence interval of this interaction term excluded zero. This interaction indicated that experienced males (i.e. two years or older) winter in the isotopic cluster two with a greater probability than experienced females (estimated probability: 0.83 ± 0.051 in experienced males, 0.64 ± 0.061 in experienced females), whereas young females winter in the isotopic cluster two with greater probability than young males (estimated probability: 0.55 ± 0.096 in young females; 0.38 ± 0.106 in young males; Fig. 2). Birds differing in body condition did not differ in their probability to winter in either isotopic cluster, as shown by the confidence interval for this predictor including zero (Table 2). The origin of the birds ('hatched at the study colonies' or 'hatched outside the study colonies') did not affect the probability to winter in either isotopic cluster, as shown by the confidence interval for this predictor including zero (Table 2). Top models selected for averaging are shown in Online Supplementary Material Appendix 2, Table A2. To test if experienced individuals winter in different areas from young ones regardless of sex, we repeated this analysis of probability to winter in either area excluding the interaction term between age and sex. This analysis confirmed that age showed a significant difference between wintering areas [estimate = –1.57, SE = 0.71, relative importance (RI) = 0.95, 95% CI = (–2.97, –0.18)]. Thus, experienced house martins winter in the isotopic cluster two with a higher probability than young ones (estimated probability: 0.73 ± 0.041 for experienced individuals and 0.49 ± 0.072 for young individuals). In the latter analysis, sex did not correlate with the probability to winter in the isotopic cluster two [estimate = –0.38, SE = 0.36, RI = 0.37, 95% CI = (–1.09, 0.33)].

Table 2. Summary results after model averaging of the relationship between age and sex of house martins on the probability to winter in either isotopic cluster. Sample size is 163 individuals. Parameters estimated for predictors with confidence intervals that do not include zero are highlighted in bold. The reference level of the fixed factor sex was ‘female’, for age was ‘young’ and for origin was ‘hatched at the study colonies’. RI – Relative importance.

Parameter	Estimate	SE	95% CI	RI
(Intercept)	-0.110	0.504	(-1.10, 0.88)	
Age	-1.065	0.822	(-2.68, 0.55)	1.00
Sex	0.540	0.711	(-0.85, 1.93)	0.77
Origin	0.858	0.630	(-0.38, 2.09)	0.48
Scaled body mass index	-0.063	0.394	(-0.83, 0.71)	0.25
Age*Sex	**-1.783**	**0.773**	**(-3.30, -0.27)**	**0.63**

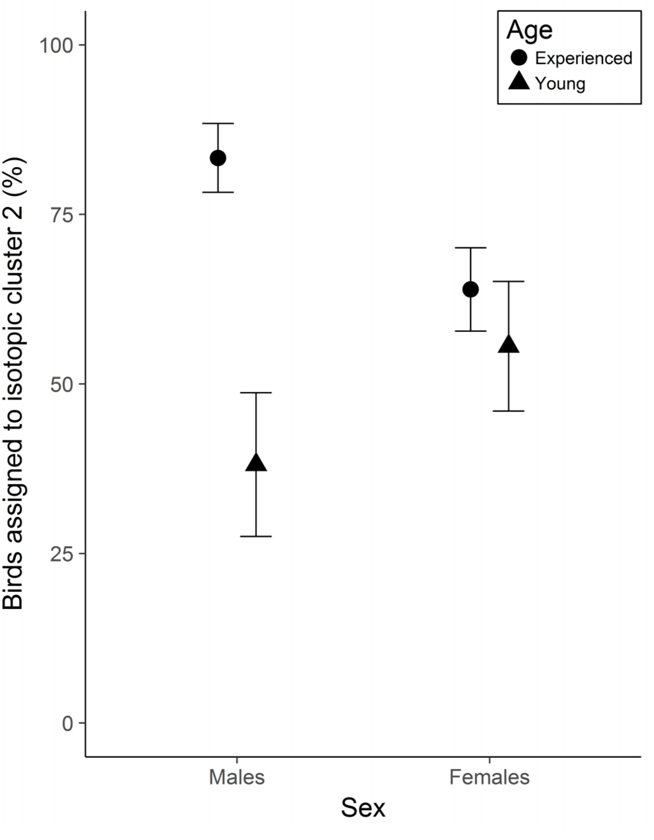

Figure 2. Relationship between the interaction term of age (young vs experienced) and sex on the probability to winter in the isotopic cluster two of West Africa for house martins *Delichon urbica* from Badajoz (southwestern Spain). Sample size is 45 experienced males, 8 young males, 39 experienced females and 15 young females wintering in the isotopic cluster two. Error bars indicate mean percentage ± 1 SE.

Third objective: carry over effects from wintering areas on reproductive performance

The final model of the analysis explaining variation in laying date showed a significant effect of the interaction term between age and isotopic cluster. The confidence interval for this predictor excluded zero (Table 3). Specifically, this interaction indicated that there was no difference in laying date between young and experienced birds wintering in the isotopic cluster two (laying date mean ± SE: 113.8 ± 3.2d for young individuals; 114.1 ± 2.9 d for experienced birds), whereas there was a difference in laying date between young and experienced birds wintering in the isotopic cluster one (Fig. 3). Specifically, young birds start to reproduce earlier than experienced ones when wintering in the isotopic cluster one (laying date mean ± SE: 118.4 ± 3.1 d for young birds; 125.8 ± 3.8 d for experienced individuals). Neither sex nor body condition were correlated with laying date, as shown by the confidence interval of these predictors including zero. Top model sets selected for averaging are shown in Online Supplementary material Appendix 2, Table B2. For the model in which age was excluded, we found that birds wintering in the isotopic cluster two bred earlier than birds wintering in the isotopic cluster one (Table 3). Specifically laying date (mean ± SE) for birds wintering in the isotopic cluster two was 114.0 ± 2.2 d, while for birds wintering in the isotopic cluster one was 121.5 ± 2.4 d. In contrast, we did not find any significant effect of winter area on clutch size nor on the number of fledglings, while controlling for laying date and body condition (Table 4). None of the predictors included in these models had a significant effect between the response variables. Mean ± SE clutch and brood size for birds wintering in the isotopic cluster two were respectively, 4.5 ± 0.1 eggs and 3.6 ± 0.1 young; whereas mean ± SE clutch and brood size for birds wintering in the isotopic cluster one were respectively 4.5 ± 0.1 eggs and 3.3 ± 0.1 young. We also introduced age effects in the global model explaining variation in clutch size and the number of fledglings. Again, none of the predictors included in these models had a significant effect with the response variables as all their confidence intervals included zero (Online Supplementary material Appendix 3, Table A3).

Table 3. Summary results after model averaging of the effects of spending the winter in either isotopic cluster on laying date in the house martin. Sample size is 196 individuals in the first model (upper section), which did not include age effects. Results for known age individuals are shown in the lower section (sample size is 112 birds). Parameters estimated for predictors with confidence intervals that do not include zero are highlighted in bold. The reference level of the fixed factor sex was 'female', for age was 'young' and for isotopic cluster was 'two'. RI – Relative importance.

Parameter	**Estimate**	**SE**	**95% CI**	**RI**
(Intercept)	4.75	0.033	(4.685, 4.816)	
Isotopic cluster	**0.082**	**0.022**	**(0.039, 0.125)**	**1.00**
Sex	-0.027	0.021	(-0.069, 0.014)	0.56
Scaled body mass index	0.014	0.018	(-0.021, 0.05)	0.32
Sex*Isotopic cluster	0.052	0.033	(-0.014, 0.118)	0.29
(Intercept)	4.744	0.049	(4.648, 4.841)	
Isotopic cluster	0.052	0.042	(-0.032, 0.136)	1.00
Sex	-0.04	0.027	(-0.095, 0.014)	0.58
Scaled body mass index	0.038	0.026	(-0.014, 0.089)	0.47
Age	0.009	0.059	(-0.106, 0.125)	0.62
Sex* Isotopic cluster	0.054	0.049	(-0.043, 0.151)	0.21
Age* Isotopic cluster	**0.114**	**0.056**	**(0.003, 0.225)**	**0.43**

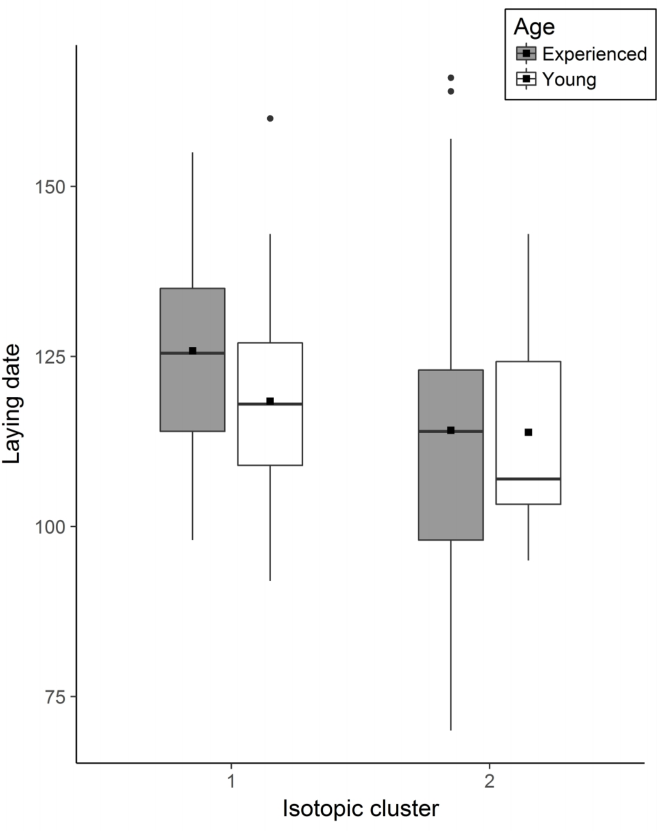

Figure 3. Relationship between the interaction term of age (young vs experienced) and isotopic cluster (1 vs 2) on laying date for house martins *Delichon urbica* from Badajoz (southwestern Spain). Black squares represent mean laying dates for each age category wintering in either isotopic cluster. Within the y-axis, ‘day 75’ corresponds to 16 March and ‘day 150’ to 30 May. Sample size is 112 adult birds.

Table 4. Summary results after model averaging of the effects of spending the winter in either isotopic cluster on clutch size and number of fledglings for house martins. Sample size is 196 individuals. Parameters estimated for predictors with confidence intervals that do not include zero are highlighted in bold. The reference level of the fixed factor sex was 'female' and for isotopic cluster was 'two'. RI – Relative importance.

Parameter	**Estimate**	**SE**	**95% CI**	**RI**
Clutch Size:				
(Intercept)	1.481	0.044	(1.39, 1.57)	
Isotopic cluster	0.007	0.073	(-0.136, 0.15)	0.27
Sex	0.044	0.069	(-0.091, 0.18)	0.31
Scaled body mass index	-0.021	0.068	(-0.155, 0.11)	0.26
Laying date	-0.106	0.069	(-0.24, 0.03)	0.54
Sex* Isotopic cluster	0.005	0.14	(-0.27, 0.28)	0.01
Nº of fledglings:				
(Intercept)	1.229	0.051	(1.13, 1.33)	
Isotopic cluster	-0.04	0.083	(-0.2, 0.123)	0.29
Sex	0.047	0.079	(-0.11, 0.202)	0.30
Scaled body mass index	0.043	0.077	(-0.11, 0.193)	0.28
Laying date	-0.081	0.078	(-0.23, 0.073)	0.36
Sex* Isotopic cluster	-0.091	0.16	(-0.40, 0.223)	0.02

Discussion

Using a multi-isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{2}H$) analysis of feathers, we predicted two primary wintering areas in West Africa (i.e. isotopic cluster one and two) for house martins breeding in southwestern Europe. We found that experienced individuals mainly winter in the isotopic cluster two. Furthermore, birds wintering in the isotopic cluster two initiated their clutches sooner than those wintering in the isotopic cluster one. We also showed that experienced males wintered in the isotopic cluster two with a greater probability than experienced females, whereas young females wintered in the isotopic cluster two with a greater probability than young males. Also, young birds from the isotopic cluster one reproduced earlier than experienced birds from the same wintering region. Given the low trans-Sahara recovery ratio for house martins ringed in the European breeding quarters, we provide a correlative approach that provides insights into the winter ecology of this species. Our results can in turn be helpful for understanding the causes of the declining population trend of this species throughout Europe.

Previous studies have linked higher winter habitat quality to higher proportion of dominant individuals (Marra & Holmes 2001), to earlier breeding phenology and to improved reproductive success (Marra et al. 1998; Norris et al. 2004). We found that the proportion of experienced males (i.e. the dominant class) is much higher in the isotopic cluster two of West Africa. Moreover, we found that birds wintering in the isotopic cluster two of West Africa reproduced earlier in the subsequent breeding season. In agreement with Evans et al. (2012) our results suggest, that for the study house martins, the quality of the isotopic cluster two (mostly savannah and tropical seasonal forest) is higher than the quality of the isotopic cluster one (predominantly moist broadleaf forest). However, we did not find any differences in body condition between birds wintering in either isotopic cluster. This could be explained because body condition was measured during the breeding season, and hence it may not reflect body condition prior to migration or upon arrival at the breeding grounds [in a sample size of 164 house martins from our study colonies, the average time between arrival and laying was 31.19 (SD = 5.26) d].

Harrison et al. (2011) suggested that in many animal systems, social status, population density and winter habitat quality are intimately linked, and they suggested that the true mechanism driving carry-over effects would be the interaction among these factors. Our results are consistent with this view and we hypothesize that dominant house martins would exclude subordinates from high quality winter habitats, reducing density in these habitats and finally experiencing a positive carry over effect in breeding phenology. Nevertheless we acknowledge that fieldwork on the wintering areas is required to completely understand the underlying mechanism driving such observed carry-over effects.

We did not find a difference in the number of eggs laid nor in the number of fledglings produced for individuals wintering in either location. However, it has long been recognized that laying date is negatively correlated with other reproductive parameters such as clutch size and number of offspring in many species (Winkler & Allen 1996; Smith & Moore 2004) and in house martins (Marzal et al. 2013). Therefore, we can consider that the delay in laying dates of birds wintering in the isotopic cluster one should carry-over an indirect cost in terms of fitness that we were not able to detect because our analyses tested only the direct effect of wintering in either area on fitness. Other statistical approaches, such as path analysis, may in turn be helpful to elucidate the variables mediating the effect from the wintering areas on reproductive success (Norris et al. 2004; Drake et al. 2013).

In many bird species, including hirundines, males arrive to the breeding grounds earlier than females because competition for mating is stronger in males than females (Møller 1992; Lozano et al. 1996; Hasselquist 1998). It is well known in birds and other animals that many phenotypic and fitness traits improve with age and decline during senescence (reviewed by Nussey et al. 2013). The mechanisms explaining within-individual improvement in fitness traits in early life are poorly understood (Forslund & Part 1995). For example, it has been suggested that individuals gain experience in performing reproductive tasks such as nest building, provisioning young, or finding a partner (Lozano et al. 1996; Hasselquist 1998). Alternatively, it is possible that in migratory species, more experienced individuals would perform better during winter by selecting better quality habitats (Marra et al. 1998), or that they would perform better during the migratory journey (Sergio et al. 2014). We have shown that experienced birds winter in the isotopic cluster two with a greater probability than young birds, and that birds wintering in the isotopic cluster two started to reproduce earlier than birds wintering in the isotopic cluster one of West Africa. Therefore, in our study system a possible cause of earlier start of reproduction with age might be explained partly by the selection of habitat used during winter. In contrast to previous studies (Møller 1994; Lozano et al. 1996; Hasselquist 1998; Smith & Moore 2004), we found that young birds wintering in the isotopic cluster one lay their eggs earlier than experienced birds from the same wintering region (7.4 d on average). A plausible hypothesis to explain this result could be that experienced birds wintering in the isotopic cluster one were of lower quality or senescent. Unfortunately, we do not have data on individual quality (i.e. body condition) measured during winter, nor do we have sufficient longitudinal observations to examine senescence effects over the range of ages observed in our studied species (i.e. 1–8 yr).

To summarize, isotopic values measured in feathers allowed us to identify winter origins and relevant age-related aspects of the winter ecology in this migratory species. We have shown that birds of different age and sex winter in different regions of West Africa, and that the selection of winter habitat may have carry-over effects to the next breeding season. Further studies are now required to understand the cost and benefits that may derive from spending the winter in either of the two wintering habitats identified within this study.

Acknowledgements

This study was funded by research projects of the Spanish Ministry of Economy and Competitiveness (CGL2012-36665, CGL2015-646509), Junta of Extremadura (PRI08A116), Junta of Andalucía (P12-RNM-2144) and Univ. of Extremadura (A7-26). AM and MR were supported by grants from the Spanish Ministry of Education and Science (JC2011- 0405, BES 2004-4886, BES-2007-15549 and BES-2010- 030295, respectively). The authors declare that they have no conflicts of interest. All the experiments comply with the current laws of Spain, where the experiments were performed. C. Gryba and G. Koehler assisted with stable isotope analyses and K. Kardynal assisted with cluster analyses. Isotope analyses were supported by an operating grant from Environment Canada to KAH.

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Chapter 3

Environmental conditions during winter predict age- and sex-specific differences in reproductive success of a trans-Saharan migratory bird

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López-Calderón et al. *Scientific Reports* (2017),
doi: 10.1038/s41598-017-18497-2

Abstract

Previous studies have linked winter habitat with subsequent breeding phenology and physical condition of migratory birds, but few have found delayed effects of winter habitat on subsequent reproductive success. The aim of this study was to test if African winter habitat is related to subsequent reproductive success of house martins (*Delichon urbicum*) breeding at a colony in Spain. We measured stable isotope (δ^2H, δ^{13}C, δ^{15}N) values from feathers moulted in West Africa and used confirmatory path analysis to test if isotopic values of winter-grown feathers were related to reproductive success through the mediation of breeding phenology and body condition. We conducted separate analyses for males, females and age classes (yearlings *vs* ≥ 2 years old). Experienced males wintering in habitats of higher rainfall (as inferred from lower feather δ^2H values) were in better body condition and produced more offspring during the subsequent breeding season. In contrast, we did not find any effect of winter habitat on reproductive success of young males or females. These findings provide evidence consistent with a complex causal link between winter habitat quality and subsequent breeding success of long-distance migratory songbirds.

Introduction

In an ecological context, carry-over effects occur when the previous history and experience of an individual explains its current performance in a given situation (O'Connor et al. 2014). Many studies of migratory birds have found that environmental conditions experienced during winter have carry-over effects in terms of subsequent body condition and breeding phenology (e.g., Marra et al. 1998; Bearhop et al. 2004; Studds & Marra 2005; Balbontín et al. 2009; Marzal et al. 2013; López-Calderón et al. 2017). However, few studies have found carry-over effects of environmental conditions from winter areas on final reproductive success (Saino et al. 2004, 2017; Norris et al. 2004; Gunnarsson et al. 2005; Drake et al. 2013). For example, older barn swallows (*Hirundo rustica*) arrived earlier to their breeding grounds after winters with favorable environmental conditions and, as a result of increased frequency of second broods, their reproductive success was higher in those years (Saino et al. 2004).

Given the difficulty in tracking migratory songbirds with exogenous instruments or markers, analyses of stable isotopes on feathers can provide an alternate or complementary means of studying carry over effects (Webster et al. 2002; Harrison et al. 2011). For instance, populations of godwits (*Limosa limosa islandica*) wintering in ^{13}C-enriched (i.e., high quality marine) habitats have higher reproductive success than populations wintering in ^{13}C-depleted (lower quality terrestrial) habitats (Gunnarsson et al. 2005).

Environmental conditions experienced in winter may first affect physical condition during winter, timing of departure from wintering sites and timing of arrival to breeding areas (Marra et al. 1998; Studds & Marra 2005). Then, arrival time to breeding areas could determine the start of breeding and this in turn directly influence the number of offspring produced (Winkler & Allen 1996; Smith & Moore 2004; Marzal et al. 2013). In addition, physical condition upon arrival and during the breeding season could be related to breeding performance (e.g. Balbontín et al. 2012). Structural Equation Models (hereafter: SEM) and path analysis (a kind of SEM with only measured variables) provide a powerful statistical method to study complex natural systems, since multiple predictor and response variables can be analyzed in a single causal network (Shipley 2016; Lefcheck 2016). Specifically, these kinds of analyses can be especially useful in dealing with carry over effects in migratory birds since the above-mentioned variables flow in a time series fashion from wintering to breeding period and vice versa (Norris et al. 2004). However, despite the great potential of SEM to detect carry-over effects in migratory birds, it has rarely been used for this purpose. For instance, an indirect carry-over effect of winter habitat quality (inferred from δ^{13}C measurements) on reproductive success in American redstart (*Setophaga ruticilla*), was elegantly disentangled by the use of path analysis (Norris et al. 2004). In that study, the use of path analysis was essential for discerning that arrival, laying and fledging date, as mediated by the effect of winter habitat quality, determined the number of fledglings finally reared during the subsequent breeding season. A similar result was found for yearling female yellow warblers (*Setophaga petechia*) overwintering in Mexico and breeding in Canada, utilizing general linear models in a path-like analysis (Drake et al. 2013). Recently, the complex associations among phenology, winter ecology and breeding performance in the barn swallow have been analyzed by means of partial least squares path modeling (which is related to SEM) (Saino et al. 2017).

The house martin (*Delichon urbicum*) is a small colonial hirundine distributed across Europe, Asia and Africa. In the Palearctic, this species breeds across Europe and winters throughout Africa south of 20ºN (Turner 2004; Szép et al. 2017), where the single complete annual moult occurs (Cramp 1988; Turner & Rose 1989). House martins breeding in Western Europe tend to winter in Western Africa and those breeding in Eastern Europe tend to winter in Eastern Africa (Hobson et al. 2012a). Like many aerial insectivores and long-distant migrants, the Palearctic population of house martin has declined during 1990-2000 and the global population is also declining (Sanderson et al. 2006; Nebel et al. 2010; Birdlife International 2012). However, causes of these declines and the degree of migratory connectivity in this species are poorly understood (Hobson et al. 2012a). We previously analyzed stable isotope (δ^2H, δ^{13}C, δ^{15}N) values from feathers of house martins breeding in southwestern Spain and proposed two winter areas in West Africa with different environmental conditions (savannah and broadleaf forest) (López-Calderón et al. 2017). We also found that experienced males winter in savannahs at higher probability than females, whereas young males winter in savannahs at lower probability than females. By contrast, experienced and young females winter in both areas with similar probabilities. Furthermore, we showed that winter area choice was related to clutch initiation date in the subsequent breeding season. However, we did not detect a final carry-over effect on the number of fledglings produced.

The aim of this study was to investigate the relationship between winter habitat of house martins wintering in West Africa and subsequent reproductive success in the temperate breeding area in southwestern Spain. We used as a proxy of winter habitat the isotopic values of feathers moulted at the wintering grounds, and used confirmatory path analysis to discriminate between some of the likely mediator variables involved in this migratory system. We predicted that environmental conditions in winter areas, as reflected by multiple feather isotopes (δ^2H, δ^{13}C, δ^{15}N), indirectly affect the subsequent reproductive success of individuals through effects on subsequent breeding phenology and body condition. Specifically, we hypothesized that open habitats dominated by C4 vegetation with high seasonal rainfall are more suitable for our study species to overwinter than equatorial closed forests dominated by C3 vegetation (Evans et al. 2012; López-Calderón et al. 2017). This is caused by higher rainfall being correlated with higher abundance of flying insects (Denlinger 1980; Cumming & Bernard 1997; Frampton 2000), but also because open habitats may provide a better habitat for aerial insectivores to forage. Therefore, we expected that lower feather δ^{2}H and higher feather δ^{13}C and δ^{15}N values would be correlated with earlier breeding phenology, improved physical condition and thus higher reproductive success in the subsequent breeding season. We separately analyzed young (i.e., one-year old birds migrating for the first time) from experienced birds (i.e., two-years or older birds that have previously migrated successfully), and males from females, since we predicted such carry-over effects operate differently according to age and sex (Drake et al. 2014; López-Calderón et al. 2017). Each breeding season, we performed more than one hundred capture sessions from March to July, capturing more than 90% of the colony. Breeding dispersal is negligible for house martins, while recruitment and first reproduction occurs at the age of one year (de Lope & da Silva 1988; Marzal et al. 2016). Thus, we could assign the age of individuals accurately, assuming un-ringed adult birds being yearlings at first capture originating from outside the study area, and assuming that disappearance of ringed breeders from the colony indicated mortality rather than dispersal.

Materials and methods

Field procedures

We studied a single colony of house martins in Badajoz, Southern Spain (38º53'N 7º01'W), during 2005-2013 (excluding 2006). From February to July, we followed reproductive events every second day to determine laying date (i.e., the date the first egg is laid) and brood size (i.e., the number of fledglings in the first brood). Adult house martins were captured and identified with numbered metal rings. Our original sample size was 195 adults and we only took one observation per individual. We categorized our original data in two different age-classes: young birds (i.e., one-year old individuals that have migrated for the first time that year; *n* = 129), and experienced birds (i.e., two-years or older individuals that were at least in their second migration year; *n* = 66). We categorized as young birds, individuals ringed as nestlings/fledglings that were recaptured in the next year (*n* = 41), and also individuals ringed for the first time as adults (*n* = 88). We categorized as experienced birds, individuals ringed as nestlings/fledglings that were recaptured two years or more after their first capture (*n* = 20), and also individuals ringed for the first time as adults that were recaptured in subsequent years (*n* = 46). From each adult we removed the outermost rectrix for isotopic analysis, recorded tarsus length with a digital caliper to the nearest 0.01 mm and body mass with a Pesola spring balance to the nearest 0.5 g. To estimate body condition we used the scaled mass index, which standardizes body mass at a fixed value of a linear body measurement (tarsus) based on the scaling relationship between mass and body length (Peig & Green 2010).

Stable isotope analysis

All feathers were cleaned of surface oils in 2:1 chloroform:methanol solvent rinse and prepared for δ^{2}H, δ^{13}C and δ^{15}N analysis. Deuterium abundance in the non-exchangeable hydrogen of feathers was determined following standard procedures (Wassenaar & Hobson 2003), and using three calibrated keratin hydrogen-isotope reference materials (CBS = -197‰; SPK = -121.6‰; KHS = -54.1‰). Deuterium measurement was performed on H_2 gas derived from high-temperature (1350°C) flash pyrolysis of 350±10 µg feather subsamples and keratin standards. Measurement of the three keratin laboratory reference materials, corrected for linear instrumental drift, were both accurate and precise with typical within-run (n=5) SD values of < 2‰. For feather δ^{13}C and δ^{15}N analyses, between 0.5 and 1.0 mg of feather material was combusted online using a Eurovector 3000 elemental analyzer (Eurovector, Milan, Italy). The resulting CO_2 and N_2 was separated by Gas Chromatograph (GC) and introduced into a Nu Horizon (Nu Instruments, Wrexham, UK - www.nu-ins.com) triple-collector isotope-ratio mass-spectrometer via an open split and compared to CO_2 or N_2 reference gas. Using previously calibrated internal laboratory C and N standards [powdered keratin (BWBIII; $\delta^{13}C$ = -20‰; $\delta^{15}N$ = 14.4‰) and gelatin (PUGEL; $\delta^{13}C$ = -13.6‰; $\delta^{15}N$ = 4.73‰)], within run (n=5), precisions for $\delta^{15}N$ and $\delta^{13}C$ measurements were ~ ± 0.15*‰*. Stable isotope ratios are reported in standard delta (δ) notation relative to VSMOW for $\delta^{2}H$, VPDB for $\delta^{13}C$, and AIR for $\delta^{15}N$ analyses.

Environmental conditions in the wintering areas of house martins

House martins have feather $\delta^{2}H$ values reflecting long-term, amount-weighted, average $\delta^{2}H$ from precipitation prior to moult according to established calibration equations (Bowen et al. 2005; Hobson et al. 2012b), while $\delta^{13}C$ and $\delta^{15}N$ values are associated more directly from inorganic and organic sources to primary production following isotopic discrimination (Vanderklift & Ponsard 2003; Hobson et al. 2012b). Feather keratin is metabolically inert after synthesis (Hobson 1999) and so isotopic values in feathers reflect the environmental conditions where they were grown (i.e. the wintering areas). Considerable literature has emphasized that foodweb $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ values are strongly influenced by climate (reviewed by West et al. 2010). Lower values of $\delta^{2}H$ in rain are linked to higher amount of precipitation in tropical latitudes (i.e. the so-called “amount effect”; Dansgaard 1964; Clark and Fritz 1997). Higher values of $\delta^{13}C$ are associated with environments dominated by C4 plants as well as C3 plants adapted to hydric stress (Ehleringer 1989). Finally, xeric/cultivated habitats tend to be relatively enriched in ^{15}N relative to mesic/uncultivated habitats (Pardo & Nadelhoffer 2010).

The winter areas we previously identified for house martins breeding in southwestern Spain (López-Calderón et al. 2017) were defined by multi-isotopic clusters described for Africa (Hobson et al. 2012b). These isotopic clusters were closely associated with different African biomes, broadleaf forest in the so-called “Cluster 1” and savannah in “Cluster 2” (Fig. 1). Values of feather δ^2H and δ^{13}C differed markedly between the two areas of West Africa (López-Calderón et al. 2017), reflecting foodwebs in savannahs relatively enriched in ^{13}C and depleted in ^{2}H. In this wintering area, the savannah receives more rainfall during the rainy season than the broadleaf forest to the south (Sultan & Janicot 2003). Thus, we expect the foodweb to be relatively enriched in ^{13}C here due to expected C4-dominated grasslands (Ehleringer 1989), but depleted in ^{2}H due to greater seasonal rainfall (Dansgaard 1964; Clark and Fritz 1997). Indeed, that pattern was shown previously in the multi-isotopic cluster analysis for Africa (Hobson et al. 2012b).

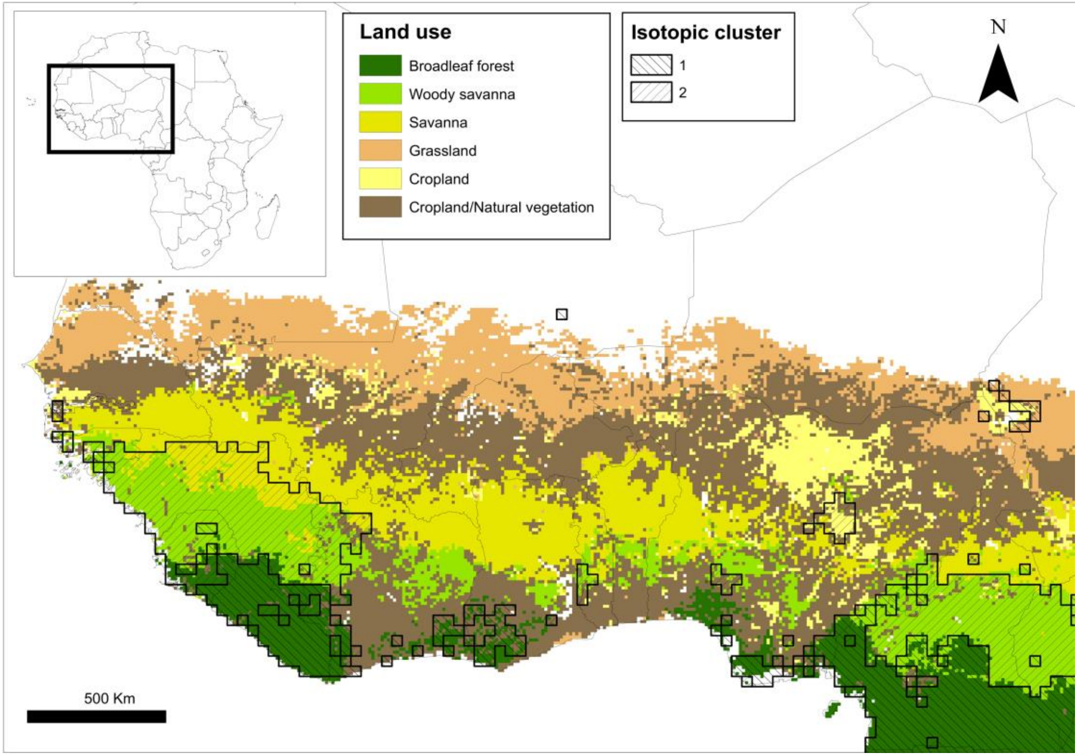

Figure 1. Assigned wintering areas for house martin *Delichon urbicum* breeding at Badajoz (southwestern Spain). African isotopic clusters were generated in ArcGis 10.2.2., following previously described methods (Hobson et al. 2012b). Land cover classification layer was obtained from freely available images (Allen 2016).

Confirmatory path analysis

Structural equation models (SEMs) are probabilistic models that hypothesize a causal network with multiple variables that can appear as both predictor and response variables (Lefcheck 2016). SEMs are usually represented as path diagrams in which one-headed arrows represent causal direct relationships from the independent variable to the dependent variable. Path analysis is a type of SEM that only takes into account observed variables; omitting latent variables (i.e. not measured variables; Grace et al. 2012; McDonald & Ho 2002). We used confirmatory path analysis to examine how feather isotope values reflecting winter habitats could explain reproductive success in the subsequent breeding season through mediation of breeding phenology and body condition. We refer to “confirmatory path analysis” as directed acyclic, or piecewise, SEM based on applications from graph theory (Shipley 2016; Lefcheck 2016). In piecewise SEM, the path diagram is translated to a set of linear equations (e.g. linear mixed models), which are then evaluated individually thus allowing a wide range of distributions and sampling designs. Another advantage of this methodology is that it allows fitting models with relatively small sample sizes. In addition, the goodness of fit of the entire causal network can be quantified by a directed separation test (”d-separation test”), which tests the assumption that all variables are conditionally independent (i.e. that there are no missing relationships among unconnected variables) (Shipley 2016).

To control the effect of age and sex on breeding success, we developed different path models for each age and sex category. We split our original data set in four subsets: experienced males (*n* = 38), experienced females (*n* = 28), young males (*n* = 52) and young females (*n* = 77). We included six variables in our models: feather δ^{2}H, δ^{13}C, δ^{15}N, body condition, first-egg laying date and number of fledglings in the first brood. Isotopic values were considered independent (exogenous), while the rest were considered dependent (endogenous) variables. Every observed variable included in our models had the same sample size (i.e. listwise deletion).

The structure of the path analysis was designed based on previous knowledge of the migratory ecology of our study population (López-Calderón et al. 2017; Norris et al. 2004), but also on hypotheses to be tested. Exploratory analyses indicated that δ^{13}C and δ^{15}N values were negatively correlated for young males. We found in a previous study that breeding phenology (i.e., first egg laying date) depends on wintering area inferred from stable isotope analysis (López-Calderón et al. 2017). It is also well known that breeding phenology is related to reproductive success in migratory passerines (Norris et al. 2004; Marzal et al. 2013). Different studies have found an effect of wintering habitat (inferred from δ^{13}C values) on body condition before departure to the breeding areas (Marra et al. 1998; Studds & Marra 2005), and during spring migration (Bearhop et al. 2004). Therefore, we included as hypotheses to be tested causal paths from winter-grown feather δ^2H, δ^{13}C and δ^{15}N to body condition during the breeding season. We measured body condition when we first captured the individual. Within our original data set, 140 birds were first captured after they had already started breeding, whereas 55 birds were first captured before they started breeding. For this reason, we assumed correlated errors between laying date and body condition, i.e. a relationship that is bidirectional and assumed to be caused by a shared underlying driver (Lefcheck 2016). For instance, arrival time to breeding areas could determine the start of breeding but also the physical condition during the breeding season. The R library *piecewiseSEM* implements a crude approximation of correlated errors by excluding them from the basis set (since there is no presumed direction of causality), and then running a simple test of significance on the bivariate correlation (Lefcheck 2016). Finally, body condition may influence the potential foraging of adults and hence the quantity of prey delivery they could afford for their progeny, and this, in turn, may influence the number of fledglings reared. In all cases, we captured adults and measured body condition before we recorded the number of fledglings. Each of our path analyses was made up of three linear mixed models, one for each dependent variable. Isotopic values were the independent variables of the linear mixed models built for body condition and first-egg laying date, while these were in turn the independent variables of the linear mixed model built for number of fledglings. In every linear mixed model we included “year” as the random effect, to statistically control for inter-annual effects. Finally, we examined the “qq plots” to visually test for normality of residuals in every individual linear mixed model. We conducted all our analyses with the library *piecewiseSEM* (Lefcheck 2016) using R version 3.3.1 (R Core Team 2016).

Results

Winter habitat features carried over to affect reproductive traits more strongly in males than in females. Breeding performance was affected by different winter habitat features depending on age class. For experienced males, confirmatory path analysis showed that rainfall amount as inferred by feather δ^{2}H had a significant direct effect on body condition [estimate (SE) = - 0.33 (0.15), $p = 0.04$], and also that body condition had a significant direct effect on the number of fledglings produced during the breeding season [estimate (SE) = 0.45 (0.16), $p = 0.01$]. For young males, confirmatory path analysis showed that vegetation type as indexed by feather δ^{13}C and δ^{15}N had significant direct effects on body condition [δ^{13}C: estimate (SE) = 0.36 (0.14), $p = 0.01$; δ^{15}N: estimate (SE) = 0.40 (0.14), $p < 0.01$] and laying date [δ^{13}C: estimate (SE) = - 0.29 (0.13), $p = 0.03$; δ^{15}N: estimate (SE) = - 0.29 (0.13), $p = 0.04$]. For females, we only found that feather δ^{15}N had a significant direct effect on the laying date of young females [estimate (SE) = 0.21 (0.09), $p = 0.03$], possibly related to diet (i.e. trophic position) or foraging microhabitat. Therefore, confirmatory path analyses showed strong differences in path coefficients across age and sex categories (Table 1, Fig. 2).

Laying date and body condition were not significantly correlated in any of the confirmatory path analyses [experienced males: estimate = 0.23, $p = 0.08$; young males: estimate = 0.19, $p = 0.09$; experienced females: estimate = - 0.13, $p = 0.75$; young females: estimate = - 0.17, $p = 0.93$]. Feather δ^{13}C and δ^{15}N values were not significantly correlated for young males (estimate = - 0.37, $p = 0.99$). We used d-separation test to quantify the goodness of fit of our models, which tests the assumption that all variables are conditionally independent (Shipley 2016). All models provided robust fit to data (experienced males: Fisher's $C = 7.7$, $df = 6$, $p = 0.26$; young males: Fisher's $C = 3.59$, $df = 6$, $p = 0.73$; experienced females: Fisher's $C = 7.65$, $df = 6$, $p = 0.26$; young females: Fisher's $C = 6.66$, $df = 6$, $p = 0.35$). We did not find any significant association among unconnected variables in any of our models. Thus, we concluded that the hypothesized relationships we examined were consistent with the data. By examining "qq plots", we determined that every single linear mixed model was well fitted with the only exception of the linear model built for the number of fledglings in experienced females.

Since for experienced males we found that feather δ^{2}H had an indirect effect on the number of fledglings mediated through body condition, we used standardized path coefficients from this model to predict how final reproductive success shifts in response to the change in feather δ^2H (Fig. 3). We quantified that the increase by one SD of δ^2H decreases the number of fledglings by 0.2 SD.

Table 1. Summary results from confirmatory path analyses built for each age and sex class. Estimates shown here are standardized path coefficients (i.e. slopes of effects). R^2 shown here is the conditional R^2, based on fixed and random effects. Sample size is 38, 28, 52 and 77 respectively for experienced-males, experienced-females, young-males and young-females. Significant effects are highlighted in bold.

Model	**Response**	**Predictor**	**estimate**	**SE**	***p***	**R^2**
Experienced males	Number of fledglings	**Scaled body mass index**	**0.455**	**0.160**	**0.010**	0.340
		Laying date	-0.261	0.162	0.136	
	Laying date	δ^{2}H	0.217	0.153	0.169	0.418
		δ^{13}C	-0.273	0.182	0.160	
		δ^{15}N	-0.021	0.163	0.899	
	Scaled body mass index	**δ^{2}H**	**-0.333**	**0.154**	**0.041**	0.338
		δ^{13}C	0.147	0.180	0.450	
		δ^{15}N	0.109	0.163	0.516	
Young males	Number of fledglings	Scaled body mass index	0.017	0.141	0.908	0.025
		Laying date	-0.161	0.141	0.299	
	Laying date	δ^{2}H	0.249	0.141	0.099	0.350
		δ^{13}C	**-0.293**	**0.132**	**0.034**	
		δ^{15}N	**-0.292**	**0.134**	**0.037**	
	Scaled body mass index	δ^{2}H	-0.122	0.145	0.430	0.340
		δ^{13}C	**0.357**	**0.136**	**0.013**	
		δ^{15}N	**0.397**	**0.139**	**0.007**	

Model	**Response**	**Predictor**	**estimate**	**SE**	***p***	**R^2**
Experienced females	Number of fledglings	Scaled body mass index	-0.034	0.204	0.896	0.002
		Laying date	0.017	0.204	0.939	
	Laying date	δ^{2}H	0.229	0.197	0.279	0.205
		δ^{13}C	-0.207	0.206	0.379	
		δ^{15}N	0.242	0.198	0.244	
	Scaled body mass index	δ^{2}H	-0.088	0.194	0.664	0.285
		δ^{13}C	-0.229	0.209	0.316	
		δ^{15}N	-0.142	0.191	0.472	
Young females	Number of fledglings	Scaled body mass index	-0.025	0.118	0.841	0.038
		Laying date	-0.133	0.123	0.369	
	Laying date	δ^{2}H	-0.010	0.100	0.923	0.477
		δ^{13}C	-0.105	0.094	0.269	
		δ^{15}N	**0.215**	**0.094**	**0.026**	
	Scaled body mass index	δ^{2}H	0.150	0.110	0.179	0.519
		δ^{13}C	0.114	0.103	0.273	
		δ^{15}N	-0.046	0.103	0.656	

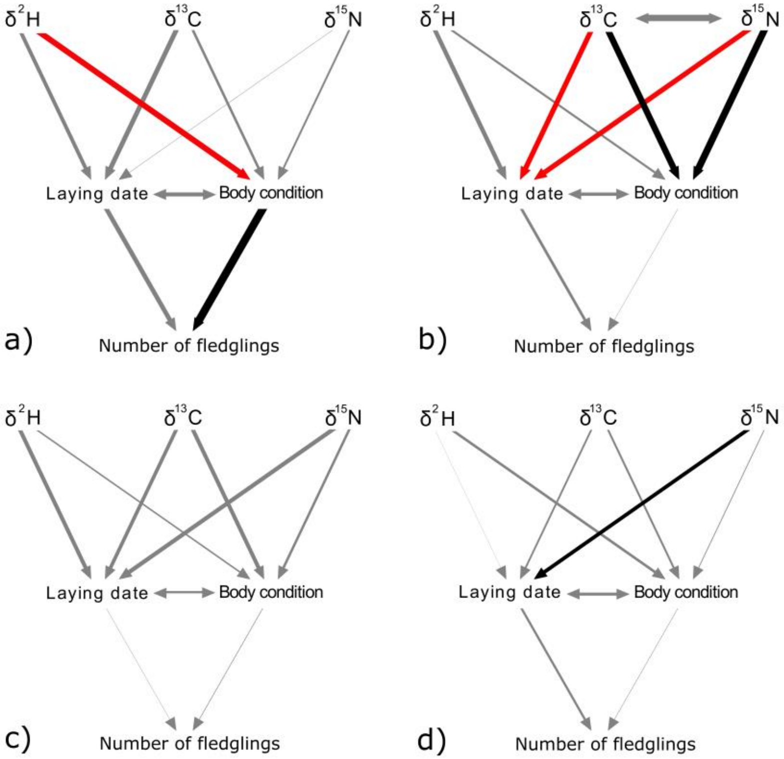

Figure 2. Path diagrams of the models developed for experienced male house martins (a), young males (b), experienced females (c) and young females (d). One-headed arrows represent a causal effect of one variable on another (i.e. a path). Double-headed arrows link variables with correlated errors. The width of the arrows reflects the magnitude of standardized path coefficients. Black arrows indicate significant positive effects, red arrows significant negative effects and grey arrows non-significant effects.

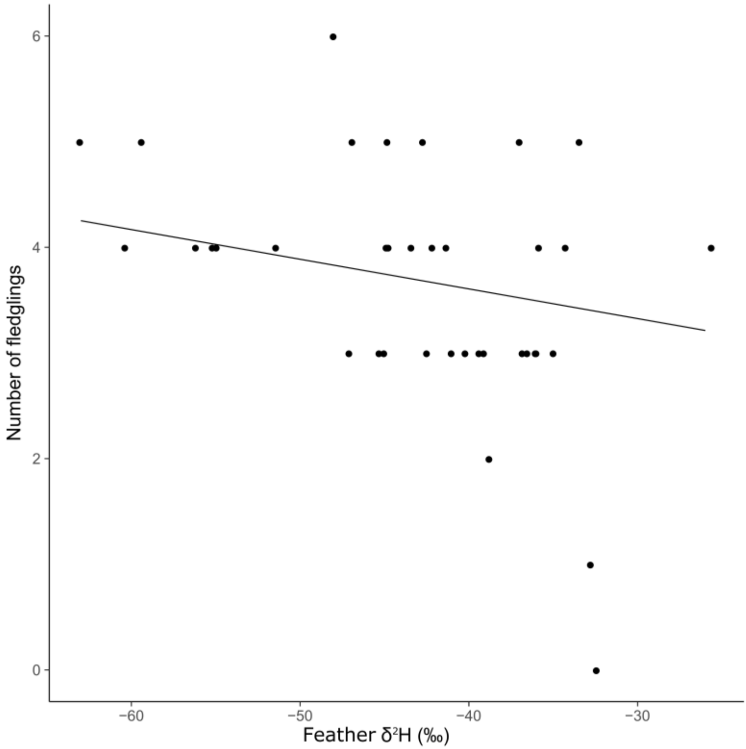

Figure 3. Relationship between feather δ^2H and reproductive success of experienced male house martins from Badajoz (southwestern Spain). Points represent observed values. The line shows predicted values obtained from the standardized path coefficients of the confirmatory path analysis fitted for experienced males. The increase across feather δ^2H range of values is predicted to decrease one fledgling reared by experienced males. Predicted values were obtained leaving δ^{13}C and δ^{15}N constant at their means, and taking into account only fixed effects.

Discussion

We found direct and indirect effects of environmental conditions experienced in the winter areas on different breeding traits in our migratory study population of house martins. Interestingly, we found that environmental conditions experienced during winter in Africa affected reproductive success depending on the age and sex of individuals. We found for experienced males that amount of rainfall, as inferred by feather δ^2H, directly affected physical condition, and that physical condition directly affected the number of fledglings produced in the first brood. This amounted to a difference of one fledgling reared across the range of feather δ^2H values. In ecological terms, experienced males wintering in habitats of higher rainfall (i.e. indicated by lower δ^2H) were in better body condition and raised a larger number of fledglings during the subsequent breeding season than experienced males wintering in drier habitats (i.e. higher δ^2H). In contrast, vegetation type as indexed by feather δ^{13}C and δ^{15}N values, affected body condition and breeding phenology of young males. In ecological terms, young males wintering in C4-dominated savannah (i.e. enriched in ^{13}C) were in better body condition during the subsequent breeding season and reproduced earlier than young males wintering in broadleaf forest (i.e. depleted in ^{13}C). However, the change in these reproductive variables did not translate into a final change in the number of fledglings. This could indicate that breeding phenology and body condition are of lower importance for reproductive success of young compared to experienced males. Actually the confirmatory path analysis built for young males revealed some effect of breeding phenology on the number of fledglings, but not sufficiently strongly to reach statistical significance. We hypothesize that young males may invest less in reproduction than experienced males, yet survival rates of young males are much lower. Characterizing winter habitats based on δ^{15}N values are inherently more difficult due to effects of both natural (e.g. climate) and anthropogenic (e.g. fertilizer use, agricultural intensity) factors (Pardo & Nadelhoffer 2010). Mean predicted δ^{15}N values were similar in both isotopic clusters described as winter areas for house martins breeding in southern Spain (López-Calderón et al. 2017). Feather δ^{15}N values also affected laying dates of young females, although in the opposite direction as in young males; young females wintering in habitats of higher δ^{15}N started to reproduce later than young females wintering in habitats of lower δ^{15}N. Because several factors can influence dietary δ^{15}N values, including climate, anthropogenic factors and plant nitrogen fixation pathways, it is difficult to predict what may be driving this pattern (Pardo & Nadelhoffer 2010). Finally, we did not find any effect of winter habitat features on the reproductive performance of experienced females.

Previous studies on Neotropical migratory passerines have found improved reproductive success (Norris et al. 2004; Drake et al. 2013) and improved physical condition associated with lower δ^{13}C values expected from more mesic environments such as wet forests or mangroves (Marra et al. 1998; Bearhop et al. 2004; Studds & Marra 2005; Smith et al. 2010). In contrast, previous studies on house martins showed that this species mainly winters in habitats enriched in ^{13}C such as open savannah and grassland (Evans et al. 2012). We found that experienced males wintering in habitats of presumed higher seasonal rainfall raised more offspring in the subsequent breeding season, and also that young males improved body condition and initiated breeding earlier when wintering in habitats of higher $\delta^{13}C$ values. Therefore, our results suggest that savannah in West Africa is a more suitable winter area for house martins than broadleaf forest. Previous studies have determined that house martins benefit from higher precipitation on their wintering grounds (Robinson et al. 2008; Ambrosini et al. 2011), and this is in agreement with our results based on feather $\delta^{2}H$ values. We interpret this in terms of higher expected insect production in wet *vs* dry areas (Denlinger 1980; Cumming & Bernard 1997; Frampton et al. 2000).

To our knowledge, only two previous studies have quantified the indirect effect of isotopic signature from the wintering areas on the number of fledglings reared by a small passerine in the subsequent breeding season, and these were both in the New World. The effect of winter habitat on the number of fledglings was stronger for female than for male American redstarts (Norris et al. 2004), while only young female yellow warblers experienced this carry-over effect

(Drake et al. 2013). We also found that environmental conditions from winter areas affected house martins differently according to age and sex. Specifically, a final increase of approximately one fledgling was predicted for experienced males wintering in areas with lowest δ^2H values relative to areas with the highest values. Interestingly, the final shift in breeding success we found for experienced male house martins was similar to the previous studies on Neotropical migrants (Norris et al. 2004; Drake et al. 2013).

Few studies have identified the specific mechanism underlying carry-over effects (O'Connor et al. 2014). We found previously for house martins breeding in Spain that experienced males winter with a much higher probability in high-quality areas (i.e. savannahs) than young males (López-Calderón et al. 2017). However, females winter in high quality areas with similar probabilities regardless of their age category. This finding is consistent with the notion that competition over winter habitats occurs among male house martins, and that it is linked to habitat characteristics important to male reproductive success. Previous studies found that competition at the winter grounds or selective pressure to arrive earlier at the breeding areas are stronger for males than for females (Møller et al. 1992; Lozano et al. 1996; Hasselquist 1998; Studds & Marra 2005; Bearhop et al. 2004), which could explain the differences we found among ages and sexes.

We showed a seasonal carry-over effect in a migratory passerine, where environmental conditions experienced in the tropical wintering areas had an indirect effect on reproductive success. Interestingly, we found this carry-over effect to be operating differently according to age and sex, where only the reproductive success of experienced males is sensitive to isotopic signatures of wintering area foodwebs. Confirmatory path analysis allowed us to identify body condition as a mediator for the observed carry-over effect on reproductive success. The analysis of stable isotopes from feathers has proven very useful for understanding the migratory ecology of this long-distant aerial insectivore, which is difficult or impossible to track by other methods. Further research, ideally involving the ground-truthing of feather isotope values compared to model predictions is recommended.

Acknowledgements

This study was funded by research projects of the Spanish Ministry of Economy and Competitiveness (CGL2015-64650-P), Junta of Extremadura (IB16121), Junta of Andalucía (P12-RNM-2144) and University of Extremadura (A7-26). CLC was supported by an operating grant from the Junta of Andalucía (P12-RNM-2144). AM and MR were supported by grants from the Spanish Ministry of Education and Science (JC2011-0405, BES 2004-4886, BES-2007-

15549 and BES-2010-030295, respectively). Isotope analyses were supported by an operating grant from Environment Canada to KAH. We want to thank C. Gryba and G. Koehler who assisted with stable isotope analyses.

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Chapter 4

Rainfall at African wintering grounds predicts age-specific probability of haemosporidian infection in a migratory passerine bird

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López-Calderón et al. *Ibis* (2018), doi: 10.1111/ibi.12680

Abstract

In migratory species breeding in temperate zones and wintering in tropical areas prevalence of blood parasites may be affected by migratory strategies and winter habitat choice. We explored if African winter habitat was linked to the probability of haemosporidian infection in the House Martin *Delichon urbicum* breeding in Spain, and tested for potential differences between age classes. As a proxy for winter habitat features, we analyzed stable isotope (δ^2H, δ^{13}C and δ^{15}N) values of winter-grown feathers moulted in tropical Africa. Rainfall at the African winter grounds was related to the probability of being infected with haemosporidians, and this effect differed among age classes. We found that haemosporidian prevalence was similar for young and experienced birds wintering in habitats of higher rainfall (^{2}H-depleted) whereas we found great differences in winter habitats of lower rainfall (^{2}H-enriched), with young having much higher prevalence than experienced birds. Likewise, experienced birds wintering in habitats of higher rainfall had higher probability of haemosporidian infection than experienced birds wintering in habitats of lower rainfall. By contrast, young birds wintering in habitats of lower rainfall had higher probability of haemosporidian infection than young birds wintering in habitats of higher rainfall. These outcomes highlight age interacts with haemosporidian infection in the migratory ecology of the House Martin, which may drive carry-over effects in this long-distance aerial insectivore.

Introduction

In response to changes in environmental settings, many bird species perform seasonal journeys between their breeding areas and distant locations looking for favourable ecological conditions (Gauthreaux 1982). Numerous studies have shown that these periods are linked because previous conditions experienced can have carry-over effects on breeding performance and other life history traits of migratory birds (Norris & Marra 2007; Harrison et al. 2011). In addition to known mediators of carry-over effects such as winter habitat, immune defense and parasite pressure also play important roles in migratory strategies (Piersma 1997; Møller & Erritzøe 1998; Altizer et al. 2011; Hill et al. 2012; Clark et al. 2016). In this line, it has been shown that for some migratory birds, the diversity of their haemosporidian parasites is higher in the tropical wintering grounds than in the temperate breeding areas (review in Shegal 2015). Thus, migratory behaviour may have evolved as a mean to escape from this higher pressure of parasites at the wintering grounds (Møller & Szép 2010). Alternatively, the exposure to a higher risk of parasitism and/or a more diverse parasite fauna could be seen as a cost to migration for birds breeding in temperate latitudes and wintering in the tropics (Figuerola & Green 2000; Waldenström et al. 2002). Furthermore it is well known that certain habitat features such as temperature, rainfall or vegetation cover are intimately linked with distribution and abundance of parasite vectors and hence with the prevalence of haemosporidians throughout the world (Mendes et al. 2005; Lourenço et al. 2011; Cornault et al. 2013; Oakgrove et al. 2014; Roiz et al. 2015; Sehgal 2015).

One powerful means of inferring winter habitat characteristics has been the measurement of naturally occurring stable isotope ratios in feathers grown at the wintering grounds and comparing these to known isotope patterns or 'isoscapes' in the environment (Hobson 1999). Birds have feather δ^2H values reflecting long-term, amount-weighted, average δ^2H from precipitation prior to moult according to established calibration equations (Bowen et al. 2005; Hobson et al. 2012), while feather δ^{13}C and δ^{15}N values are associated more directly with those values in primary production following isotopic discrimination (Vanderklift & Ponsard 2003). Feather keratin is metabolically inert after synthesis (Hobson 1999), and thus isotopic values in feathers reflect the environmental conditions where they were grown (i.e. the wintering areas). Considerable literature has emphasized that food web δ^2H, δ^{13}C and δ^{15}N values are strongly influenced by climate (reviewed by West et al. 2010). Lower values of δ^2H in rain are linked to higher amounts of precipitation in tropical latitudes (i.e. the so-called 'amount effect'; Dansgaard 1964; Clark & Fritz 1997). Higher values of δ^{13}C are associated with environments dominated by C4 plants as well as C3 plants adapted to hydric stress (Ehleringer 1989). Finally, xeric/cultivated habitats tend to be relatively enriched in ^{15}N relative to mesic/uncultivated habitats (Pardo & Nadelhoffer 2010).

This stable isotope approach can be combined with blood assays to link parasite prevalence with winter habitat features (Yohannes et al. 2008; von Rönn et al. 2015). Avian malaria and related haemosporidians are known to reduce survival and reproductive success of their hosts (Valkiūnas 2005; Marzal et al. 2005). Because many haemosporidians infecting migratory birds are transmitted in tropical Africa (Bensch et al. 2009; Hellgren et al. 2007), winter habitat choice may affect probability of infection, and this in turn may have carry-over fitness effects in the subsequent breeding season.

The House Martin *Delichon urbicum* is a small migratory passerine that suffers high prevalence of haemosporidian infection (Marzal et al. 2005; Piersma & van der Velde 2012; van Rooyen et al. 2014; Marzal et al. 2016). House Martins moult in their tropical wintering areas (Cramp 1988), where they acquire most of their blood infections (Piersma & van der Velde 2012; García-Longoria et al. 2015). We have previously identified the most-probable wintering areas of House Martins breeding in Spain by means of multi-isotopic clusters (Hobson et al. 2012). We assigned sample feathers as being grown in the isotopic clusters one and two from West Africa (López-Calderón et al. 2017a, 2017b), which are closely associated with different habitats (broadleaf forest and savannah respectively).

Savannahs were enriched in ^{13}C and depleted in ^{2}H compared to broadleaf forest, because of assumed C4-dominated grasslands and greater seasonal rainfall provided by the West African monsoon (Sultan & Janicot 2003). Thus, for our House Martin population, we are able to use δ^{2}H values as an indicator of habitat use and not just larger-scale provenance in Africa (Hobson et al. 2014). Furthermore, the probability of wintering in these areas is related to age and sex, and winter area choice is related to subsequent breeding success (López-Calderón et al. 2017a, 2017b).

Here we explore whether House Martins breeding at the same location in Spain and wintering in different habitats of West Africa differ in probability of haemosporidian infection (i.e. genus *Haemoproteus*, *Plasmodium* and *Leucozytozoon*). As a proxy for winter origins and habitats used during moult, we used stable isotope (δ^{2}H, δ^{13}C and δ^{15}N) measurements from winter-grown feathers. Because previous studies have revealed the importance of age for haemosporidian infection and migratory ecology in House Martins (Marzal et al. 2016; López-Calderón et al. 2017a, 2017b), we also tested for differences in probability of blood infection and winter quarters according to age class. The few studies that have previously explored the relationship between feather stable isotope values and haemosporidian infection in birds have shown mixed results. For example, for Great Reed Warblers *Acrocephalus arundinaceus* wintering in tropical Africa, higher probability of haemosporidian infection is associated with habitats dominated by C4 vegetation and C3 vegetation adapted to hydric stress (Yohannes et al. 2008). In contrast, for Barn Swallows *Hirundo rustica* wintering in tropical Africa, higher probability of *Plasmodium* infection is associated with habitats dominated by C3 vegetation (von Rönn et al. 2015). Isotopic signatures by region and habitat type across Africa are obviously complex (Hobson et al. 2012) and it is difficult to generalize expected patterns. Winter habitat features affecting parasite prevalence may also differ across host species. Consequently we could not set up *a priori* predictions explaining how probability of haemosporidian infection may be related to isotopic values in winter grown feathers of House Martins.

Materials and methods

Field procedures

We studied two colonies of House Martins in southern Spain (38º52'N, 6º58'W), during 2005-13, excluding 2006. Adult House Martins were captured and identified with numbered metal rings. From each individual we removed the outermost rectrix feather for isotopic analysis and sampled blood (50 µl) from the brachial vein. The final sample size used in this study was 302 feather and blood samples taken from 247 individuals (some of them were captured during consecutive years). Each year, we performed more than one hundred capture sessions from March to July, capturing more than 90% of the colony each breeding season. House Martins show high breeding site philopatry and recruitment occurs at the age of one year (de Lope & da Silva 1988). Breeding dispersal is negligible in our study sites, with less than 0.1% of breeding individuals among the more than 10,000 adults ringed in the study population during more than 20 years ever moving to another colony (F. de Lope, Author pers. comm.). Moreover, age at first reproduction occurred at the age of one year for the majority of individuals, since all but one of more than 500 individuals marked as nestlings were recruited as 1-year old breeding adults. Thus we could assign the age of individuals accurately, assuming un-ringed birds to be yearlings at first capture that had originated from outside the study area, and assuming that disappearance of ringed breeders from the colony indicated mortality rather than dispersal (Marzal et al. 2016). We categorized as *young* birds, individuals ringed as nestlings/fledglings that were recaptured in the next year (*n* = 51), and also individuals ringed for the first time as adults (*n* = 107). We categorized as *experienced* birds, individuals ringed as nestlings/fledglings that were recaptured two years or more after their first capture (*n* = 28), and also individuals ringed for the first time as adults that were recaptured in subsequent years (*n* = 116). Young birds constituted birds that have migrated for the first time that year, while experienced birds were at least in their second migration year.

Stable isotope analysis

All feathers were cleaned of surface oils in 2:1 chloroform:methanol solvent rinse and prepared for δ^2H, δ^{13}C and δ^{15}N analysis. Deuterium abundance in the non-exchangeable hydrogen of feathers was determined following Wassenaar & Hobson (2003), and using three calibrated keratin hydrogen-isotope reference materials (CBS = -197 ‰; SPK = -121.6 ‰; KHS = -54.1 ‰). Deuterium measurement was performed on H_2 gas derived from high-temperature (1350°C) flash pyrolysis of 350±10 μg feather subsamples and keratin standards using continuous-flow isotope-ratio mass spectrometry CFIRMS with a Eurovector 3000 (Milan, Italy - www.eurovector.it) elemental analyzer interfaced with an Isoprime (Manchester, UK) mass spectrometer. Measurement of the three keratin laboratory reference materials, corrected for linear instrumental drift, were both accurate and precise with typical within-run (n=5) SD values of < 2 ‰. Hydrogen isotopic ratio (^{2}H/H) was reported in standard delta (δ) notation, in units of per mille (*‰*), and normalized to the Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale

For feather δ^{13}C and δ^{15}N analyses, between 0.5 and 1.0 mg of feather material was combusted online using a Eurovector 3000 elemental analyzer. The resulting CO_2 and N_2 was separated by Gas Chromatograph (GC) and introduced into a Nu Horizon (Nu

Instruments, Wrexham, UK - www.nu-ins.com) triple-collector isotope-ratio mass-spectrometer via an open split and compared to CO_2 or N_2 reference gas. Using previously calibrated internal laboratory C and N standards [powdered keratin (BWBIII; $\delta^{13}C$ = -20‰; $\delta^{15}N$ = 14.4‰) and gelatin (PUGEL; $\delta^{13}C$ = -13.6‰; $\delta^{15}N$ = 4.73‰)], within run (n=5), precisions for $\delta^{15}N$ and $\delta^{13}C$ measurements were ~ ± 0.15 ‰. Stable nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) isotope ratios were expressed in delta (δ) notation, as parts per thousand (‰) deviation from the primary standards: atmospheric nitrogen and VPDB (Vienna Pee Dee Belemnite carbonate) standards, respectively.

Haemosporidian molecular detection and transmission origin

Haemosporidian parasites (*Plasmodium* spp., *Haemoproteus* spp. and *Leucocytozoon* spp.) were detected from blood samples using molecular methods (Hellgren et al. 2004). DNA from the avian blood samples were extracted in the laboratory using a standard phenol/chloroform/isoamylalcohol method (Sambrook et al. 2002). Diluted genomic DNA (25 ng/µl) was used as a template in a polymerase chain reaction (PCR) assay for detection of the parasites using nested-PCR protocols described by Hellgren et al. (2004) that amplifies part of the haemosporidian cytochrome b (cyt b) gene. In the first PCR step the three haemosporidian parasite genera are detected simultaneously. In the second round of this protocol, one PCR detects *Haemoproteus* and *Plasmodium* spp., whereas another detects *Leucocytozoon* spp. The amplifications were evaluated by running 2.5 µl of the final PCR on a 2 % agarose gel. All PCR experiments contained one negative control for every eight samples. In the very few cases of negative controls showing signs of amplification (never more than faint bands in agarose gels), the entire PCR-batch was run again to make sure that all positives were true. Parasites detected by a positive amplification were sequenced using the procedures described by Hellgren et al. (2004). The obtained sequences of 478 bp of the parasite cyt b were edited, aligned and compared in a sequence identity matrix using the program BioEdit (Hall 1999). Mixed infections were identified as either different amplifications in the second nested-PCR (co-infection of *Haemoproteus* spp. or *Plasmodium* spp. with *Leucocytozoon* spp.), or a ‘double base calling’ in the electropherogram. Parasites with sequences differing by one nucleotide substitution were considered to represent evolutionary independent lineages (Bensch et al. 2004; Ricklefs et al. 2005). For testing differences in probability of blood infection related to winter habitat, we selected only haemosporidian lineages with strict transmission in Africa. Following van Rönn et al. (2015) we classified an haemosporidian lineage as being exclusively transmitted in Africa, (i) when no record has ever been made in any juveniles or nestlings of any bird species in Europe, and (ii) when it has never been recorded in any resident bird species in Europe. Information on transmission areas was provided by MalAvi database (Bensch et al. 2009, Version 2.3.3 November 2017).

Statistical analysis

As prospective analyses, we tested if haemosporidian infection status (i.e. ‘infected or not’) differed between age or sex categories by means of Wilcoxon Test. To investigate how winter habitat features may influence probability of haemosporidian infection according to age class, we used Generalized Linear Mixed Models with binomial distribution of errors. Because the global models would not converge when including the three isotopes together as explanatory variables, we built different global models for each isotope. In each global model infection status was the response variable (i.e. ‘infected or not’), while δ-values, age class and the interaction term between them were explanatory variables. By including this interaction term, we were explicitly testing if winter habitat affected probability of blood infection differently according to age class and whether probability of blood infection differed between age classes depending on wintering habitat. We accounted for individual identity, year and colony as random effects (intercepts), thus controlling respectively for repeated measures taken on the same individual, for inter-annual and among breeding sites variation. First, we ran our global models pooling together in the response variable the three detected haemosporidian genera. In this first analysis, we assumed that infection across haemosporidian genus was affected by similar winter habitat features. Second, we ran our global models considering separately in the response variable each haemosporidian genus. In this second analysis, we assumed that infection across haemosporidian genus was affected by different winter habitat features.

We evaluated all possible candidate models that could be built with our initial global models following procedures described in Grueber et al. (2011). We first standardized the input variables entering the global models, scaling them by dividing means by two standard deviations. Therefore, parameter estimates were standardized effect sizes and were on a comparable scale (Gelman 2008; Grueber et al. 2011). The best candidate model was determined using Akaike information criterion corrected for small sample size (AICc). We calculated Akaike weight (*w*) for each candidate model (‘i’) that can be interpreted as the probability that ‘i’ is the best model, given the data and set of candidate models (Burnham & Anderson 2002). We also calculated the Relative Importance (RI) for a given variable as the sum of Akaike weights from candidate models that contained the given variable (Burnham & Anderson 2002). The final model was obtained by averaging the parameter estimates from top models at a cut-off criterion of ΔAICc < 6.0 (Richards 2008). The confidence intervals (hereafter 95% CI) were calculated from the final models using the parameter estimates (effect size) and associated standard errors (hereafter SE) obtained after model averaging. We assumed that a predictor term significantly contributed to explain the response variable when the 95% CI for the estimated parameter excluded zero (Grueber et al. 2011). Analyses were performed in R version 3.3.1 (R Core Team 2016) using the libraries *MuMIn* (Bartón 2015), *lme4* (Bates et al. 2015) and *arm* (Gelman et al. 2016).

Results

We detected 125 (42%) birds infected with haemosporidians. Among infected birds, 92 (74%) were infected by only one parasite lineage, while 33 (26%) harbored a mixed infection. The most frequent haemosporidian genus was *Haemoproteus* (133 infections), followed by *Plasmodium* (18 infections) and *Leucocytozoon* (seven infections). We found 12 haemosporidian lineages, of which seven have transmission restricted to Africa (Table 1). Subsequently, for studying the infection risk in different wintering areas, we only included in our analysis House Martins infected with parasite lineages with restricted African transmission.

Table 1. Cytochrome b lineage names, tentative parasite species, GenBank accession numbers, transmission area and number of infected birds per parasite lineage found.

Cytochrome *b* lineage	**Parasite species**	**Genbank nº**	**Transmission area**	***n***
DELURB1	*Haemoproteus hirundinis*	EU154343	Africa	65
DELURB2	*Haemoproteus spp.*	EU154344	Africa	65
DELURB3	*Haemoproteus spp.*	EU154345	Africa	1
HIRUS05	*Haemoproteus spp.*	KP696488	Africa	2
AFTRU5	*Plasmodium spp.*	DQ847263	Africa & Europe	1
SGS1	*Plasmodium relictum*	AY560372	Africa & Europe	2
GRW02	*Plasmodium ashfordi*	AF254962	Africa	10
BLUTI10	*Plasmodium spp.*	JQ434696	Europe	1
LK06	*Plasmodium spp.*	EF564179	Africa & Europe	3
LAMPUR03	*Plasmodium spp.*	EU810655	Africa	1
HIRUS07	*Leucocytozoon spp.*	KP696490	Africa	6
PARUS19	*Leucocytozoon spp.*	HM234024	Africa & Europe	1

Sample size is 125 House Martins infected with haemosporidian parasites, 92 House Martins were infected by only one parasite lineage and 33 harbored mixed infection.

Prevalence of haemosporidians differed between age classes (young: 48% infected; experienced: 31%; Wilcoxon test: $W = 9006$, $p = 0.004$), but not between sexes (males: 39.6% infected; females: 39.8%; Wilcoxon test: $W = 10489$, $p = 0.97$). Because of the low number of infections and convergence problems, we did not fit mixed models considering infection by *Plasmodium* and *Leucocytozoon* as the response variables.

We found very small differences across mixed models when pooling together the three haemosporidian genus or when considering only infection by *Haemoproteus* in the response variable (Tables 2 and 3). We did not find any parameter estimate whose 95% CI excluded zero; however, the standardized effect size for the interaction between feather δ^2H and age class on haemosporidian infection was much stronger than the rest (Tables 2 and 3). Across age-classes, this interaction term indicated that prevalence of haemosporidians was similar for both young and experienced birds wintering in habitats of higher rainfall (^{2}H-depleted), whereas prevalence of haemosporidians differed across age classes in habitats of lower rainfall (^{2}H-enriched), with young birds having much higher prevalence compared to experienced birds (Fig. 1). Across habitats, this interaction term indicated that young birds wintering in habitats of lower rainfall had higher probability of haemosporidian infection than young birds wintering in habitats of higher rainfall, whereas experienced birds wintering in habitats of higher rainfall had higher probability of

haemosporidian infection than experienced birds wintering in habitats of lower rainfall (Fig. 1). On the other hand, effect sizes of feather δ^{13}C and δ^{15}N on the probability of haemosporidian infection were both low, as well as effect sizes of their interactions with age class (Tables 2 and 3; Fig. 2 and 3). Top models used for averaging are presented in Online Supplementary Material Tables 1-6, which can be found in the published version:
https://onlinelibrary.wiley.com/doi/full/10.1111/ibi.12680.

Table 2. Summary results after model averaging the effects of each isotope ratio and age class on haemosporidian infection status (i.e. infected or not) for House Martins breeding in Spain. Three haemosporidian genus (*Haemoproteus*, *Plasmodium* and *Leucozytozoon*) were pooled in the response variable. Sample size was 294 individuals: 153 young and 141 experienced birds. Parameters estimated represent comparable effect sizes that have been standardized to two SD following Gelman (2008). The reference level of the fixed effect age class was 'experienced'. Random effects were year, colony and individual identity. RI – Relative importance.

Parameter	**Estimate**	**SE**	**95% CI**	**RI**
(Intercept)	-0.61	0.40	(-1.40, 0.17)	
Age class	0.34	0.33	(-0.31, 0.99)	0.46
δ^2H	-0.24	0.49	(-1.20, 0.72)	0.39
Age class * δ^2H	1.12	0.59	(-0.04, 2.28)	0.17
(Intercept)	-0.60	0.40	(-1.38, 0.19)	
Age class	0.31	0.33	(-0.34, 0.95)	0.38
δ^{13}C	-0.12	0.31	(-0.74, 0.49)	0.31
Age class * δ^{13}C	-0.31	0.57	(-1.42, 0.80)	0.04
(Intercept)	-0.60	0.40	(-1.38, 0.19)	
Age class	0.31	0.33	(-0.33, 0.95)	0.38
δ^{15}N	0.01	0.31	(-0.60, 0.61)	0.29
Age class * δ^{15}N	-0.34	0.58	(-1.47, 0.80)	0.04

Table 3. Summary results after model averaging the effects of each isotope ratio and age class on *Haemoproteus* infection status (i.e. infected or not) for House Martins breeding in Spain. Sample size was 278 individuals: 148 young and 130 experienced birds. Parameters estimated represent comparable effect sizes that have been standardized to two SD following Gelman (2008). The reference level of the fixed effect age class was 'experienced'. Random effects were year, colony and individual identity. RI – Relative importance.

Parameter	**Estimate**	**SE**	**95% CI**	**RI**
(Intercept)	-0.89	0.43	(-1.73, -0.06)	
Age class	0.47	0.34	(-0.20, 1.14)	0.54
δ^2H	-0.06	0.49	(-1.02, 0.91)	0.41
Age class * δ^2H	1.01	0.62	(-0.21, 2.24)	0.15
(Intercept)	-0.87	0.43	(-1.71, -0.03)	
Age class	0.43	0.34	(-0.23, 1.10)	0.47
δ^{13}C	-0.09	0.35	(-0.77, 0.59)	0.32
Age class * δ^{13}C	-0.42	0.60	(-1.60, 0.76)	0.05
(Intercept)	-0.87	0.43	(-1.71, -0.04)	
Age class	0.43	0.34	(-0.23, 1.10)	0.47
δ^{15}N	-0.06	0.34	(-0.73, 0.62)	0.31
Age class * δ^{15}N	-0.44	0.60	(-1.62, 0.74)	0.05

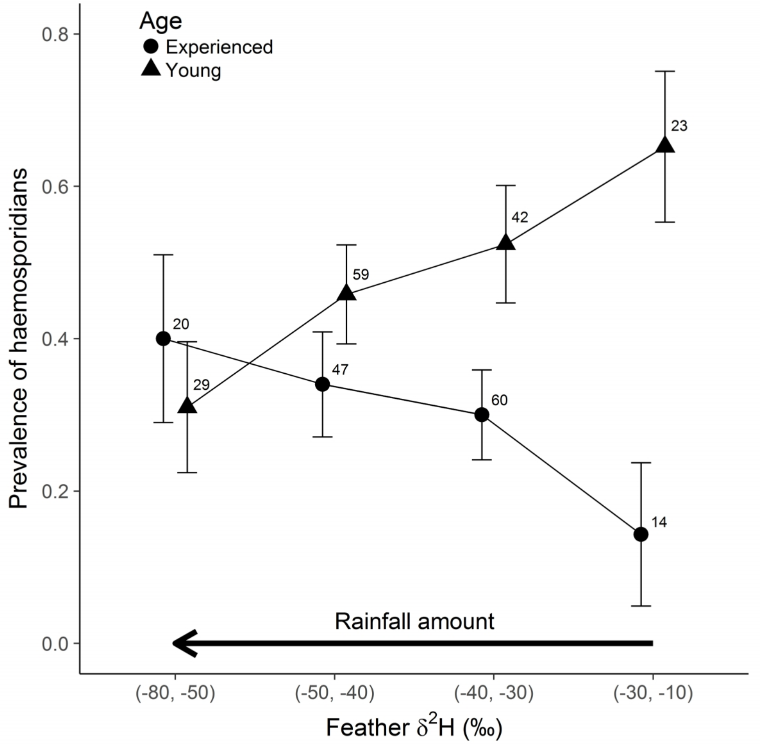

Figure 1. Relationship between δ^2H from winter-grown feathers and haemosporidian prevalence (*Haemoproteus*, *Plasmodium* and *Leucozytozoon* pooled) for House Martins breeding in Spain. Points represent observed values of haemosporidian prevalence in different categories of δ^2H (the range of δ^2H values was categorized in four levels only for depiction purposes). The arrow indicates increasing precipitation values in relationship to feather δ^2H as predicted by the 'amount effect'. Error bars indicate standard errors. Sample sizes for prevalence values are given close to each point.

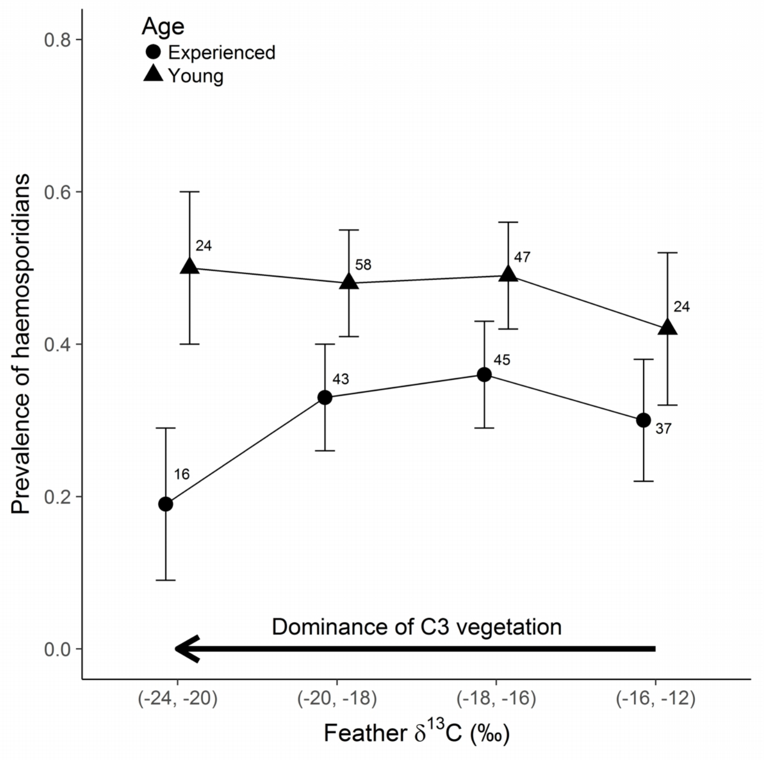

Figure 2. Relationship between δ^{13}C from winter-grown feathers and haemosporidian prevalence (*Haemoproteus*, *Plasmodium* and *Leucozytozoon* pooled) for House Martins breeding in Spain. Points represent observed values of haemosporidian prevalence in different categories of δ^{13}C (the range of δ^{13}C values was categorized in four levels only for depiction purposes). The arrow indicates the shift of vegetation in relationship to feather δ^{13}C. Error bars indicate standard errors. Sample sizes for prevalence values are given close to each point.

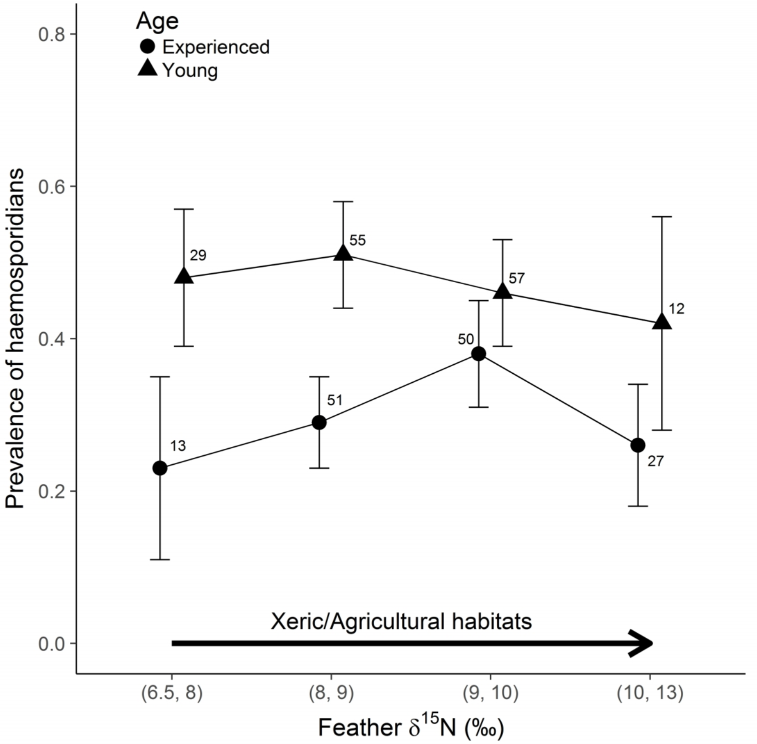

Figure 3. Relationship between δ^{15}N from winter-grown feathers and haemosporidian prevalence (*Haemoproteus*, *Plasmodium* and *Leucozytozoon* pooled) for House Martins breeding in Spain. Points represent observed values of haemosporidian prevalence in different categories of δ^{15}N (the range of δ^{15}N values was categorized in four levels only for depiction purposes). The arrow indicates the ecological interpretation of δ^{15}N values. Error bars indicate standard errors. Sample sizes for prevalence values are given close to each point.

Discussion

In this study, we combined different methods to unveil the complex relationships between winter habitat, age class and probability of haemosporidian infection in House Martins breeding in Spain. There was higher haemosporidian prevalence in young than in experienced birds, probably due to selective disappearance of infected birds (Marzal et al. 2016). The amount of precipitation (inferred by δ^2H) was a major driver of blood infection in House Martins, rather than vegetation type or any other biome character (inferred by δ^{13}C and δ^{15}N). Our results highlight the importance of age when investigating the relationship between haemosporidian infection and winter habitat. We have revealed an effect of the interaction between age class and feather δ^2H on the probability of haemosporidian infection. On one hand, this interaction indicated that the prevalence of haemosporidians was similar for both age classes wintering in habitats of higher rainfall (i.e. lower δ^2H values); however it was much higher for young than for experienced birds wintering in habitats of lower rainfall (i.e. higher δ^2H values). On the other hand, this interaction also indicated that experienced birds wintering in habitats with higher rainfall had higher probability of becoming infected than experienced birds wintering in habitats with lower rainfall. In contrast, young birds wintering in habitats with higher rainfall had lower probability of becoming infected than young birds wintering in habitats with lower rainfall.

Previous studies that have combined stable isotopes and molecular detection of haemosporidian parasites do not show a consistent general pattern. For instance, Great Reed Warblers wintering in tropical Africa suffer a higher haemosporidian prevalence associated with water-stressed habitats (Yohannes et al. 2008). In contrast, Barn Swallows wintering in tropical Africa suffer a higher prevalence of *Plasmodium* associated with moist habitats (van Rönn et al. 2015). Therefore, habitat features driving haemosporidian prevalence may depend on the study host species, specific wintering area and parasite type (i.e. genus or lineage). In this study, we have found very small differences when pooling together the three haemosporidian genus compared to using only infection by *Haemoproteus* in the response variable. This may suggest that winter habitat features related to blood infection are similar across the haemosporidian genera we detected in House Martins breeding in Spain. However, we acknowledge that our sample size of birds infected with *Leucocytozoon* and *Plasmodium* was very low, and thus further studies are necessary to better understand if winter habitat features affect probability of blood infection in the House Martin differently according to parasite genus. In addition, our results indicate that age class is another source of variance to take into account in these kinds of studies. Unlike previous studies in other bird species, we were also able to associate House Martins with specific wintering areas using assignment to isoscapes (Hobson et al. 2012;

López-Calderón et al. 2017a, 2017b) where we could better interpret climate regimes.

Experienced House Martins wintering in habitats of higher rainfall had higher probability of haemosporidian infection than experienced House Martins wintering in habitats of lower rainfall. Because higher rainfall (i.e. lower δ^2H values) correlates with higher insect abundance in Africa (Denlinger 1980; Cumming & Bernard 1997), experienced House Martins wintering in habitats of higher rainfall should have higher exposure to blood-feeding dipteran vectors and thus higher prevalence of haemosporidians. In agreement with this idea, it has been shown that increased rainfall and humidity significantly increased the abundance of vector-borne disease, because they are known to affect the reproduction, development, and population dynamics of the arthropod vectors of these diseases (Oakgrove et al. 2014; Roiz et al. 2015; see also review in Gage et al. 2008). However, we have previously shown that experienced males wintering in habitats of higher rainfall obtained a fitness advantage in terms of body condition and reproductive success (López-Calderón et al. 2017b), probably because such areas also provide more food for aerial insectivores (Ambrosini et al. 2011). Hence, there appears to be a trade-off for experienced House Martins wintering in West Africa, in which habitat quality and risk of blood infection interact with opposite selective pressure.

In contrast, young House Martins wintering in habitats of higher rainfall had lower probability of haemosporidian infection than young House Martins wintering in habitats of lower rainfall. Mounting an immune response against a new parasite challenge is costly in terms of nutrients (Klasing 2004), therefore young House Martins may be able to mount a stronger immune response in areas of higher food availability and thus eliminate the infection. Taking into account that the development of key immune defence organs occurs for migratory birds during early life and they regress after accomplishing the first migration (Møller & Erritzøe 2001), winter habitat choice should have stronger consequences for the immune system of young compared to experienced birds. In agreement with this hypothesis, Navarro et al. (2003) experimentally showed that House Sparrows *Passer domesticus* in prime body condition had faster and stronger immune response (i.e. PHA-test) than individuals in poor condition, which allowed the former to reduce haemosporidian infection. Because resource availability (i.e. nutrients) may be lower in drier habitats, young House Martins wintering in these habitats may not be able to mount an energetically costly immune response against blood parasites, thus increasing the prevalence of haemosporidians. Any shortage in food intake during the first winter could especially minimize the immune response of House Martins, while on the other hand the immune system of experienced House Martins is already developed and they should be less affected by reduced food availability. Young House Martins may compensate for the higher density of vectors in wet habitats with the higher abundance of resources necessary for mounting strong immune defences. Consequently, haemosporidian infection may occur at similar probability for both young and experienced birds wintering in habitats of higher rainfall.

Although we cannot ensure the overlap between moult period and haemosporidian infection for House Martins, and hence that the isotopic signature from moulting sites represents the habitat where the infection took place (Yohannes et al. 2008), previous studies suggest that haemosporidian infection can take place immediately after birds arrive to their tropical winter areas (Waldenström et al. 2002). Moreover, birds wintering in sub-Saharan West Africa moult feathers from October to December (Bensch et al. 1991). Given that moult is an energy-demanding process, birds wintering in West Africa may tune moult to the rainy season (Bensch et al. 1991), which ends in November (Sultan & Janicot 2003), but also vector-borne infections could be more probable during this period (Sehgal 2015).

Summarizing, we provide evidence that rainfall in the African winter grounds, inferred from δ^2H in winter-grown feathers, is related to probability of haemosporidian infection in House Martins depending on age class. This interaction has never previously been described. Analyses of stable isotopes conducted in this and previous studies have identified age as a key variable in the migratory ecology of House Martins. These findings can be useful for developing future conservation efforts for House Martins and other long-distance aerial insectivores with declining population trends.

Acknowledgments

We thank two anonymous reviewers and Rebecca Kimball for their comments and editorial assistance that substantially helped to improve our manuscript. This study was funded by research projects of the Spanish Ministry of Economy and Competitiveness (CGL2015-64650-P) and *Junta de Extremadura* (IB16121). CLC was supported by an operating grant from *Junta de Andalucía* (P12-RNM-2144) and University of Seville (international mobility grant, *Plan Propio Investigación V*). SM was supported by a grant from the Spanish Ministry of Education and Science. C. Gryba and G. Koehler assisted with stable isotope analyses. Funding for isotope analyses were provided in part by an operating grant from Environment Canada to KAH.

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Chapter 5

Age-dependent carry-over effects in a long-distance migratory bird

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Abstract

Migratory birds usually respond to climate change by modifying wintering areas or breeding earlier. In addition, changes in winter habitat use or breeding phenology could have important carry-over effects on subsequent breeding success. Here, we studied age- and sex-dependent carry-over effects from wintering to the breeding stage of a small aerial insectivorous long-distance migratory bird, the barn swallows (*Hirundo rustica*) breeding in Denmark during 1984-2013. First, we used stable isotope analyses combined with ringing recoveries to identify wintering areas. Second, we found that environmental conditions as inferred by Normalized Differential Vegetation Index (NDVI) have deteriorated at the wintering grounds. Third, we used confirmatory path analysis to quantify the indirect effect of winter conditions on subsequent breeding success. Interestingly, females advanced onset of breeding, laid more eggs and raised more fledglings in the first brood when ecological conditions during the previous winter improved. This response was age-dependent, since yearlings did not respond to this environmental cue but the response was increasingly stronger as females aged. Males showed a similar response to winter conditions, although not statistically significant. These results highlight the importance of studying carry-over effects within the context of climate change, especially in relation to age of individuals.

Introduction

Many species of birds, especially those living at high latitudes, have adapted to perform regular annual movements between breeding and non-breeding areas, often involving thousands of kilometers (e.g. Newton 2008). Migratory behavior is important because the previous history or experience of a given individual may explain its performance in the current situation (i.e. "carry-over effect"; O'Connor et al. 2014). Hence, in migratory birds, what occurs during winter and the migratory journey could have consequences for subsequent reproductive success back at the breeding areas, which in turn, may result from a response to previous environmental conditions (Norris & Marra 2007; Harrison et al. 2011). Furthermore, carry-over effects from the winter to the breeding stage may change according to age and sex classes (Saino et al. 2004a; Balbontín et al. 2007, 2009a, 2012; Drake et al. 2013; Sergio et al. 2014; López-Calderón et al. 2017a, 2017b). Environmental conditions experienced in winter should first affect physical condition during winter, then timing of departure from wintering sites and ultimately timing of arrival to breeding areas (e.g. Marra et al. 1998; Studds & Marra 2005). In addition, arrival time to breeding areas usually determines the start of breeding, and this in turn influences the number of offspring produced (Winkler & Allen 1996; Smith & Moore 2004; Marzal et al. 2013). Because all these variables flow sequentially in a time series fashion, Structural Equation Models (hereafter: SEM) provide a powerful and elegant approach to investigate carry-over effects in migratory birds (Norris et al. 2004; López-Calderón et al. 2017b; Saino et al. 2017a, 2017b; Briedis et al. 2018).

Differential migration among age and sex groups may arise because of the different role of each sex in reproduction, timing of other events during the annual cycle (e.g. moult) or factors related to body size and behavioral dominance (review in Cristol et al. 1999; Newton 2008). For instance, males usually arrive at breeding areas earlier than females, a consequence of sexual selection through female choice. Because females usually select partners that are established in the most suitable nesting habitats, competition for earlier arrival to breeding areas is higher among males, and they can develop different strategies for this purpose. For instance, males can stay closer to breeding areas during winter, advance departure date from winter areas, travel more quickly during spring migration or compete more aggressively for food. In addition, young adults breeding for the first time usually arrive later to the breeding areas and have lower breeding success than more experienced adults. Age-related migratory behavior may differ for several reasons. For example, dominant (e.g. older) individuals could displace (e.g. younger) subordinates from better winter or stopover areas. In such cases, older individuals require less time to acquire fuel reserves or to complete moult, allowing older individuals to depart earlier from the wintering areas or to speed up the return journey (the so-called dominance hypothesis; e.g.

Gauthreaux 1978; Newton 2008). On the other hand, individuals should gain competence through experience as they age, allowing them to perform the migratory journey better or to respond more efficiently against environmental conditions found in the wintering areas (the so-called constraint hypothesis; e.g. Curio 1983; Forslund & Pärt 2001). There is evidence supporting both hypotheses that are not mutually exclusive. Alternatively, a third explanation for variation in migratory behavior among age classes is the disappearance of poor quality individuals from the population, such as the disappearance of later-than-average individuals at various stages prior to arrival on the breeding grounds (the so-called selection hypothesis; e.g. Curio 1983; Forslund & Pärt 2001).

Many different climate variables (e.g. temperature and precipitation) are changing rapidly worldwide and are predicted to continue to change in future years (IPCC 2013). The use of biotic indices are especially powerful to understand and predict the indirect effect of climate change on organisms; such is the case of the Normalized Differential Vegetation Index (hereafter: NDVI; reviews in Pettorelli et al. 2005, 2011). NDVI integrates the effect of climate on vegetation and vegetation dynamics in turn are linked to animal distribution and performance. For example, NDVI is linked to abundance and diversity of insects (Lassau & Hochuli 2008; Lourenço et al. 2011; Roiz et al. 2015). Associations between NDVI and climatic variables shift throughout the world. For instance, in tropical latitudes lower temperature and higher rainfall are usually correlated with higher NDVI (Ichii et al. 2002). Therefore, lower temperature and higher rainfall may imply greener vegetation, higher abundance of insects (Denlinger 1980; Cumming & Bernard 1997; Lassau & Hochuli 2008), and thus favorable conditions for aerial insectivores wintering in the tropics, whereas higher temperatures and lower precipitation may imply the reverse (Saino et al. 2004a, 2004b, 2017a; Balbontín et al. 2009a, 2009b).

The barn swallow (*Hirundo rustica*) is a small passerine (*c.* 20 g) that breeds semi-colonially and feeds on insects while flying. The barn swallow is a long-distance migrant that breeds north of the Tropic of Cancer and winters south of this latitude throughout the world (e.g. Ambrosini et al. 2009; but see García-Pérez et al. 2013). For example, the Danish breeding population covers >6000 km to reach Central and South Africa (Ambrosini et al. 2009). Barn swallows provide excellent longitudinal data because once recruited to the breeding population, individuals almost always return to the same colony to breed every year. In addition, age at first reproduction occurs at the first year, and therefore, a high trapping effort each year allows the estimation of age with accuracy in this species (for details, see Møller 1992; Balbontín et al. 2009a).

In this study, first we used both stable isotope analyses and ringing recoveries to determine the winter areas used by our study populations of barn swallows breeding in Denmark. Second, we tested if winter conditions based on satellite-derived NDVI have changed during the last three decades corresponding to the data available on our barn swallow study populations (i.e. 1984-2013). Third, we focused on how environmental conditions experienced in wintering areas, based on NDVI, affected subsequent breeding date and reproductive success measured as clutch size and number of fledglings in the first brood. We were interested to determine how the response to external factors affected these life history traits during the lifetime of known-aged individuals over the 30-year period (see Table 1 for sample sizes).

To explain age-related difference in response to conditions found at winter areas and subsequent carry-over effects during the breeding period, we set *a priori* predictions from established hypotheses as described above. If we assume that individuals depart from their wintering areas as soon as possible after acquiring the appropriate body condition to start the migratory journey, then under the competition hypothesis we should expect a smaller age-related difference in breeding date in good compared to poor years. This outcome is expected because during good years competition is relaxed, and there should be less difference in breeding date amongst individuals of different ages. If it is the inexperience of young individuals that causes a delay in the onset of breeding, then environmental conditions experienced during winter should be less important, and we would expect similar age-related differences in breeding date with individuals breeding earlier as they age (i.e. constraint hypothesis).

Table 1. Sample size across age class for each sex. All our dependent variables of interest had the same sample size.

Age (years)	**Females**	**Males**
1	1414	1273
2	349	403
3	144	175
4	44	64
5	23	23
6	6	8
7	1	3
Total:	1981	1949

Materials and methods

Field procedures

We monitored 40 breeding colonies of barn swallows at Kraghede (57.22ºN, 9.97ºE), Denmark, during 1984-2013. This 45 km^2 study area consists of scattered farms and houses interspersed by meadows and fields where the main crops are wheat and potatoes. APM captured barn swallows with mist nets at least once weekly throughout the breeding season, assuring that more than 95% of all adults are captured and measured annually. All individuals were provided with both metallic and colour rings. We assigned an age of 1 year to all barn swallows when captured the first time as adults. This assumption is supported because breeding dispersal is negligible in the study species. Only three of more than 5000 adults ever moved from one breeding colony to another, and then only a short distance of < 750 m (Møller 1994). Therefore, we assumed that adult disappearance from the breeding population indicated mortality rather than dispersal (Saino et al. 2004a; Balbontín et al. 2009b). Moreover, age assignment was also supported because age at first reproduction was at the age of one year for 100% of recruits. Fieldwork showed that from 315 recruits, all were captured for the first time as an adult when one year old, implying that no recruit was captured the first time when aged two or more years. Therefore, because capture effort was high every year, all recruits without metal rings should be yearlings coming from outside the study sites.

All captured birds were weighed with a Pesola spring balance to the nearest 0.1 g, and tail length was measured to the nearest mm with a ruler. We estimated breeding date of females as the date when they laid the first egg during the first brood, whereas breeding date of males was the date their mate's laid their first egg. Laying date for females was assigned during weekly visits to nests assuming that one egg was laid daily. Males were assigned to their female patners by regular observations from a hide of colour ringed swallows at their nests using binoculars (8 x 30 Zeiss). We estimated reproductive success as the number of eggs laid and the number of fledglings raised during the first breeding attempt, because we expected that carry-over effects from the winter period would be easily detected during the first part of the breeding stage. Reproductive success at the first brood correlated well with total reproductive success (i.e. including subsequent broods), and so is a good proxy for breeding performance in this multi-brooded species (Møller 1994).

During the breeding seasons 2000 and 2016, we collected respectively 84 and 119 feather samples for later stable isotope analyses to identify winter origins. Feather samples from 2000 were taken from the red throat badge, whereas feather samples from 2016 were taken from the rectrix (i.e. outermost feather). Barn swallows usually moult their feathers in the African winter areas (Cramp 1988;

Jenni & Winkler 1994), and furthermore they moult feathers continuously during almost the whole wintering stay (A. P. Møller unpublished data from South Africa, Namibia and Ghana). Because feather keratin is metabolically inert after synthesis (Hobson 1999), we were confident that our feather samples represent the isotopic values derived from the winter areas.

Stable isotope analysis

All feathers were cleaned of surface oils in 2:1 chloroform:methanol solvent rinse and prepared for δ^2H, δ^{13}C and δ^{15}N analysis. Deuterium abundance in the non-exchangeable hydrogen of feathers was determined following Wassenaar & Hobson (2003), and using three calibrated keratin hydrogen-isotope reference materials (CBS = -197 ‰; SPK = -121.6 ‰; KHS = -54.1 ‰). Deuterium measurement was performed on H_2 gas derived from high-temperature (1350°C) flash pyrolysis of 350±10 µg feather subsamples and keratin standards. Measurement of the three keratin laboratory reference materials, corrected for linear instrumental drift, were both accurate and precise with typical within-run (n = 5) SD values of < 2 ‰. On the other hand, δ^{13}C and δ^{15}N measurements were performed on CO_2 and N_2 gases resulted from combustion of 0.5-1.0 feather material. Using previously calibrated internal laboratory C and N standards [powdered keratin (BWBIII; δ^{13}C = -20‰; δ^{15}N = 14.4‰) and gelatin (PUGEL;

$\delta^{13}C$ = -13.6‰; $\delta^{15}N$ = 4.73‰)], within run (n = 5) precisions for $\delta^{15}N$ and $\delta^{13}C$ measurements were ~ ± 0.15 ‰. Stable isotope ratios are reported in standard delta (δ) notation relative to VSMOW-SLAP (Vienna Standard Mean Ocean Water – Standard Light Antarctic Precipitation) for $^{2}H/H$, VPDB (Vienna Pee Dee Belemnite carbonate) for $^{13}C/^{12}C$ and atmospheric nitrogen for $^{15}N/^{14}N$.

Combination of ringing recoveries and isotope analysis to identify winter areas

In June 2018, we requested access to all ringing recoveries of Palearctic barn swallows from The European Union for Bird Ringing (EURING). Every ringing scheme, except for the Copenhagen Bird Ringing Centre, agreed to share their data. We originally obtained a sample 2333 individuals that were ringed and recaptured across the barn swallow range (du Feu et al. 2009). We selected both kinds of recoveries of birds that were either dead or alive. We only retained records of birds captured either in the European breeding areas (from 36° to 66°N and from 9°W to 42°E) during May-August, or in the sub-Saharan wintering range (from 15°N to 35°S and from 17°W to 42°E) during November-February. Finally, because directions followed during migration depend on the longitude of breeding location, we filtered our data subset leaving only individuals whose breeding location was between 5ºE and 15ºE (i.e. a buffer zone in longitude around Denmark). Following this data filtering, our final sample size for ringing data was 253 individuals ringed in Europe and recaptured in sub-Saharan Africa (or vice versa).

To incorporate ringing recoveries within the probabilistic assignment of origins based on isotope analysis, we followed the method described in Van Wilgenburg & Hobson (2011). From our final subset of ringing recoveries (Fig. 1D), first we calculated bearings from breeding to wintering location. Second, we extracted the mean (circular) direction and "kappa" (the concentration parameter, an inverse measure of dispersion). Then, using these parameters, we fit a von Mises probability density function to predict the likelihood of any possible direction (from 1° to 359° separating by 2°). These probability densities were normalized with respect to the sum and were used to create a raster (f) that was used as a prior probability surface (Fig. 1F).

We evaluated the likelihood of origin for a given sample feather by employing a multivariate normal probability density function as first described by Royle & Rubenstein (2004) and used in subsequent studies (e.g. Hobson et al. 2014). By applying Bayes' Rule (Van Wilgenburg & Hobson 2011), we incorporated the prior probability surface (f) in assessing the posterior probabilities of origin (f) based on feather δ^2H, δ^{13}C and δ^{15}N. Geographic locations that were consistent with the upper 67% of the spatially explicit probability density maps for each individual (f) were coded as 1; all others were coded as 0 (Hobson et al. 2009). Thus, for each individual being assigned to a moult origin we obtained one binary map, and we depicted the population likely origin by summing over all individual binary surfaces (Fig. 1G). Finally, to select the most-probable wintering areas used by our study populations, we clipped the population likely origin surface at three different threshold values, leaving only pixels that were assigned to be the moult origin for >50, >60 and >70% of our sample (Fig. 1H). We used this cutoff criteria to extract NDVI values at three different spatial scales.

All analyses were conducted in R version 3.3.1. (R Core Team 2016) with packages raster (Hijmans 2016a), geosphere (Hijmans 2016b), graticule (Sumner 2016), CircStats (Lund & Agostinelli 2012) and mvnmle (Gross & Bates 2012).

Environmental conditions from wintering areas

We extracted NDVI values for the wintering areas previously identified using the public dataset developed by ECOCAST (available at https://ecocast.arc.nasa.gov/data/pub/gimms/3g.v0/). This data base provided bimonthly NDVI measures at 0.83º resolution, during the period 1981-2013. We used as a proxy of environmental conditions during winter of a given year "i", the averaged NDVI measures from

November (year "i-1") to March (year "i"). The selection of this time window (November-March) was chosen because this is the average period that barn swallows breeding in Denmark spend wintering in sub-Saharan Africa (A. P. Møller unpublished data). It has been found that winter areas in southern Africa have moved northwards during 1912−2008 at a rate of 9 km/yr for the Palearctic barn swallow (Ambrosini et al. 2011). Consequently, during our study (1984-2013), barn swallow wintering areas may have shifted approximately 290 km northwards.

Statistical analysis

To investigate how NDVI from wintering areas may influence subsequent breeding date and reproductive success, we used a structural equation modeling approach. SEMs are probabilistic models that hypothesize a causal network with multiple variables that can appear as both predictor and response variables (Lefcheck 2016). We used “confirmatory path analysis” or piecewise SEM, based on applications from graph theory (Shipley 2016). In piecewise SEM, the causal network is translated to a set of linear equations (e.g. linear mixed models), which are then evaluated individually thus allowing a wide range of distributions and sampling designs. In addition, the goodness of fit of the entire causal network can be quantified by a directed separation test (”d-separation test”), which tests the assumption that all variables are conditionally independent (i.e. that there are no missing relationships among unconnected variables).

We conducted separated confirmatory path analyses for males and females. In this way, different responses across sex are indicated by differences in path coefficients from both causal models. We included eight variables in the model built for females: NDVI from winter areas, age, the interaction term between age and NDVI, tail length, body mass, breeding date, clutch size and number of fledglings in the first brood. For males, we excluded clutch size from the causal model, because this variable should be determined by the female only. Reproductive parameters were considered dependent (endogenous) variables, while the rest were considered independent (exogenous). Every observed variable included in our models had the same sample size (i.e. listwise deletion).

The path analysis built for females was made up of three linear mixed models, and that for males was made up of two linear mixed models (i.e. one for each dependent variable). The structure of the path analyses was designed based on previous knowledge of migration ecology (e.g. Norris et al. 2004; López-Calderón et al. 2017b). The first linear model in both path analyses, tested the effects of NDVI from winter areas, age and their interaction term on breeding date, while controlling for the confounding effect of tail length (Møller 1992, 1994). The second linear model for females, tested the effect of breeding date on clutch size while controlling for age and tail length. The third linear model for females tested the effect of clutch size on number of fledglings while controlling for age and tail length. The second linear model for males tested the effect of breeding date on number of fledglings while controlling for age and tail length. Individual (ring) identity, breeding colony and year were included as cross-random intercepts in all linear models, thus controlling for repeated measures in the dependent variable taken on the same individual, and also for among breeding site and inter-annual variation. We used normal distributions of errors and the identity link function for all response variables. Residuals of each linear model were visually inspected for deviation from normality using normal "qqplots". Finally, we also controlled for the confounding effect of body mass on each reproductive parameter (Balbontín et al. 2012). Our dataset contained measures of body mass before, after or during the first breeding attempt. Thus, in our full dataset, we do not know the direction of causality between body mass and the reproductive parameters. Consequently, to maintain a high sample size, we assumed correlated errors (i.e. a relationship that is bidirectional and assumed to be caused by a shared underlying driver) between body mass and every reproductive parameter.

Finally, we repeated our analyses using the different spatial scales defining wintering areas to determine if spatial scale could influence the results obtained. Thus, we conducted the above described analyses for NDVI extracted at different sizes of wintering areas, as defined by pixels assigned to be the origin for >50, >60 or >70% of our sample. All these analyses performed at different spatial scales provided the same results. Thus, for simplicity, we only present results for NDVI extracted from wintering areas defined as pixels assigned to be the origin for >60% of our sample.

We conducted the confirmatory path analyses with library *piecewiseSEM* ver. 1.2.1. (Lefcheck 2016) and linear mixed models with library *lme4* (Bates et al. 2015) using R ver. 3.3.3. (R Core Team 2016).

Results

Wintering areas

All the processes we followed to combine ringing data with stable isotope values are summarized in Fig. 1. The prior surface derived from movement directions effectively narrowed the potential moult origin of our population of barn swallows. Specifically, Fig. 1E represents the potential origins based only on the multivariate assignment of δ^2H, δ^{13}C and δ^{15}N, but after having incorporated the prior surface within the calculation of the spatially explicit probability density maps for each individual (f), the potential origins for the population are narrowed from 10ºE to 30ºE (Fig. 1G and 1H). Wintering areas were largely spread through Cameroon, Central

African Republic, Gabon, Congo, Democratic Republic of Congo, Angola, Zambia, Zimbabwe and some patches across Botswana and South Africa (Fig. 1H). These winter areas that we identified perfectly matched with previous ringing recoveries for barn swallows breeding in Denmark (Bønløkke et al. 2006). Total surface of the wintering area was 1,370,090 km^2, 492,257 km^2 and 97,621 km^2 for our different spatial scales (respectively pixels assigned to be the origin for >50, >60 and >70% of our sample). African biomes dominating the wintering areas were mainly equatorial broadleaf forest and tropical savannahs (Allen 2016).

Fig. 1A

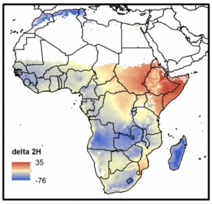

Fig. 1B

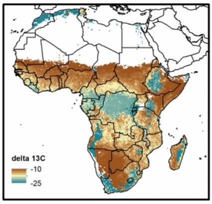

Fig. 1C

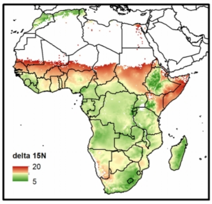

Fig. 1D

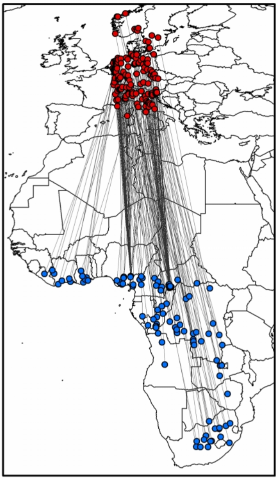

Fig. 1E

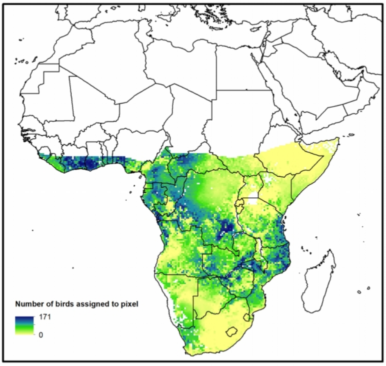

Fig. 1F

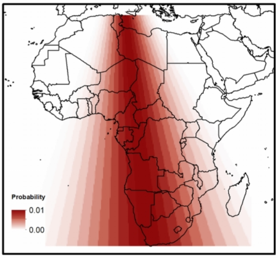

Fig. 1G

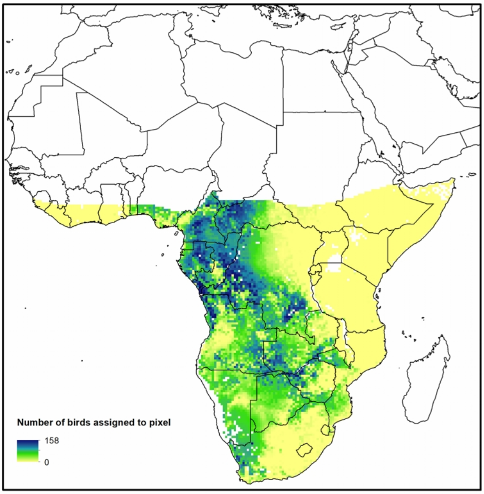

Fig. 1H

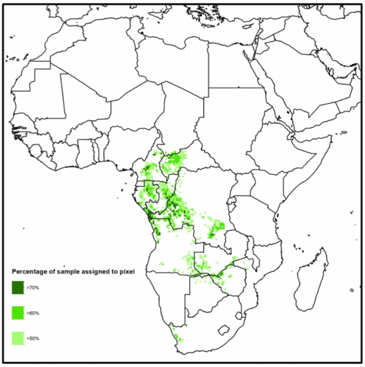

Figure 1. Summary of the full process developed to identify the wintering areas of our barn swallow populations breeding in Denmark. Figures A, B and C represent δ^2H, δ^{13}C and δ^{15}N feather isoscapes (Hobson et al. 2012). Fig. D represents breeding (red circles) and wintering (blue circles) locations for a given individual breeding between 5ºE and 15ºE longitude (du Feu et al. 2009). Fig. E represents the population likely origin of our sample individuals based only on the multivariate assignment of δ^2H, δ^{13}C and δ^{15}N. Fig. F represents the prior surface obtained from fitting a von Mises probability density function to bearings connecting breeding and wintering locations. Fig. G represents the likely origin population of our sample of individuals after incorporating the prior surface into the multivariate assignment of δ^2H, δ^{13}C and δ^{15}N. Fig. H represents the pixels that were assigned to be the likely origin for >50, >60 and 70% of our sample. These three spatial scales were then used to extract NDVI values. All maps were generated in ArcGis 10.2.2., but raster computations were conducted in *R*.

Temporal trends in environmental conditions from wintering areas

Temporal trends in winter environmental conditions (i.e. NDVI) showed a decline after 1995 (Fig. 2). We found a positive main effect of year and a negative quadratic effect of year on NDVI values extracted from pixels that were assigned to be the likely origin for >50% of our sample (year: estimate = 0.747, SE = 0.359, $p = 0.046$; year^2: estimate = -0.0002, SE = 0.0001, $p = 0.046$; $R^2 = 0.133$), >60% of our sample (year: estimate = 0.829, SE = 0.386, $p = 0.040$; year^2: estimate = -0.0002, SE = 0.0001, $p = 0.040$; $R^2 = 0.156$) and 70% of our sample (year: estimate = 0.879, SE = 0.393, $p = 0.033$; year^2: estimate = -0.0002, SE = 0.0001, $p = 0.033$; $R^2 = 0.144$).

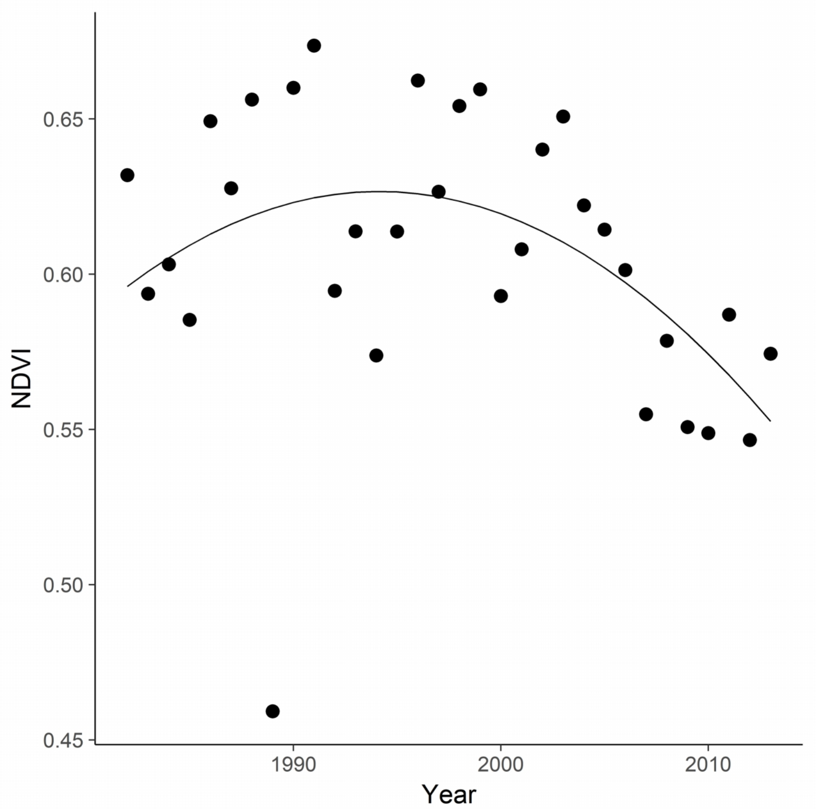

Figure 2. Temporal trend in NDVI extracted from the previously defined wintering areas during the period 1981-2013 (pixels that were assigned to be the likely origin for >60% of our sample). Points represent average NDVI from November (year "i-1") to March (year "i"). Average NDVI values were obtained from bimonthly data developed by ECOCAST. The continuous line indicates the predicted NDVI values given by the linear model fitted with NDVI as a function of year and second degree of year using Ordinal Least Squares.

Winter conditions *vs* subsequent breeding phenology and reproductive success

Environmental conditions from the winter areas carried over to affect subsequent reproductive traits, with effects stronger in females compared to males (Table 2; Fig. 3). Confirmatory path analysis revealed that winter conditions affected breeding phenology of females differently according to age class. The direct effect of the interaction term between age and NDVI on breeding date of females was significant [estimate (SE) = -0.712 (0.323), $p = 0.028$]. In addition, breeding date negatively affected clutch size of females [estimate (SE) = -0.289 (0.022), $p < 0.001$]. Furthermore, clutch size positively affected the number of fledglings produced by females [estimate (SE) = 0.484 (0.019), $p < 0.001$]. Because all these direct effects were significant, the effect of the interaction between age and NDVI on breeding date was ultimately translated to affect the number of fledglings raised, and this indirect effect was calculated by multiplying the standardized path coefficients connecting the former and latter variable. Thus, females advanced laying date (Fig. 4), layed more eggs (Fig. 5) and produced more fledglings (Fig. 6) when NDVI during the previous winter was higher and the slope of this response was increasingly stronger as they aged. For example, considering a shift on NDVI values from bad winter years (i.e. NDVI = 0.5) to good winter years (i.e. NDVI = 0.7), yearling females advanced breeding date only 3 days (from day 144 to day 141), whereas five year-old females advanced breeding date by about 18 days (i.e. from day 108 to day 90). Likewise, for yearling females the path analysis predicted a shift of 0.07 eggs (i.e., from 4.95 to 5.02) and 0.06 fledglings (i.e., from 4.12 to 4.18) when comparing predicted values from bad winter years to good winter years, whereas for five year-old females the path analyses predicted a shift of 0.34 eggs (i.e., from 5.64 to 5.98) and 0.32 fledglings (i.e., from 4.76 to 5.09). On the other hand, setting winter conditions constant, the shift in breeding date across age was much stronger. Specifically, the predicted values obtained for breeding date in response to the age of females (i.e. from 1 to 5 years) accounted for a difference in 36 days (i.e., from day 144 to day 108) under bad winter conditions (i.e. NDVI = 0.5), whereas the difference in breeding date accounted for 50 days (i.e., from day 140 to day 90) under good winter conditions (i.e., NDVI = 0.7). Likewise, setting winter conditions constant, predicted values obtained for age-related change in clutch size and number of fledglings also showed a large shift. Specifically, under bad winter conditions our analyses predicted a change of 0.68 eggs (i.e., from 4.95 eggs of one year-old females to 5.63 eggs of five year-old females) and 0.65 fledglings (i.e., from 4.12 fledglings of one year-old females to 4.77 fledglings of five year-old females), whereas under good winter conditions our analyses predicted a larger change of 1 egg (i.e., from 5.02 eggs of one year-old females to 5.98 eggs of five year-old females) and 1 fledgling (i.e., from 4.18 fledglings of one year-old females to 5.09 fledglings of five year-old females).

For males, the direct effect of the interaction term between age and NDVI on breeding date was similar to that found for females, but not statistically significant [estimate (SE) = -0.569 (0.339), p = 0.094]. In addition, breeding date negatively affected the number of fledglings raised by males [estimate (SE) = -0.222 (0.023), $p < 0.001$]. Consequently, carry-over effects operating in males were similar to that of females, but weaker in strength. Confirmatory path analyses also revealed a significant negative effect of tail length on breeding date for both females [estimate (SE) = -0.060 (0.022), p = 0.006] and males [estimate (SE) = -0.097 (0.022), $p < 0.001$]. Therefore, barn swallows with longer tail feathers started to reproduce earlier and ultimately raised more fledglings. In addition, body mass was only significantly correlated with breeding date of females (estimate = 0.042, p = 0.029), indicating that females which started to reproduce later were heavier (because most of body mass measures were taken after breeding date).

We used d-separation test to quantify the goodness of fit of our models, which tests the assumption that all variables are conditionally independent (Shipley 2016). Both path analyses provided robust fit to data (females: Fisher's C = 15.3, df = 10, p = 0.121; males: Fisher's C = 2.2, df = 4, p = 0.699). Thus, we concluded that the hypothesized causal relationships we examined were consistent with the data. By further inspection of "qq plots", we determined that every single linear mixed model was adequately fitted.

Table 2. Summary results from confirmatory path analyses built for each sex class. Estimates shown here are standardized path coefficients (i.e. slopes of effects). R^2 shown here is the conditional R^2, based on fixed and random effects. Significant effects are highlighted in bold.

Model	**Response**	**Predictor**	**Estimate**	**SE**	***p***	$\mathbf{R^2}$
Females	No. fledglings	**No. eggs**	**0.484**	**0.019**	**< 0.001**	0.318
		Age	0.036	0.019	0.066	
		Tail lenght	0.026	0.019	0.179	
	No. eggs	**Breeding date**	**-0.289**	**0.022**	**< 0.001**	0.142
		Tail	-0.005	0.021	0.826	
		Age	0.003	0.022	0.879	
	Breeding date	**Tail lenght**	**-0.060**	**0.022**	**0.006**	0.197
		Age * NDVI	**-0.712**	**0.323**	**0.028**	
		Age	0.617	0.322	0.056	
		NDVI	0.125	0.081	0.129	
Males	No. fledglings	**Breeding date**	**-0.222**	**0.023**	**< 0.001**	0.167
		Tail lenght	0.020	0.022	0.348	
		Age	0.016	0.022	0.456	
	Breeding date	**Tail lenght**	**-0.097**	**0.022**	**< 0.001**	0.134
		NDVI	0.134	0.078	0.092	
		Age * NDVI	-0.569	0.339	0.094	
		Age	0.466	0.336	0.167	

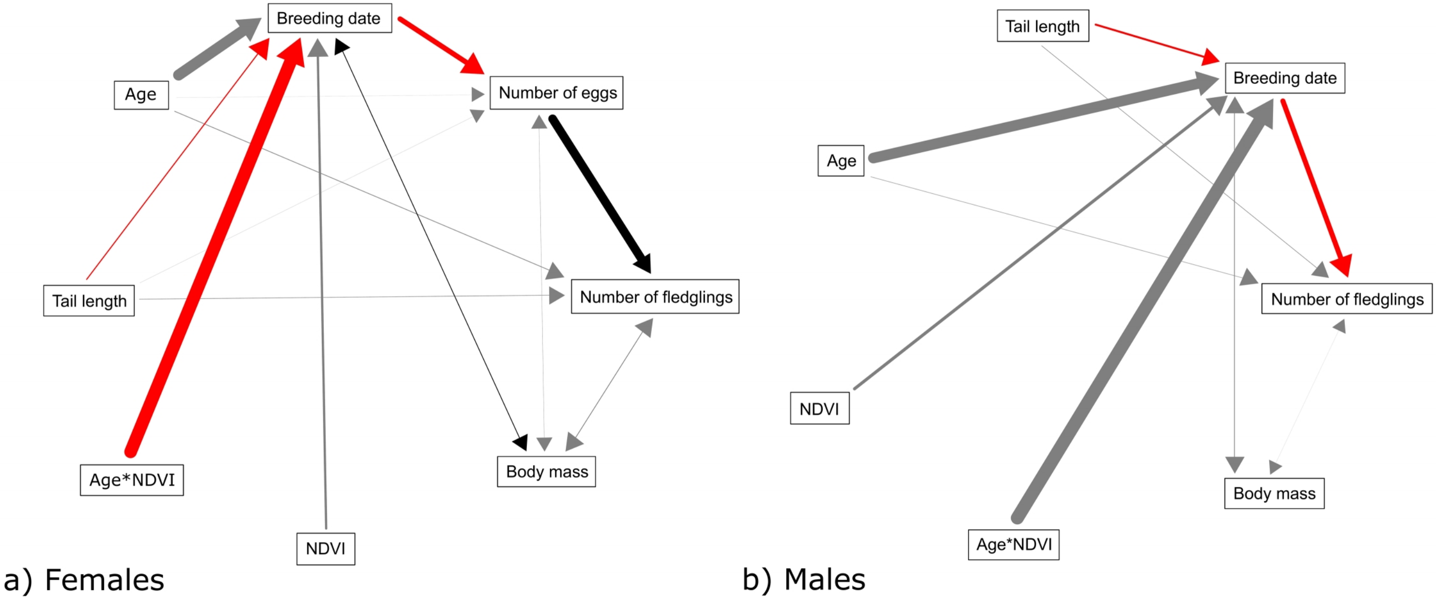

Figure 3. Path diagrams of the causal models developed. One-headed arrows represent a causal effect of one variable on another (i.e. a path). Double-headed arrows link variables with correlated errors. The width of the arrows reflects the magnitude of standardized path coefficients. Black arrows indicate significant positive effects, red arrows significant negative effects and grey arrows non-significant effects.

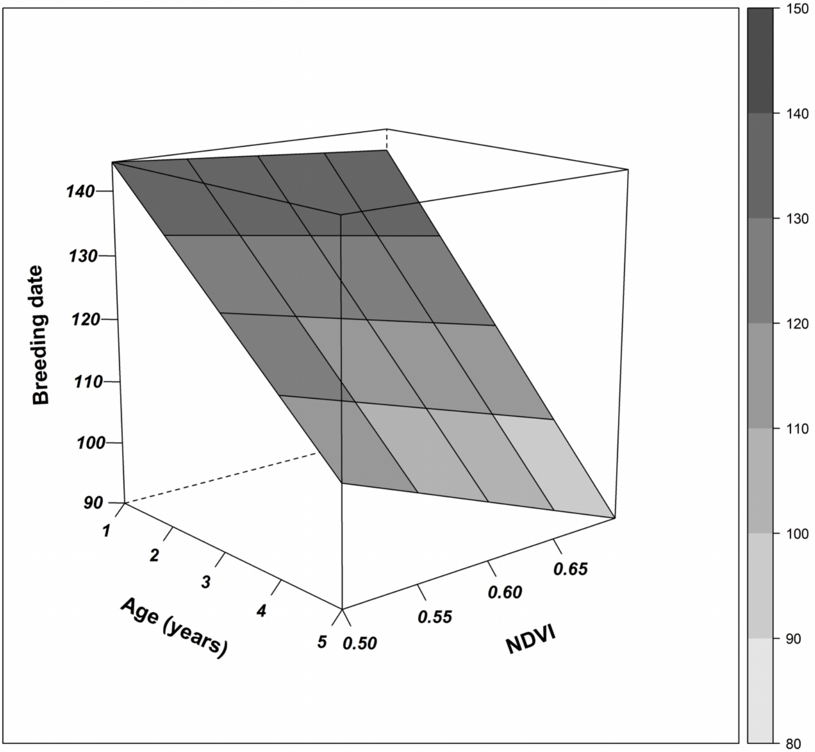

Figure 4. Three-dimensional surface plot showing the relationship between age, NDVI from winter areas and breeding date of females. Lines and grey scale represent predicted values obtained from the linear model fitted for breeding date (using the standardized path coefficient). A value of zero for breeding date corresponds to 1st January.

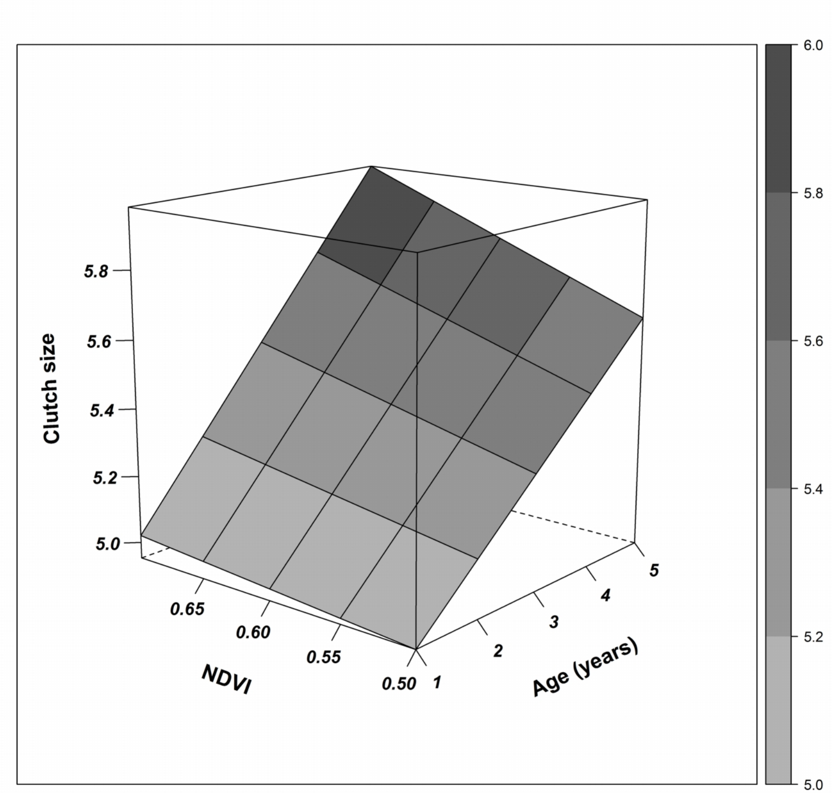

Figure 5. Three-dimensional surface plot showing the relationship between age, NDVI from winter areas and clutch size of females. Lines and grey scale represent predicted values obtained after calculating the indirect effect of the interaction between age and NDVI on clutch size. This indirect effect was calculated by multiplying standardized path coefficients connecting the interaction term with the number of eggs laid.

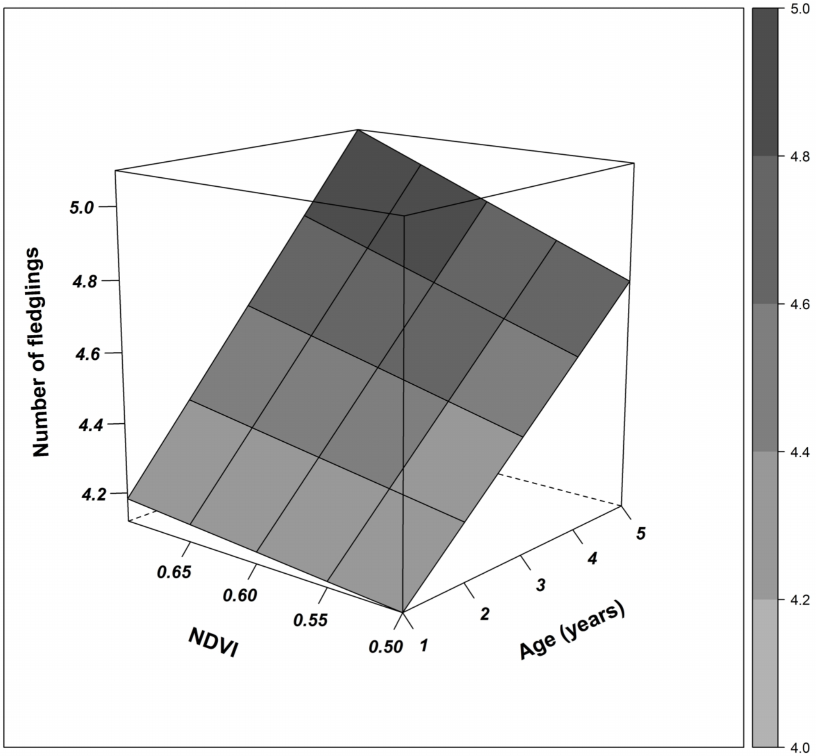

Figure 6. Three-dimensional surface plot showing the relationship between age, NDVI from winter areas and the number of fledglings produced by females at the first brood. Lines and grey scale represent predicted values obtained after calculating the indirect effect of the interaction between age and NDVI on the number of fledglings. This indirect effect was calculated by multiplying standardized path coefficients connecting the interaction term with the final reproductive success.

Discussion

We determined with reasonable precision the wintering areas of barn swallows breeding in Denmark, being distributed from Central to South Africa. This is the first study in which carry-over effects from wintering areas on subsequent reproduction have been analyzed at such large spatial and temporal scales, taking into account age- and sex-dependent variation. Environmental conditions in African wintering areas have changed during the study period showing a steep decline after 1995. We found that barn swallows advanced breeding date, produced larger clutches and raised more fledglings when experiencing good conditions during the previous winter. Interestingly, we found sex- and age-dependent carry-over effects in the annual cycle from wintering to breeding stage. Environmental conditions experienced during winter affected subsequent reproduction more strongly in females than in males, although both sexes similarly responded to this environmental cue. Individuals of different age responded differently to conditions experienced in winter quarters. In general, there was not a clear response of yearlings to conditions experienced during winter, whereas the response gradually increased as individuals aged. Additionally, our results were not confounded by other variables known to affect breeding performance, such as body mass and tail length. Finally, the analyses performed were not biased by the spatial scale used to define winter areas.

There is increasing evidence showing that environmental conditions experienced during winter are related to subsequent spring migration, breeding phenology or reproductive success of migratory birds (Marra et al. 1998; Saino et al. 2004a, 2004b, 2017a; Norris et al. 2004; Balbontín et al. 2009a; Drake et al. 2013; López-Calderón et al. 2017a, 2017b). Such carry-over effects need to be fully understood because they have important consequences for population dynamics and evolutionary processes (Saino et al. 2017a). We found that barn swallows advanced breeding date in response to favorable winter conditions, and that individuals responded differently depending on their age. In agreement with our findings, barn swallows breeding in northern Italy have also been shown to advance arrival and breeding in response to good winter conditions when studied at the population level (Saino et al. 2004a, 2004b), but the opposite outcome was found at the individual level (Saino et al., 2017a). These authors argued that when studied at the population level, individuals on average acquired body condition earlier during good winter years (i.e. high NDVI) and should depart sooner from winter areas or perform faster migration than during poor winter years (i.e. low NDVI). However, when studied at the individual level, individuals may experience different winter conditions within years and those individuals that experienced poor winter conditions (i.e. low NDVI) should depart earlier from winter areas because of seasonal deterioration of winter conditions taking place within years (Saino et al., 2017a). Therefore, both spatial and temporal variation in winter conditions could influence carry-over effects between the wintering and breeding stage. Hence, the outcome could differ when the same population of the same species is studied either at the individual or population level.

Ecological conditions from the wintering grounds usually affect subsequent breeding success more strongly in females than in males (Norris et al. 2004; Drake et al. 2013; Saino et al. 2017a; but see López-Calderón et al. 2017b). Specifically, Saino et al. (2017a) found carry-over effects from winter conditions on timing of breeding and reproductive success for female but not for male barn swallows. We found the same carry-over effects operating in both sexes, but in agreement with the latter study, the response was stronger for females. This difference found between sexes could be explained because females may be more constrained than males in the time needed to start breeding after arrival to their breeding grounds. Along this line, the variance in time elapsed between arrival and breeding was smaller for females compared to males (Saino et al. 2017a). Alternatively, the acquisition of an appropriate physical condition after arrival to breeding areas could be more important to determine breeding date for females than for males, because females must meet the physiological demands associated with egg production. Otherwise, males are more prone to arrive earlier than females to breeding grounds because males that arrive earlier usually mate sooner and have greater reproductive success (Møller 1994). Because, we lacked arrival date in our path analyses, we could not make many inference about the causes of small difference we found in carry over effects between sexes.

It is well known that avian migratory behavior depend on the age of individuals (Cristol et al. 1999; Newton 2008). The carry-over effects we found in this study should have been mediated by the effects of different winter conditions on migratory behavior such as departure date from winter areas, flight speed on route, number and duration of stopovers and arrival date to breeding areas. Most previous studies investigating migratory behavior and carry-over effects (e.g. Saino et al. 2004a; Balbontín et al. 2009a: Drake et al. 2013; López Calderón 2017a, 2017b) have discriminated between two age classes (i.e. juveniles *vs* adults) and consequently age-related migratory behavior based on actual known age marked-individuals has been reported less frequently (but see Sergio et al. 2014). Difference amongst age classes in migratory behavior is hence a less studied phenomenon (Gromadzka & Serra 1998; Yosef et al. 2003; Markovets et al. 2008).

We found different responses to winter conditions with the age of individuals. Our results support the constraint hypothesis and reject the competition hypotheses to explain age-related differences in timing of reproduction and breeding success. We found the highest difference in reproductive traits across age when winter conditions were favorable. If the competition hypothesis was operating in our study system and if older individuals were dominant over young ones, greater difference in reproductive traits across age were expected after experiencing poor winter conditions. This is because competition among individuals should be exacerbated under low resource availability. However, we found the opposite, and we hypothesize that the higher reproductive success of older individuals compared to yearlings could be due to swallows acquiring competence via experience. For instance, gaining experience or skills could help older individuals to select better quality wintering habitats or make better use of them than yearlings (Forslund & Pärt 1995). Our findings are consistent with another study performed with barn swallows in southern Europe, in which experience explained age-related difference in spring migration and reproductive success (Balbontín et al. 2007). However, we acknowledge that we cannot discard the selection hypothesis as a possible explanation in our case.

Our results have implications for conservation and management of migratory insectivorous birds which are declining (Sanderson et al. 2006; Nebel et al. 2010, BirdLife international 2016). In this study, we have linked reproductive success of barn swallows breeding in north Europe with environmental changes occurring in the African wintering areas. Temperature is predicted to increase across Africa, while precipitation is predicted to increase in Central Africa and decrease in South Africa (IPPC 2013). Considering that the correlation of NDVI with these climatic variables differs spatially throughout the winter areas (Ichii et al. 2002), further complex analyses would be necessary to predict trends in NDVI for these winter areas. Because NDVI has been established as a crucial tool for assessing the effects of climate change on organisms (Pettorelli et al. 2011), and specifically migratory birds (Saino et al. 2004a, 2004b), our results are also appropriate for understanding and predicting the potential adaptation to climate change of organisms throughout their life spans.

Summarizing, we identified winter areas of barn swallows breeding in Denmark by combining a multi-isotope assignment with prior information from ringing data. We have also determined, using satellite-derived NDVI values, that environmental conditions at the African wintering grounds have deteriorated in recent years. In addition, we have shown that changes in NDVI during winter were related to subsequent reproductive parameters, and that age and sex determined the strength of these carry-over effects. The increasing collection of long-term individual data could shed light on the ability to predict future adaptation of long-distance migrants to current climate change. We highlight that our findings are compiling evidence of the great importance of age on migratory ecology, with relevant fitness consequences.

Acknowledgements

We are grateful to the European Union for Bird Ringing (EURING), which made the recovery data available through the EURING Data Bank and to the many ringers and ringing scheme staff who have gathered and prepared the data. This study was funded by *Junta de Andalucía* (research project: P12-RNM-2144). The corresponding author was supported by an operating grant from *Junta de Andalucía* (P12-RNM-2144). The program "Erasmus +" provided additional funding necessary for the corresponding author to develop a research stage. We sincerely thank farm owners and volunteers that helped in this long-term field study. Finally, we thank Steven Van Wilgenburg for his comments regarding probabilistic assignment of origins.

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General discussion

In this thesis we have addressed several topics considered as priority research areas in ornithology, such as the identification of migratory routes, quality and distribution of non-breeding habitats, migratory schedules, carry-over effects, factors influencing migratory behaviour, and migratory connectivity (Webster & Marra 2005; Faaborg et al. 2010; Norris & Marra 2007; Harrison et al. 2011). Furthermore, we have approached other issues of relevance under the current scenario of global change, such as the importance of winter area choice on blood parasite infection, the environmental deterioration of tropical wintering areas, and responses of migratory birds to global change (Gordo et al. 2007; Altizer et al. 2015; Kramer et al. 2018). Our results have provided important insights within theoretical migration ecology that can be in turn helpful for conservation of declining species (Sanderson et al. 2006; Nebel et al. 2010; Patchet al. 2018). We have used both stable isotope analyses and light-level geolocators as our tracking techniques, which are considered the best options to study movements of small-sized birds (Hobson & Wassenaar 2008; McKinnon et al. 2013). In addition, we have incorporated other sources of information such as ringing recoveries (du Feu et al. 2009) and satellite-derive biological indexes (Pettorelli et al. 2005, 2011), completing the toolbox required to meet our goals. Moreover, we have adopted some of the most updated statistical procedures in our research areas; such as novel methods to reconstruct migratory routes from raw light data (Rakhimberdiev et al. 2015; 2017), the use of Structural Equation Models to detect carry-over effects (Shipley et al. 2016; Lefcheck et al 2016), as well as new concepts for the study of migratory connectivity (Finch et al. 2017).

We used light-level geolocators to analyse in detail the migration system of barn swallows breeding in southwestern Spain. Beyond the large amount of descriptive information provided here, we found some interesting results that may help to explain carry-over effects from the winter to the breeding stage. First, we found that migration schedules in spring were more variable among individuals than those of autumn migration. Second, we found that the range of stopover locations and the variance in the number of stopovers within individuals were larger in spring than in autumn migration. Third, we found that departure date from wintering areas significantly predicted arrival date to breeding grounds. On one hand, these results suggest that individuals should experience different environmental conditions during winter and spring migration, and thus some individuals arrive to their breeding areas much earlier than others do, which in turn can have subsequent fitness effects (Saino et al 2017). On the other hand, these results highlight that wintering and breeding stages are linked by means of migration schedules in spring (McKinon et al. 2013; Lemke et al. 2013; Szép et al. 2017; Cooper et al. 2017).

We have also found complementary results using stable isotope analyses. For instance, house martins breeding in Spain and wintering in West Africa at the isotopic cluster associated with savannahs, started to breed earlier than those wintering at the isotopic cluster associated with broadleaf forest. In addition, young male house martins wintering in habitats of higher δ^{13}C and δ^{15}N (indicating open habitats dominated by C4 vegetation and C3 plants adapted to hydric stress) initiated reproduction earlier than young males wintering in habitats of lower δ^{13}C and δ^{15}N (indicating closed or moist habitats dominated by C3 vegetation). Furthermore, barn swallows breeding in northern Denmark and wintering in Central Africa, advanced onset of breeding after years of higher NDVI during winter, being this response increasingly stronger as they aged. Unfortunately, we lack estimates of arrival date to breeding areas for our analyses based on stable isotope analyses. Nevertheless, arrival time to breeding areas usually determines the start of reproduction (e.g. Smith & Moore 2004). We also found other mechanisms driving fitness carry-over effects from the winter to the breeding stage, such as body condition and infection by blood parasites. Interestingly, both could be operating with opposite selective pressures, since experienced male house martins improved body condition and breeding success, but at the same time suffered a hig her risk of haemosporidian infection when wintering in habitats of higher rainfall (i.e. lower δ^{2}H). Therefore, we have shown different ways to propagate fitness carry-over effects from the winter to the breeding stage, which may also interact among them. Considering also the different responses that we found across age and sex classes, this thesis provides some evidence to unveil the complexity of the Palearctic-Afrotropical migration system.

In accordance with the objectives of each chapter and body size restriction, we used either stable isotope analyses or light-level geolocators as tracking technique. To the best of our knowledge, house martins have been tagged with geologgers only in one study (Szép et al. 2017), and apparent survival rates of marked birds were much lower than that of control birds (i.e. 12.5% compared to 38.9%). Therefore, due to both ethical and economic reasons, we did not fit house martins with geolocators. Nevertheless, the negative effect of tag deployment on apparent survival rates of barn swallows was proven smaller than that of house martins, yet 25.31% of tagged swallows survived to the next year compared to 38.60% of control ones (Scandolara et al. 2014). The relatively low cost of stable isotope analyses allowed us to work with a much higher sample size for house martins compared to barn swallows breeding in Spain (~300 vs 24 birds, respectively). In addition, we believe that any relationship between wintering habitats and risk of infection by blood parasites would be more easily detected in house martins than in barn swallows, because the former suffers much higher prevalence (von Rönn et al. 2015; Marzal et al. 2016). Thus, in our case, stable isotope analyses were necessary to identify fitness carry-over effects mediated by blood parasite infection. We were also interested in long-term responses of barn swallow populations breeding in Denmark to environmental changes occurring at their wintering areas. In order to maximize sample size to enhance the inference we made for wintering areas, we needed to work with stable isotope analyses rather than light-level geolocators.

We suggest that stable isotope analyses have an additional advantage compared to geologgers, which is found in long-term studies. Many ornithologists have been sampling feathers during the last decades for different purposes. Stable isotope measurements from these collected feathers through the years can provide information related to migration ecology before the development of solar geolocation. We count with a large data set of ~ 4,000 individually identified barn swallows monitored over thirty years in Denmark. This large data set, in combination with stable isotope analyses and ringing recoveries, allowed us to highlight the importance of age in shaping the strength of carry-over effects and thus the response of migratory birds to global change. Interestingly, this is in agreement with the previous results we found for house martins, which always revealed the importance of age in migration ecology.

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Conclusions

1º) Migratory behavior of barn swallows breeding in Spain have been described in detail for the first time using light-level geolocators. All but one of our studied individuals spent the winter in West Africa, mainly in Ivory Coast and surrounding countries. One single individual migrated in autumn to the British Islands, an exception to the rule.

2º) Tracking barn swallows with geolocators highlighted the importance of spring migration as the main link between the winter and the breeding stage. Specifically, migration schedules and stopover behavior in spring differed strongly among individuals, and barn swallows that departed earlier form the winter grounds arrived earlier to breeding areas. Breeding colony and year were additional sources of variance during spring migration.

3º) Stable isotope analyses combined with ringing recoveries could be used to identify the potential wintering areas of house martins breeding in Spain. These winter areas were associated with two African biomes across West Africa, savannah and broadleaf forest.

4º) Winter area choice in house martins depend on age and sex. We have found that experienced males spent the winter in savannahs with higher probability than young males. In addition, house martins wintering in savannahs started to reproduce earlier in the following breeding season than house martins wintering in broadleaf forests. Therefore, open savannah-like habitats should be of higher quality to overwinter for the house martins than close moist forests.

5º) Environmental conditions during winter affect subsequent breeding success of house martins depending on age and sex. Specifically, experienced males wintering in habitats of higher rainfall were in better body condition and raised more fledglings than experienced males wintering in habitats of lower rainfall.

6º) Experienced house martins wintering in habitats of higher rainfall suffered a higher probability of haemosporidian infection than these wintering in habitats of lower rainfall. Hence, habitat quality and risk of blood infection interact with opposite selective pressures for experienced house martins.

7º) Young house martins wintering in habitats of higher rainfall had a lower probability of haemosporidian infection than young house martins wintering in habitats of lower rainfall. Differences in responses across age classes may arise because immune defenses develop during early life, and thus any shortage in food intake at the drier habitats could especially reduce the immune response at the first winter.

8º) Stable isotope analyses combined with ringing recoveries identified the potential wintering areas of barn swallows breeding in

Denmark. These winter areas were mostly associated with savannahs and broadleaf forests across Central Africa.

9º) Age and sex of barn swallows determine the strength of seasonal carry-over effects. Females advanced breeding date, produced larger clutches and raised more fledglings when experiencing favorable ecological conditions during the previous winter. There was not a clear response of yearlings, whereas the response gradually increased as females aged.

10º) Because environmental conditions in these winter areas have deteriorated during the last three decades, we could expect important consequences in subsequent reproduction and population trends of migratory insectivorous birds.

Watercolours by Cosme López Calderón

Supplementary Materials

The following pages illustrate migration tracks for each individual barn swallow tagged. Migratory routes were reconstructed with *FlightR* (Rakhimberdiev et al. 2017) and final maps were elaborated in ArcGis 10.2.2. Coloured points indicate medians of estimated positions (by latitude and longitude) for each twilight. The colour of each point corresponds to the specific month (see legend). Black line connects medians of estimated positions chronologically. Grey straight lines represent upper and lower limits for 95% credible intervals for estimated positions.

* Indicates that *FlightR* failed to estimate credible intervals for certain periods. We noticed that fault when raw light data was especially noisy.

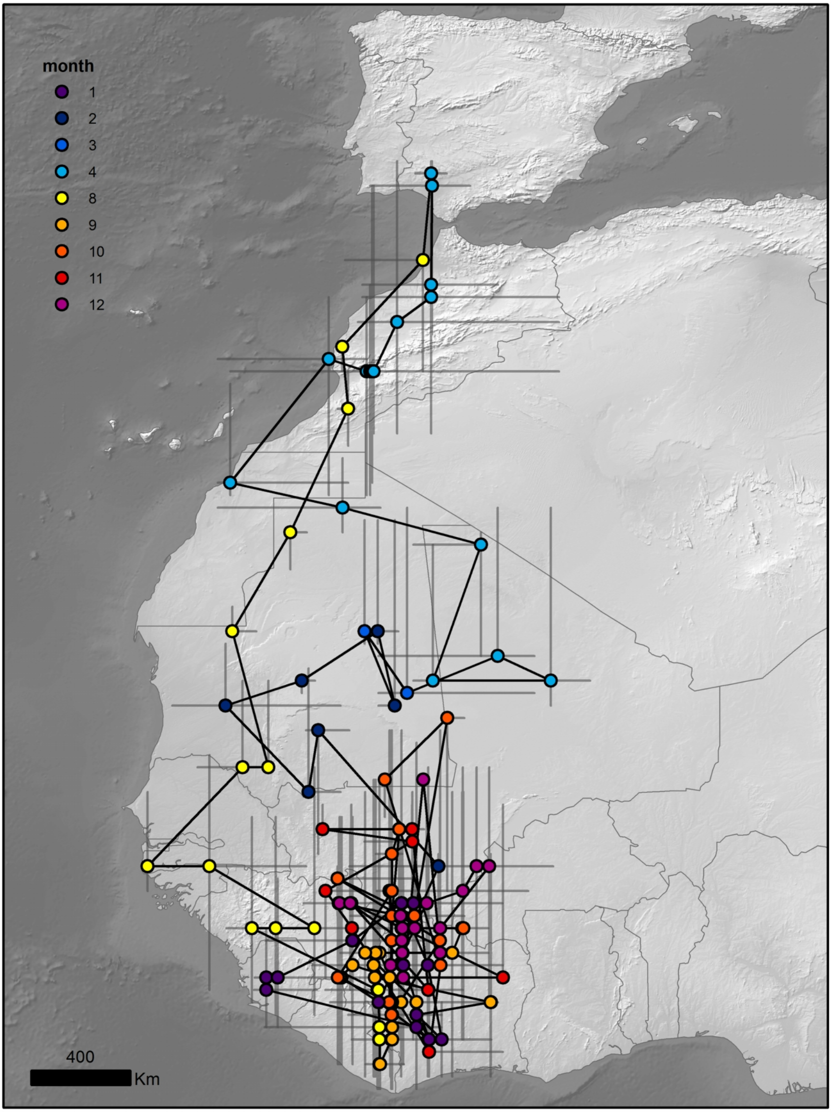

Figure 1. Migration track inferred with light-level geolocators for the bird ringed Y66266 (female breeding in "Las Coladas").

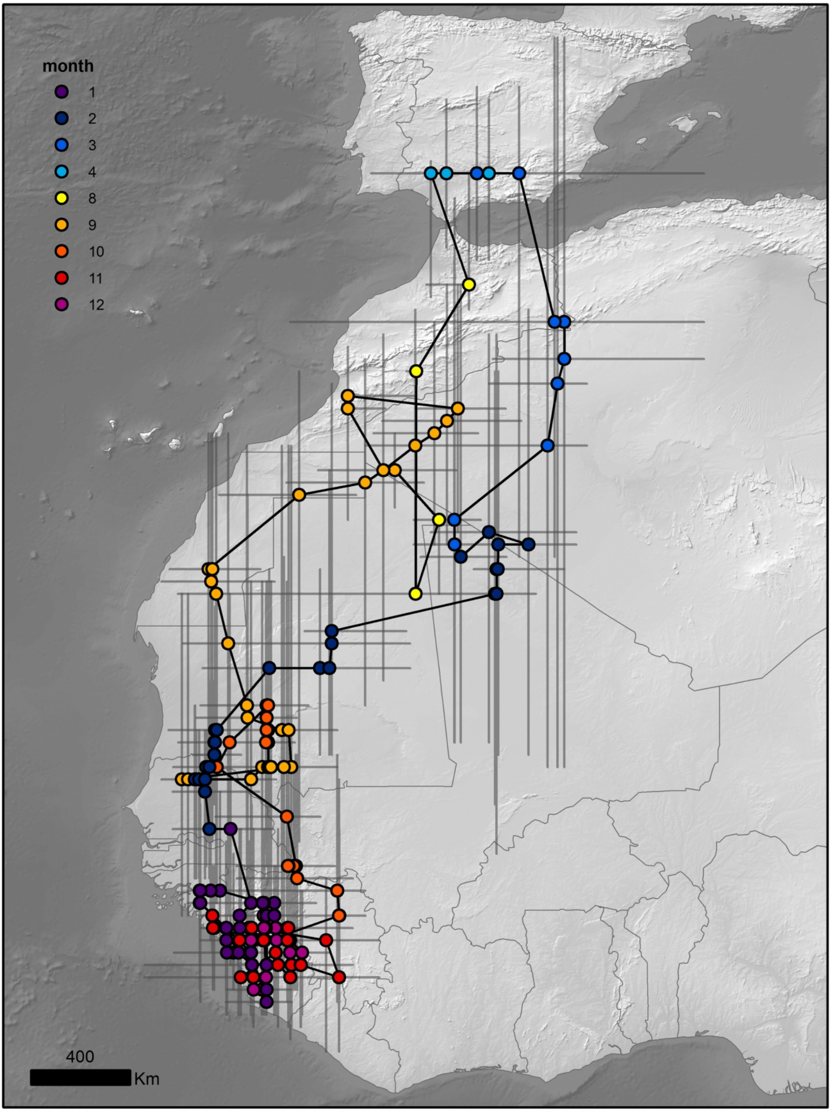

Figure 2. Migration track inferred with light-level geolocators for the bird ringed Y66029 (female breeding in "Las Coladas").

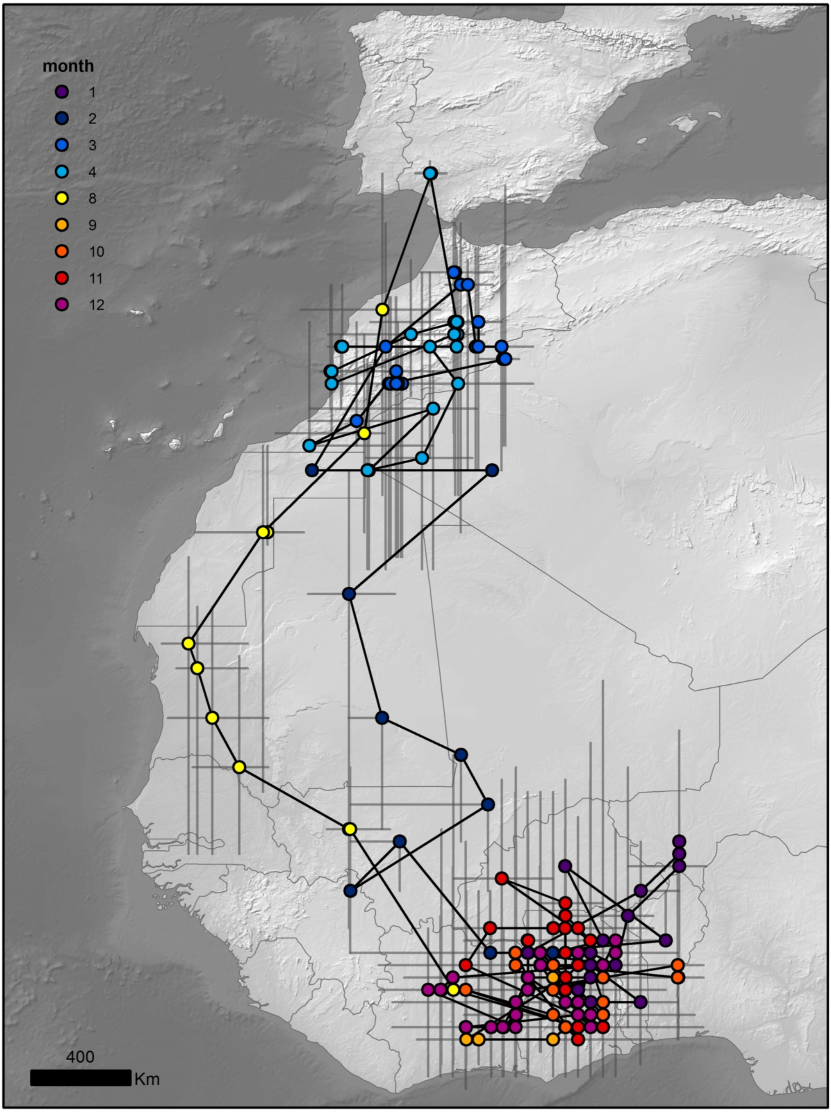

Figure 3. Migration track inferred with light-level geolocators for the bird ringed Y66103 (male breeding in "Las Coladas").

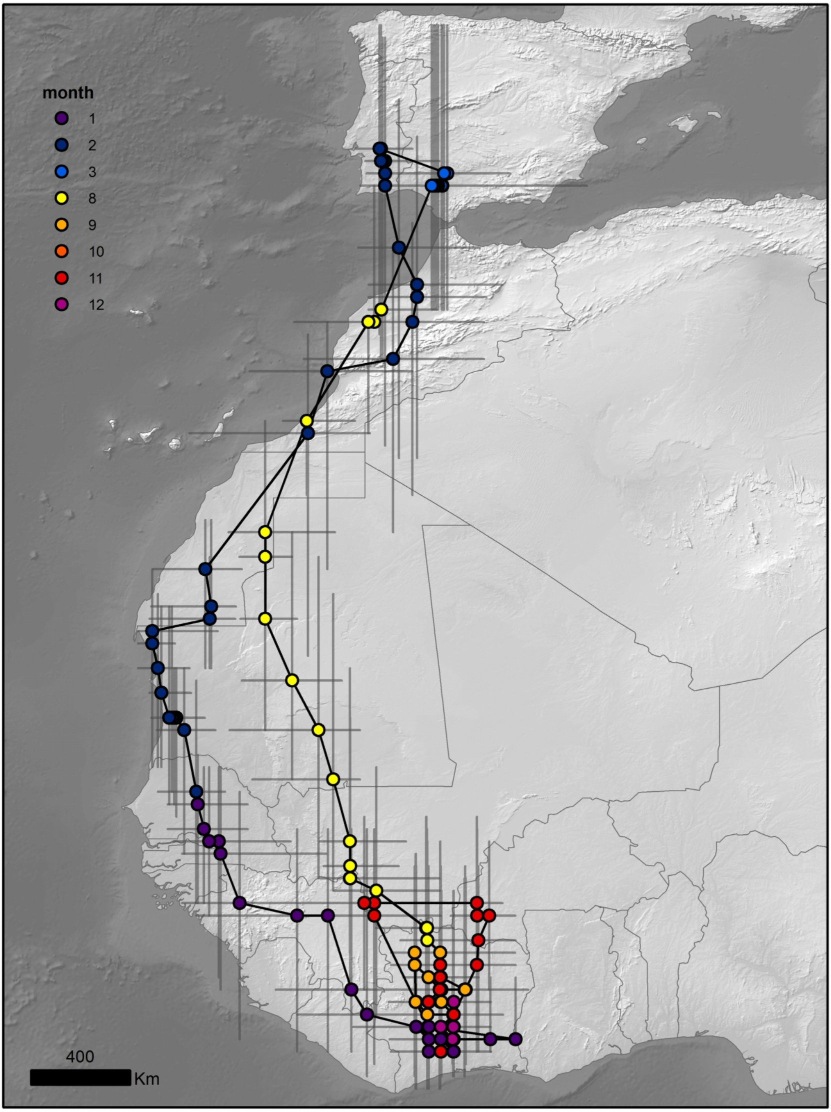

Figure 4. Migration track inferred with light-level geolocators for the bird ringed 2L12365 (male breeding in "La Calera").

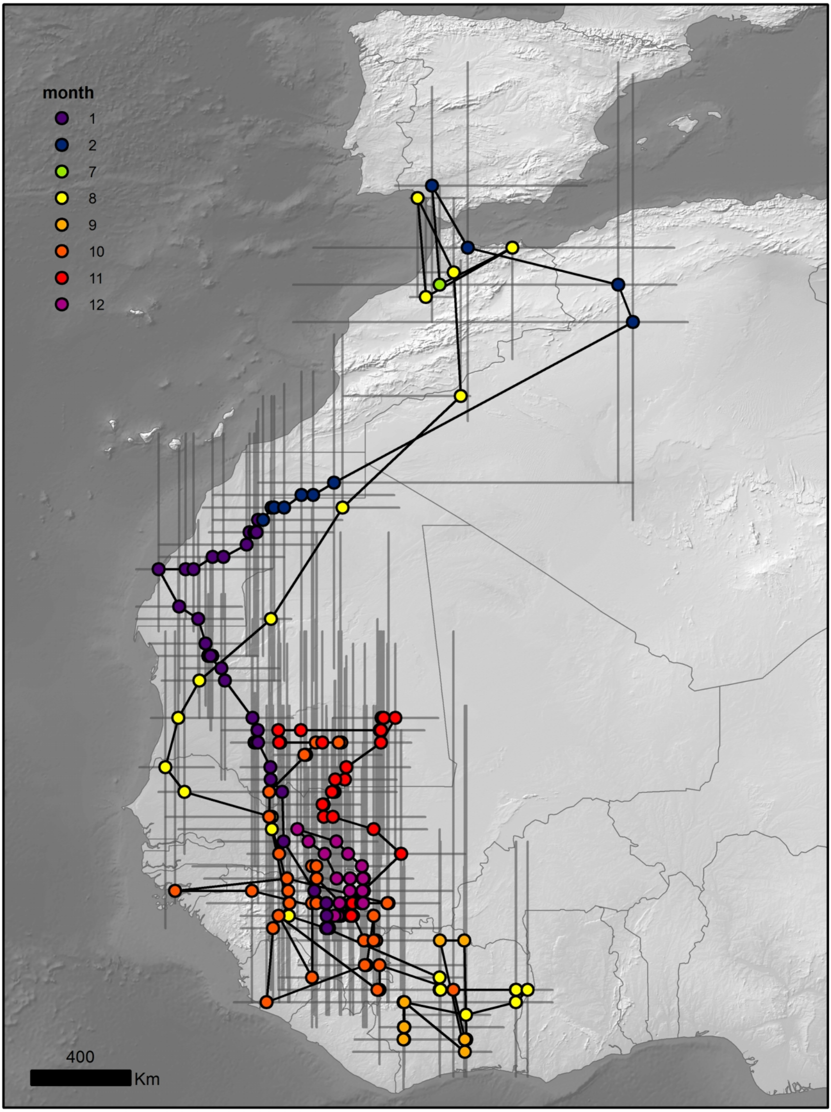

Figure 5. Migration track inferred with light-level geolocators for the bird ringed Y65845 (female breeding in “La Calera”).

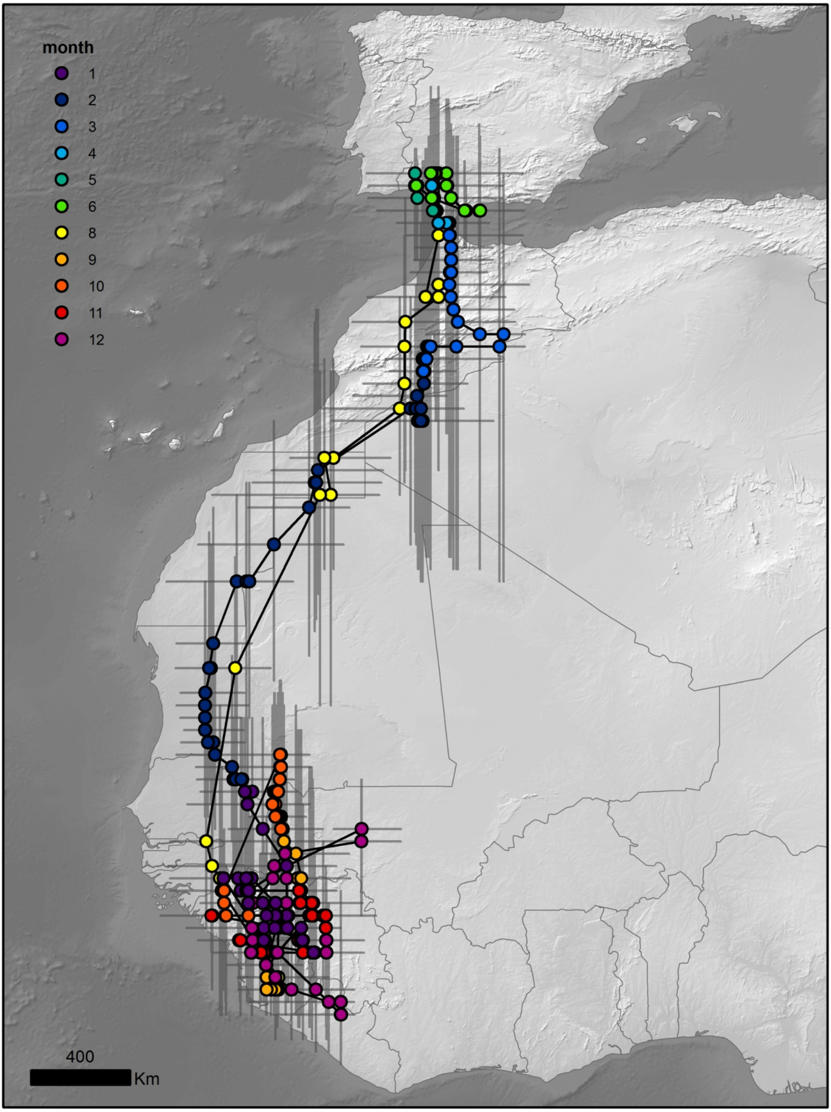

Figure 6. Migration track inferred with light-level geolocators for the bird ringed 1L92850 (male breeding in "Las Coladas").

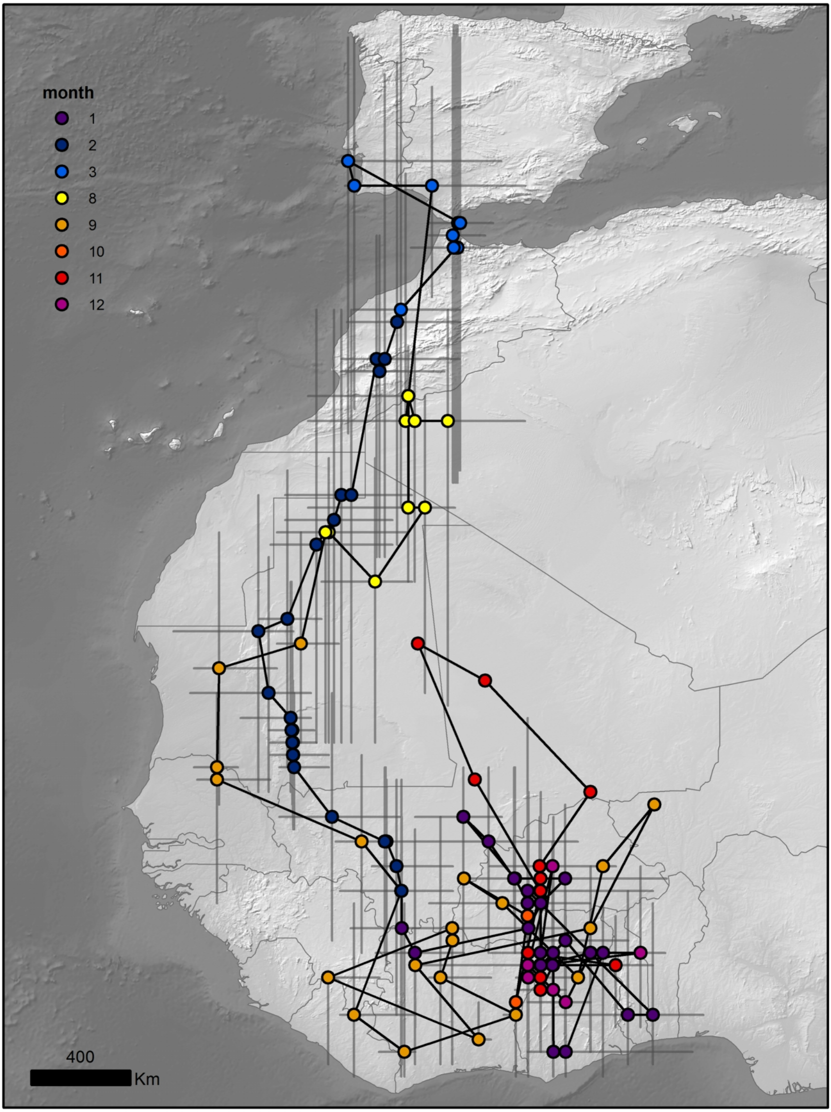

Figure 7. Migration track inferred with light-level geolocators for the bird ringed Y66049 (female breeding in "La Calera").

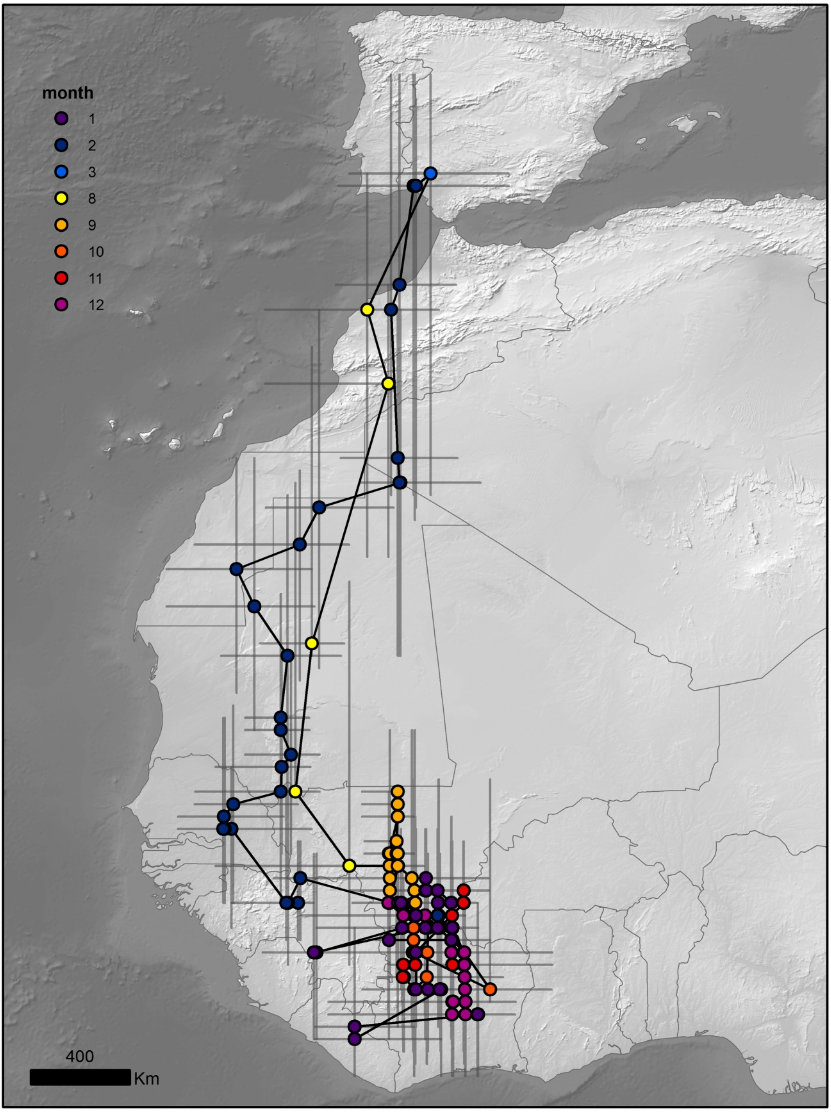

Figure 8. Migration track inferred with light-level geolocators for the bird ringed Y65702 (male breeding in “Las Coladas”).

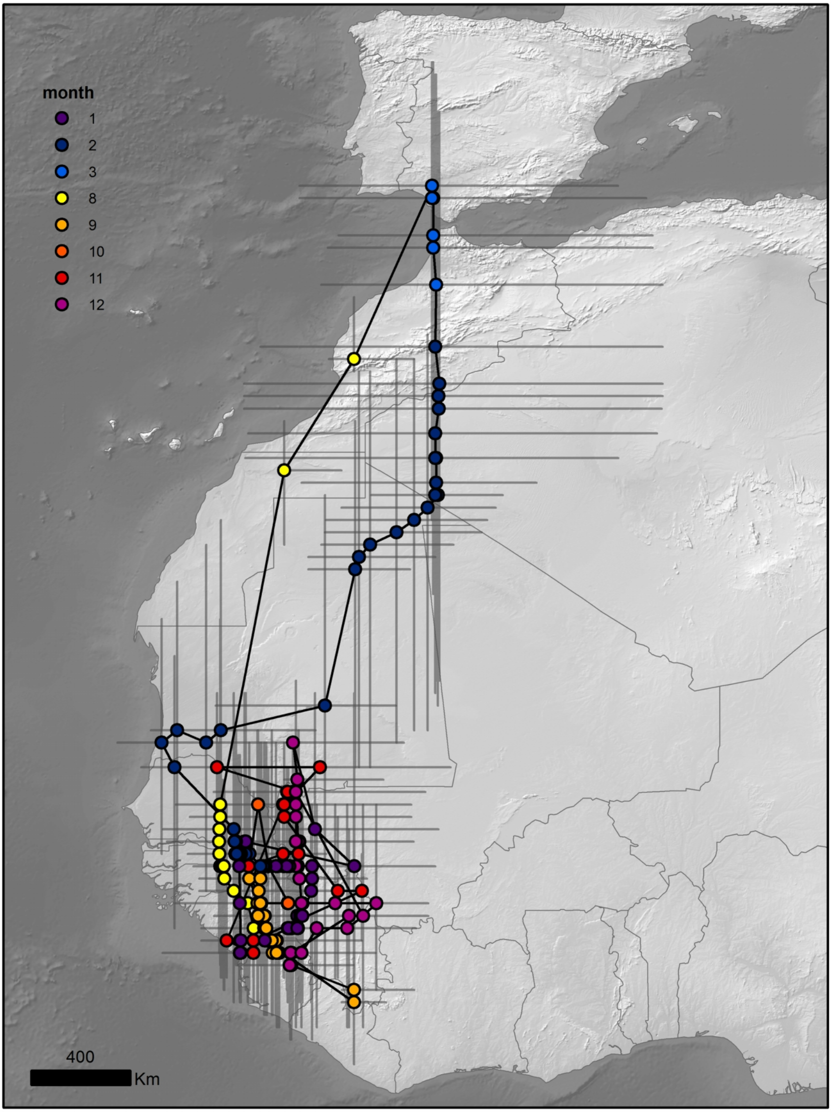

Figure 9. Migration track inferred with light-level geolocators for the bird ringed Y65848 (female breeding in "La Calera").

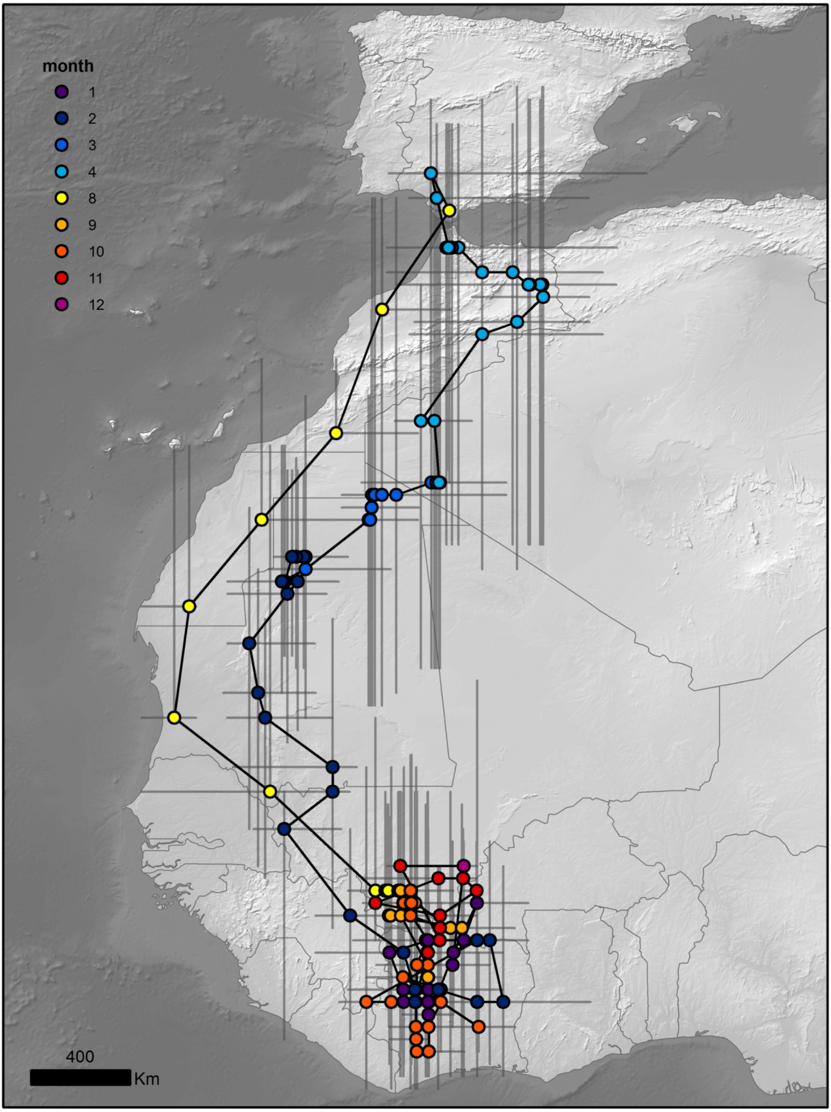

Figure 10. Migration track inferred with light-level geolocators for the bird ringed Y66105 (male breeding in "Las Coladas").

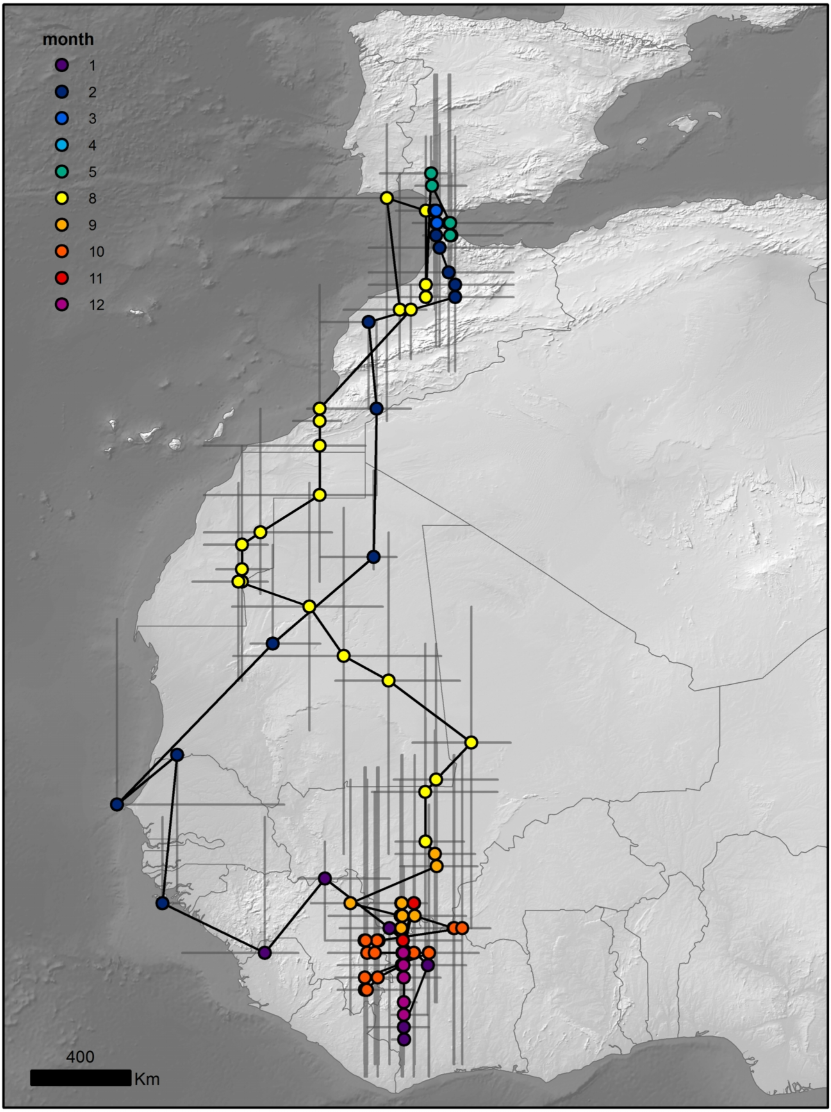

Figure 11. Migration track inferred with light-level geolocators for the bird ringed 1L92855 (female breeding in "Las Coladas").

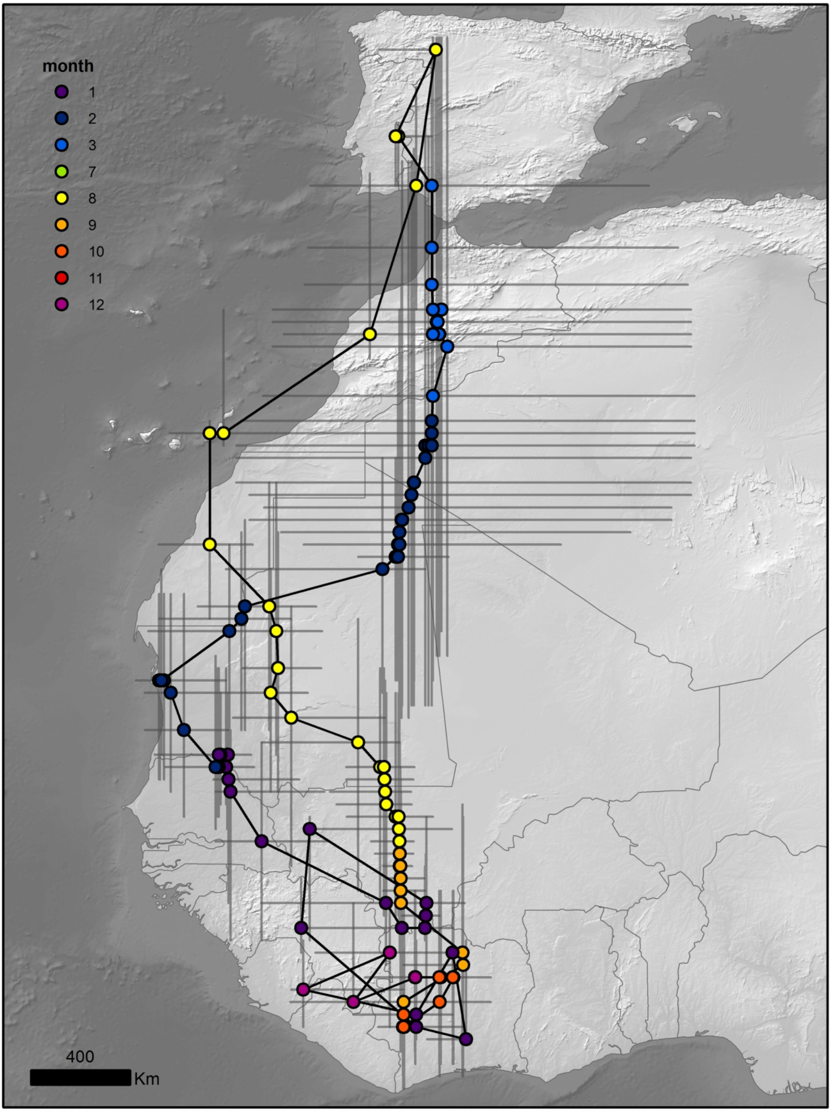

Figure 12. Migration track inferred with light-level geolocators for the bird ringed Y66194 (female breeding in "La Alegría").

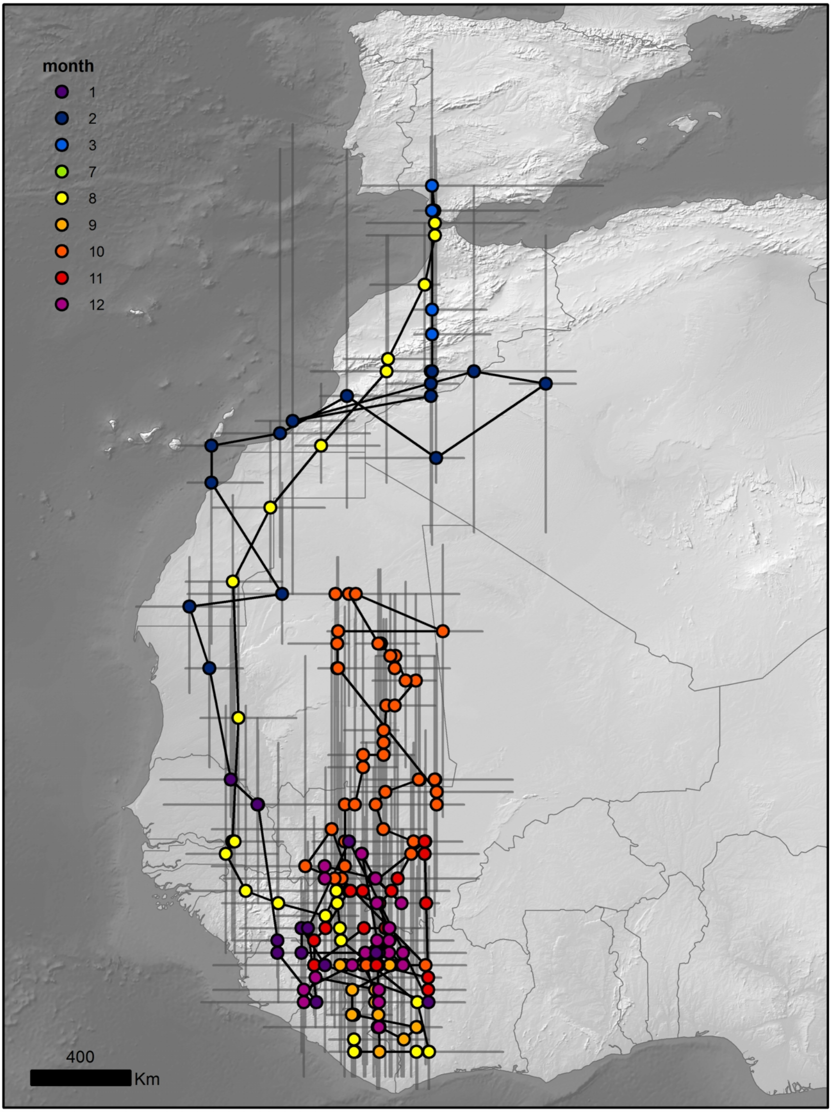

Figure 13. Migration track inferred with light-level geolocators for the bird ringed Y66247 (female breeding in "La Alegría").

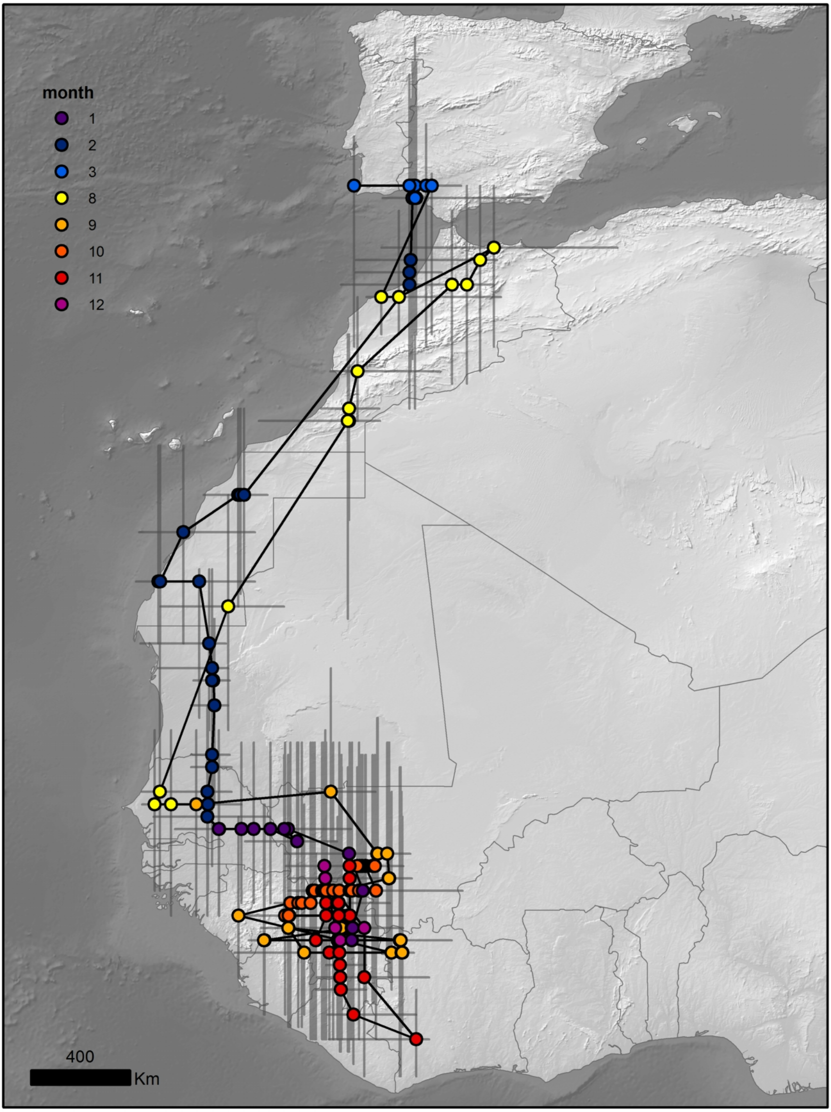

Figure 14. Migration track inferred with light-level geolocators for the bird ringed Y66245 (male breeding in "La Alegría").

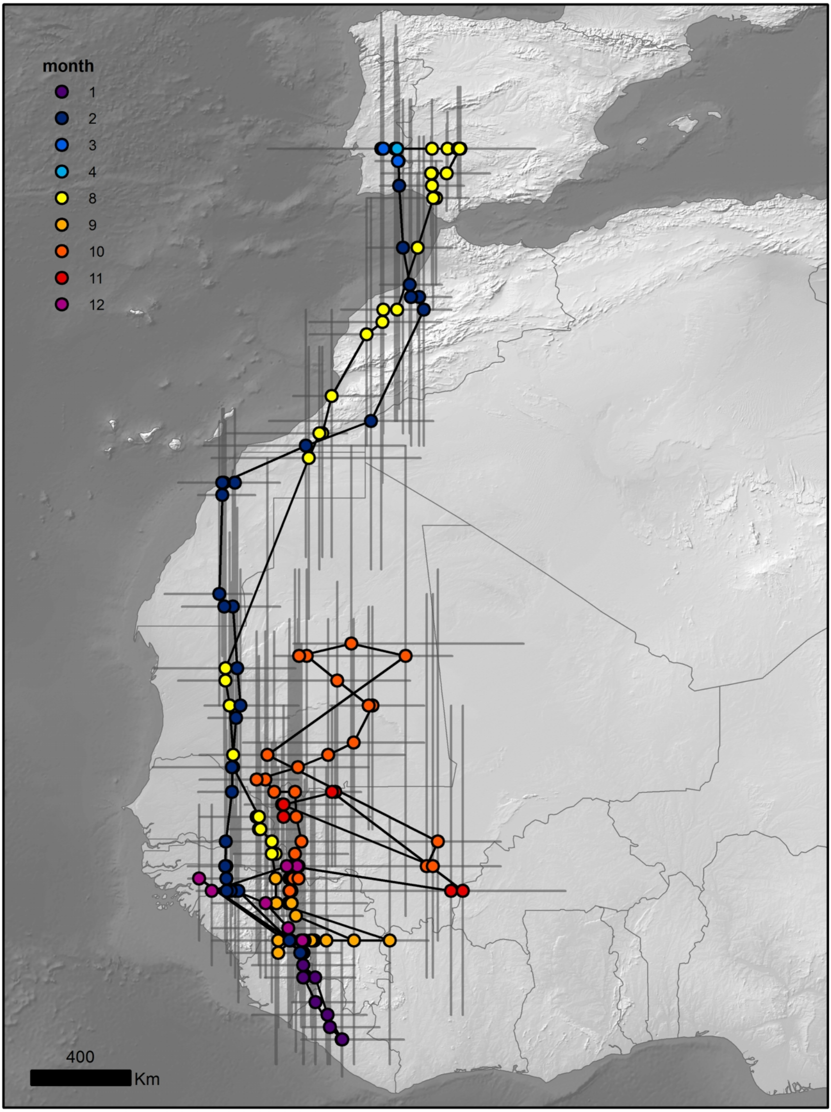

Figure 15. Migration track inferred with light-level geolocators for the bird ringed 2L12736 (female breeding in "Asesera").

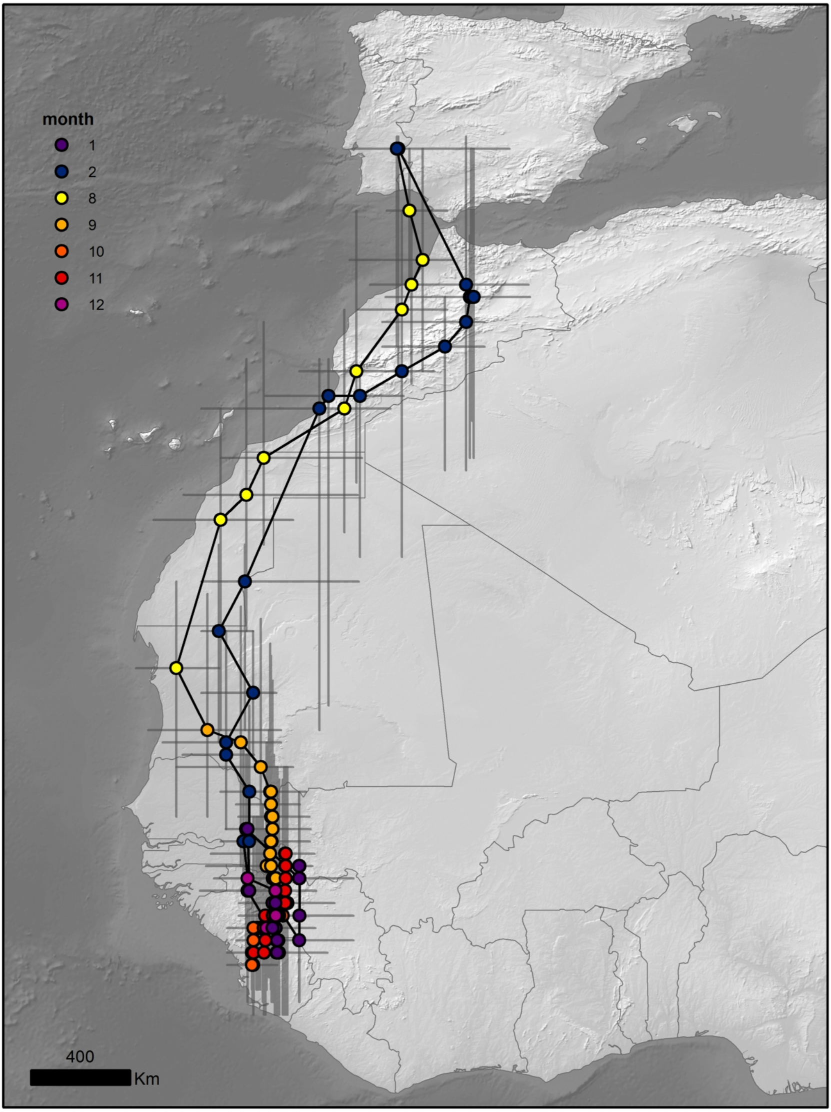

Figure 16. Migration track inferred with light-level geolocators for the bird ringed 2L12738 (female breeding in "Asesera").

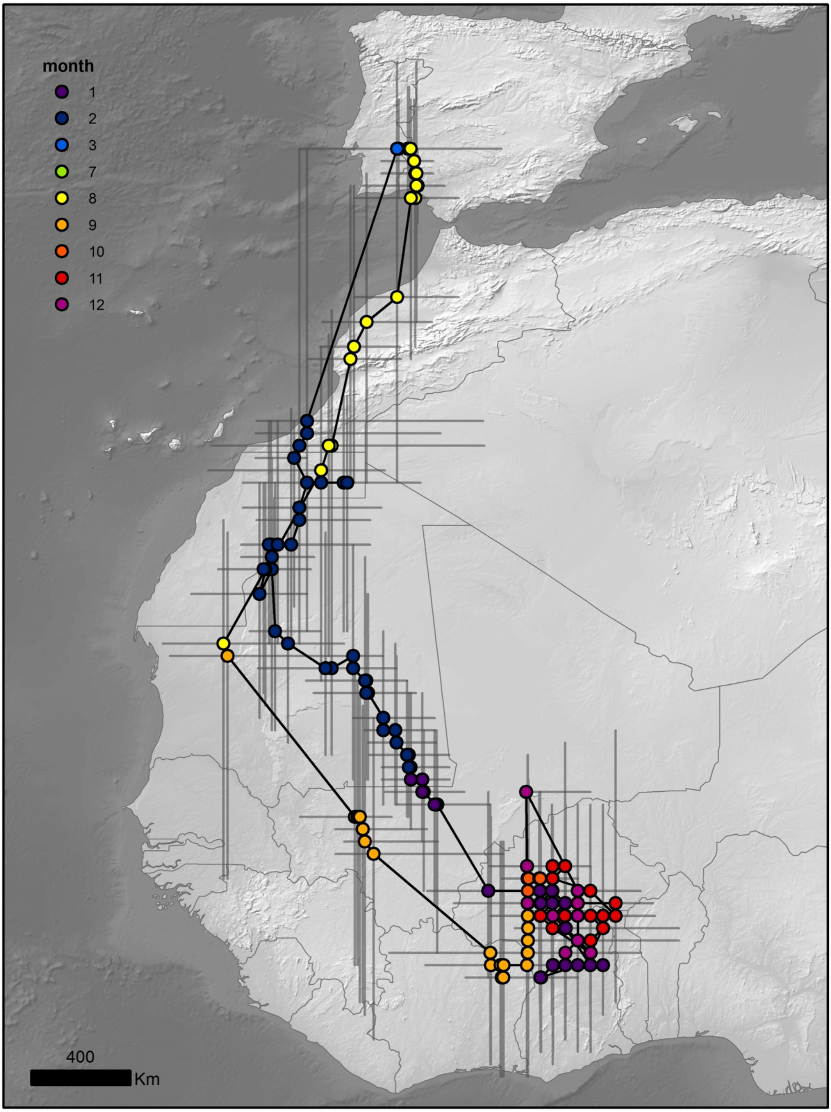

Figure 17. Migration track inferred with light-level geolocators for the bird ringed 2L12735 (female breeding in "Asesera").

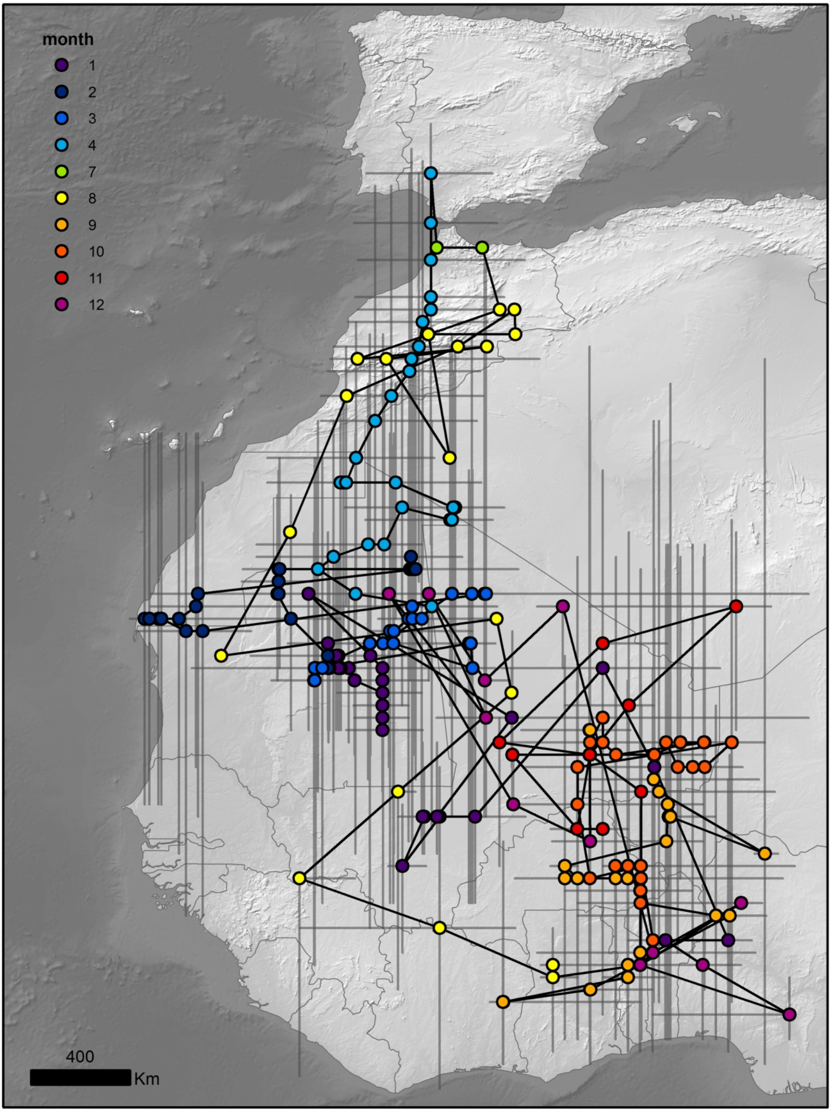

Figure 18. Migration track inferred with light-level geolocators for the bird ringed Y65905 (male breeding in "Las Coladas").*

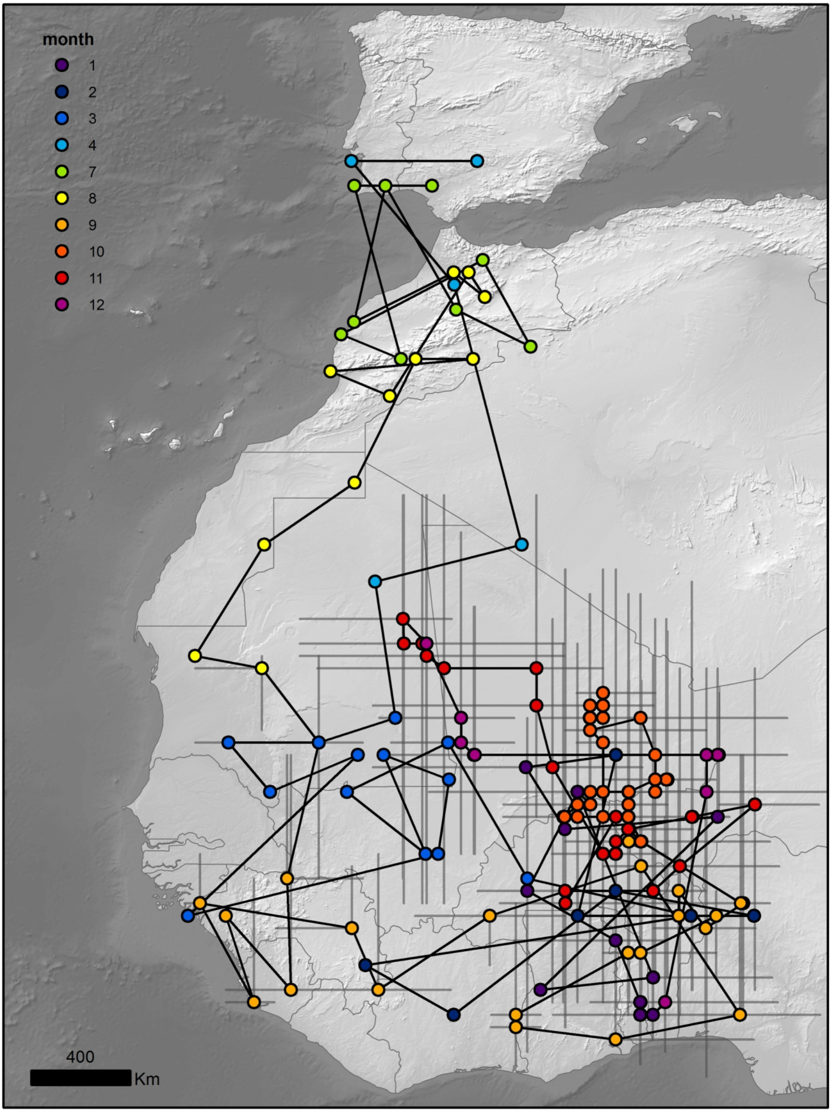

Figure 19. Migration track inferred with light-level geolocators for the bird ringed Y66251 (female breeding in “La Alegría”).*

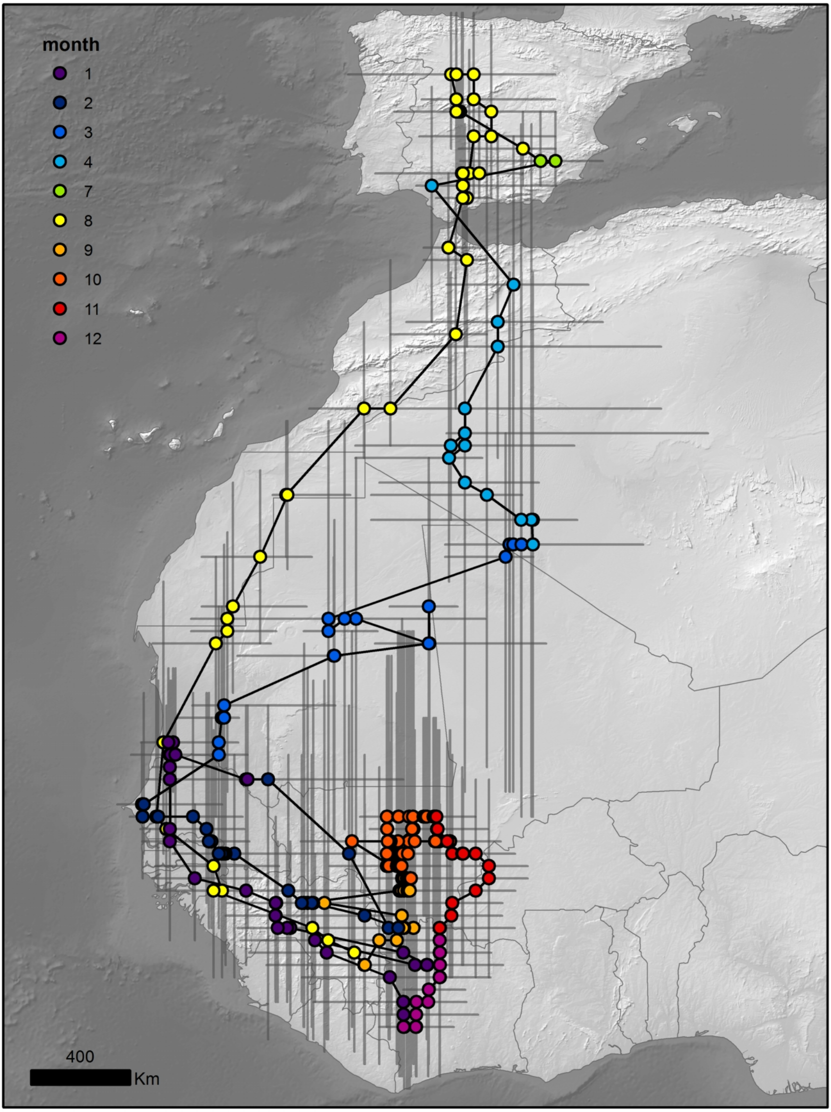

Figure 20. Migration track inferred with light-level geolocators for the bird ringed Y66220 (male breeding in "La Alegría").

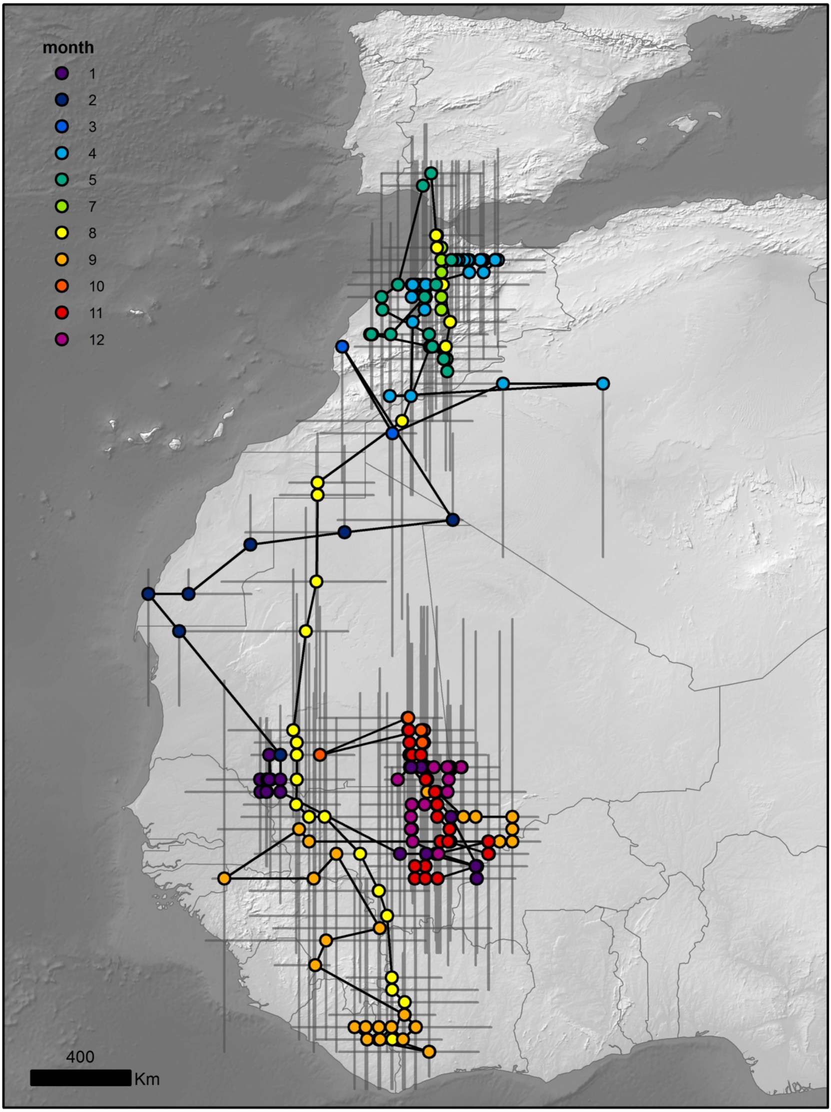

Figure 21. Migration track inferred with light-level geolocators for the bird ringed Y65702 (male breeding in "Las Coladas").

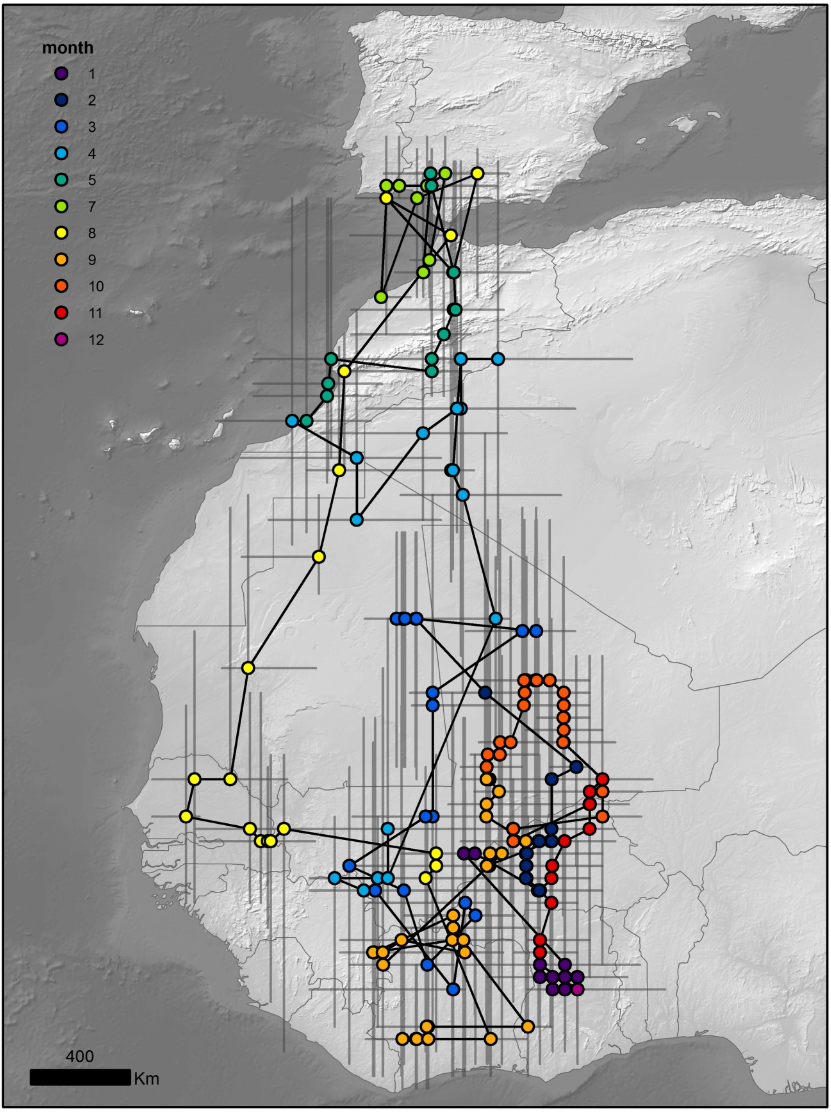

Figure 22. Migration track inferred with light-level geolocators for the bird ringed 1Y83478 (male breeding in "Las Coladas").

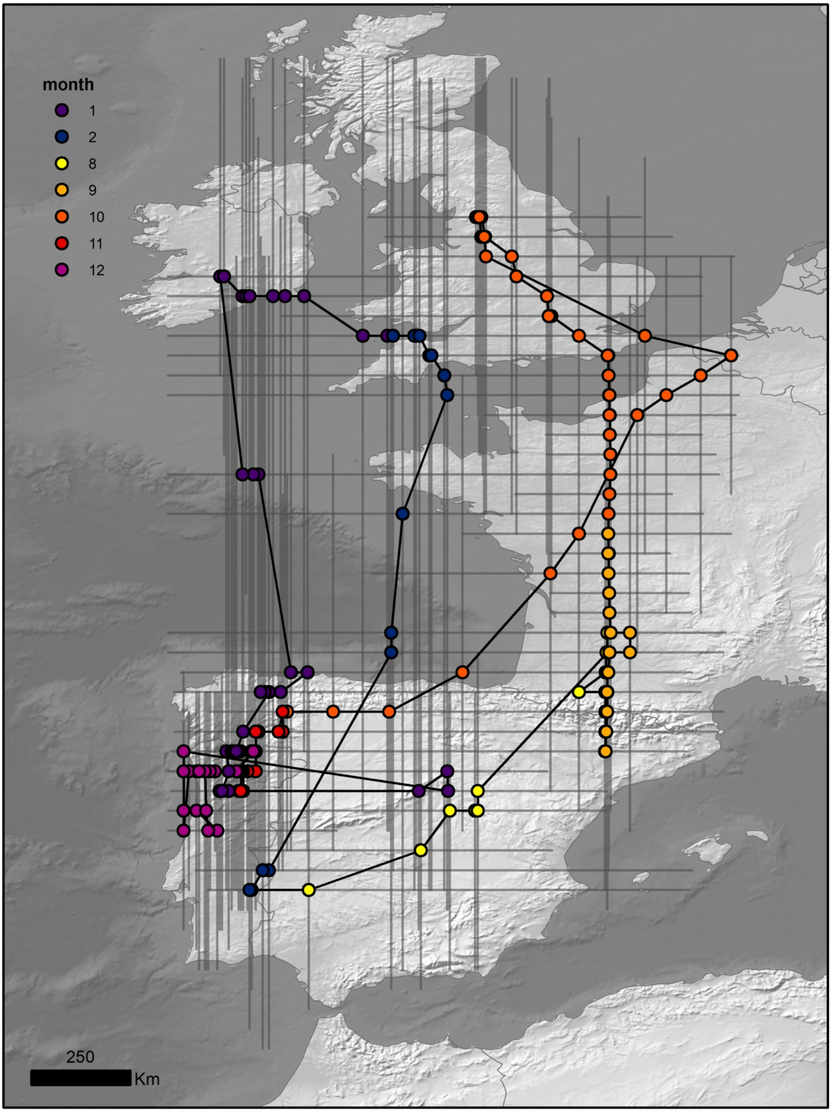

Figure 23. Migration track inferred with light-level geolocators for the bird ringed 2L12590 (male breeding in "Asesera").

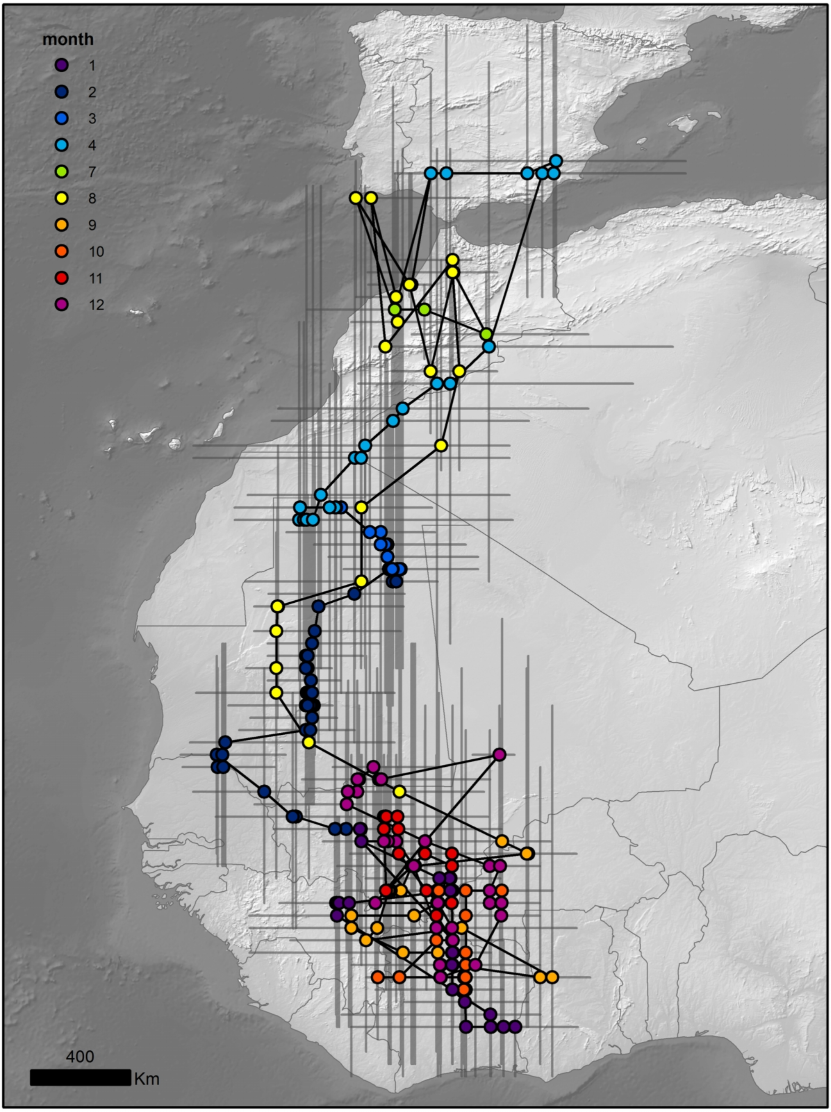

Figure 24. Migration track inferred with light-level geolocators for the bird ringed Y66027 (female breeding in "Las Coladas").

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