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Aves remores: responses of migratory birds to climate change and habitat alteration

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Abstract

Climate change and habitat modification are the main anthropogenic causes of the alarming loss of the worldwide biodiversity. Birds are considered excellent indicators of the consequence of the ongoing global environmental change because they are highly visible due to diurnal lifestyle, mobile, and widespread in almost all habitats. Migratory birds, in particular, are considered particularly sensitive to these changes because they experience divergent patterns of change in ecological conditions in the areas where they spend different phases of their annual life-cycle.

The present dissertation investigates the multifaceted effects of climate change and habitat alteration on migratory birds. In particular, it provides empirical evidence of how migrants are affected by and react to variability in the ecological conditions at non-breeding grounds and during migration journeys.

First, I propose a novel automatized method for representing migration routes through the analyses of mark-recaptures data. The procedure tested on the Barn swallow ring-recoveries was able to reconstruct patterns of migration consistent with previous knowledge on this species. This study thus discloses the possibility to use the method on other less-studied species and emphasized the often-unexploited informative values of ringing data.

Second, I investigate how general ecological conditions (summarized by indices of primary productivity) experienced by Barn swallows in their sub-Saharan non-breeding ranges and during migratory journeys affect the population dynamic of the species. Results indicate that the arrival at the non-breeding ground and at a key passage area along spring migration route were the most critical phases of the annual life-cycle of this long-distance migrant. In a further study based on ring recoveries, I find that, in the partial migrator European robin, the proportion of individuals that migrate, and the distance travelled by birds from migratory population decrease during milder winter temperatures at the breeding grounds. These studies indicate that environmental conditions during non-reproductive season may strongly affect migratory behaviour of both long and short-distance migrants with significant impact on demographical trends and distributions. Moreover, a degree of variability of phenological responses among populations is observed.

Third, through a quantitative meta-analysis of the existing literature, I investigate the effect of livestock farming on farmland birds, a group of birds that are suffering sharp declines in Europe in recent decades. This study confirms that abandonment of livestock has negative effects on Barn swallow abundance and reproduction, but not on other farmland birds. An experimental test of food deprivation on Barn swallow nestlings further confirms that resources availability at breeding sites can alter nestling begging behaviour and intra-brood interactions with potential consequences on their quality.

Finally, I contribute to the characterization of the gut microbiome of the Barn swallow and of the plumage bacteria of the Common swift.

Results suggest that studies of bird microbiomes, despite they are still in their infancy, may be relevant for investigating the ecology of migration.

Riassunto

Il cambiamento climatico e le alterazioni degli habitat sono ritenuti i principali fattori antropici che contribuiscono alla preoccupante perdita di biodiversità osservata a livello globale. Gli uccelli vengono considerati un eccellente indicatore delle conseguenze del cambiamento climatico in atto, a causa della loro estrema visibilità (causata allo stile di vita diurno), della loro mobilità e della loro diffusione in vari ambienti. Gli uccelli migratori, nello specifico, sono ritenuti particolarmente sensibili ai cambiamenti ambientali, poiché, trascorrendo le diverse fasi del loro ciclo di vita annuale in aree differenti, subiscono gli effetti di diversi pattern di cambiamento delle condizioni ecologiche.

Questo lavoro esplora i molteplici effetti del cambiamento climatico e delle alterazioni degli habitat sugli uccelli migratori. In particolare, fornisce prove empiriche di come gli uccelli migratori siano influenzati dalla variabilità delle condizioni ecologiche incontrate sia negli areali non riproduttivi che durante i viaggi migratori e di come vi reagiscano. Si elencano di seguito i temi principali trattati nel lavoro di tesi.

Innanzitutto, si propone un innotivativo metodo automatizzato per rappesentare le rotte migratorie attraverso l'analisi di dati di cattura e ricattura. La procedura è stata applicata su dati di inanellamento delle Rondini e ha consentito di ricostruire modelli migratori coerenti con le conoscenze pregresse di questa specie. Questo studio dimostra, inoltre, la possibilità di applicare il metodo di analisi ad altre specie di migratori meno studiate ed enfatizza il valore informativo dei dati di inanellamento, valore spesso sottostimato.

Secondariamente, si valuta come le condizioni ecologiche generali esperite dalla Rondine negli areali non riproduttivi sub-sahariani e durante i viaggi migratori (condizioni sintetizzate dagli indici di produttività primaria) influenzino le dinamiche demografiche di questa specie. I risultati indicano che l'arrivo nei quartieri non riproututtivi e nelle zone di sosta durante la migrazione primaverile sono le fasi più critiche del ciclo di vita annuale di questo migratore a lungo raggio. In un ulteriore studio basato sui dati di inanellamento del Pettirossi, un migratore parziale, si dimostra che la proporzione degli individui che migrano e la distanza percorsa dagli individui di popolazioni interamente migratrici diminuisce con l'aumento delle temperature invernali nei quartieri riproduttivi. Questi studi indicano che le condizioni ambientali esperite dagli uccelli durante la stagione non riproduttiva possono influenzare notevolmente i comportamenti migratori si a corto e lungo raggio, con un impatto significativo sui trend demografici e sulla loro distribuzione. Inoltre, si osserva un diverso grado di variabilità nelle risposte fenologiche tra diverse popolazioni geografiche.

Si indagano, inoltre, gli effetti delle pratiche di allevamento sugli uccelli di habitat rurali, un gruppo di uccelli che, negli utlimi decenni, stanno subendo declini demografici in diversi paesi Europei. Questo studio viene condotto attraverso una meta-analisi quantitativa della letteratura scientifica disponibile sull'argomento. I risultati di questo studio confermano che la dismissione di pratiche di allevamento ha effetti negativi sull'abbondanza e la riporoduzione delle Rondini, ma non su altre specie di uccelli. Un test sperimentale condotto su pulcini di Rondine sottoposti ad un'alterazione controllata della disponibilità di cibo ha ulteriormente confermato che la disponibilità di risorse nei quartieri riproduttivi può alterare significativamente il comportamento di *begging* dei pulcini e le interazioni sociali entro nido, con potenziali conseguenze sulla loro fitness.

Infine, sono presentati i risultati di due studi innovativi sulla caratterizzazione molecolare del microbioma intestinale delle Rondini e del piumaggio dei Rondoni. I risultati suggeriscono che gli studi sul microbioma degli uccelli possono avere un ruolo determinante nell'aumentare la conoscenza della biologia migratoria degli uccelli.

General introduction

Global biodiversity is declining at an unprecedented rate (Pimm and Raven 2000). Researchers agree that the ongoing climate change and the habitat alteration are the major causes of this alarming process (Parmesan and Yohe 2003, Thomas et al. 2004, IPCC 2014). Among vertebrates, several European migratory birds are suffering alarming demographical declines unequivocally ascribed to the ongoing environmental changes occurring in their habitats (Donald et al. 2001, 2006, Sanderson et al. 2006).

The present dissertation investigates the multifaceted effects of climate change and habitat alteration on bird migratory behaviour. In particular, it provides empirical evidence of how migratory birds are affected by and react to variability in the ecological conditions in the non-breeding grounds and during migration journeys. The focus of the dissertation is on the responses of both long and short-distance migrants, respectively the Barn swallow (*Hirundo rustica*) and European robin (*Erithacus rubecula*), while a further work investigates a different aspect of the biology of a long-distance migrant, the Common swift (*Apus apus*).

In the following paragraphs of this introduction, I will briefly summarize the current knowledge about some aspects of the migration ecology of birds, with are relevant for contextualizing the studies proposed in the next chapters, which constitute the main part of this PhD thesis.

Chapter 1 - General introduction

1.1 Bird migration

Migration is a fascinating natural phenomenon that arouses the interest of observers and researchers since ancient times (Berthold 2001). Although widespread in the animal kingdom (Dingle 2006, 2014), migration is particularly common in birds because of their ability to fly, cross geographical barriers and cover long-distances quickly. Newton (2008) distinguished six kinds of bird movements on the base of 1) the distance covered, 2) the direction(s) followed, 3) the calendar dates and 4) the possibility of a returning journey. The term ‘migration’ often refers to an intentional, bidirectional and seasonal movement of individuals between two specific destinations − the breeding and the non-breeding grounds − that may have very different extent, spanning from very small areas (e.g. a small island) as well as the entire globe (Newton 2008). Each year migratory species move in response to seasonal changes in food abundance and environmental conditions, thus typically breeding at higher latitudes and spending the non-breeding periods at lower ones (Newton 2008).

Migratory birds are commonly classified as ‘short-distance’ migrators − which remain within one biogeographical region throughout their annual cycle − and ‘long-distance’ migrators − which move between different biogeographical regions, often for thousands of kilometres, crossing biogeographical barriers like deserts or seas (Newton 2008). However, a clear distinction between long and short-distance migrants, and even between migratory and resident species is difficult because large variability in migration propensity, distance, timing of migration, and migration routes exists both between and within populations of the same species (Pulido 2007, Newton 2008). Different morphological (e.g. wing length and shape), physiological (e.g. fat accumulation) or behavioural traits (e.g. orientation and navigation skills) contribute to determine the propensity to migrate or stay (Piersma et al. 2005). Hence, the migrant-resident dichotomy should be re-interpreted as a cline in the amount of migratory activity where the resident and long-distance migrators are at the two extremes (Pulido et al. 1996).

As suggested by Newton (2007), the occurrence of migratory species and populations follow different geographical patterns, such as the easily detectable latitudinal trend of increase of migratory birds from equatorial to polar regions in response to increasing seasonal fluctuation of resources. Nevertheless, in some species, a large variability of migratory behaviours is evident among different geographical populations as well as among individuals of different sex and age. Within-species variability in migration behaviour can be roughly divided in differential and partial migration.

‘Differential migration’ can be defined as “that situation in which migration in some distinguishable classes of individuals (ages, sexes, races) differs with respect to timing, distance or both” (Terrill and Able, 1988, p. 206). It is observed in different taxa, particularly in those where the two sexes contribute very differently to reproduction, for example, when the care of offspring imposes to just one parent a long stay at the breeding ground (Newton 2008). Differential migration may regard also the timing of migration, for instance, when older males depart from their wintering grounds before females or younger males in ordered to reach the breeding ground and settle in the best territories. In some species, a differential departure from the breeding grounds of individuals of different sex or age seems to be strictly related to moult timing (Svesson and Hedentrom 1999, Jenni and Winkler 2011). As largely shown for passerines, in the species that complete their moult in the breeding grounds, juveniles replace a relative small number of feathers and then migrate before adults that change the whole plumage. In contrast, in the species that migrate on longer distances and postpone the moult in the wintering ranges, adults advance the autumn migration compared to young individuals, which remain in natal areas for some weeks and often disperse after fledging, until they are ready for their first migration (Svesson and Hedentrom 1999).

The co-existence of migratory and non-migratory (resident) individuals in the same population at the breeding grounds is an interesting example of how bird migratory behaviour may differ within the same species. ‘Partial migration’ is peculiar of birds, especially of those breeding in temperate zones at medium latitudes with mild winters (e.g. the European robin) (Newton 2008). It is possible to distinguish ‘obligate’ and ‘facultative’ partial migrators. In the former case, some individuals of a population always migrate, while other always remain in the breeding grounds throughout their live, independently of the environmental conditions at the breeding grounds during one particular winter. Such condition implies that migratoriness is under genetic control in these species (Berthold 1991, Pulido 2007), and several studies have attempted to demonstrate that the payoffs of the two migratory behaviours are equal, and that natural selection determines the coexistence of the two genotypes in the population over long periods of time (Berthold 1991).

In contrast, in facultative partial migratory species, individuals can shift from being migrators to being residents according to environmental conditions faced in each year. In this scenario, the migration is a ‘condition dependent’ strategy and is interpreted as an individual response to a depletion of food resources and to a resulting increase of intra-species competitions (Pulido et al. 1996, Newton 2008, Pulido 2011). Subordinate individuals (often juveniles or females) take advantage from migrating instead of staying in the breeding ground, where dominant individuals (typically old males) would ‘defeat’ them. In this case, the payoffs of residents and migrators are not equal: the survival probability and reproductive success are usually greater for individuals who do not migrate. Nevertheless, the migratory trait persists in the population because the migration is the better option for subordinate individuals when the selective pressure increases (Pulido et al. 1996, Newton 2008). From an evolutionary point of view, partial migration is a stable strategy (ESS) where advantages of two behavioural traits (residency and migratoriness) are balanced and maintained at population level, despite different selective pressures act on the two category of individuals resulting in not equal payoffs (see: Lundberg 1987, Berthold 1999, Chapman et al. 2011, Pulido 2011 for a review on this topic).

In recent decades, the debate on the evolution of bird migration has focused on ‘partial migration’ (Berthold 1999, Pulido 2007, 2011). Indeed, as suggested by Berthold (1999), partial migration may represent a transition phase between ‘migratoriness’ and ‘sedentariness’. According to his ‘comprehensive theory of bird migration’, the propensity to migrate or to be resident are not mutually exclusive and persist in a population as a gradient instead as a dichotomy. Several evidences support the idea that migratory propensity is mainly under genetic control, while external factors (i.e. intraspecific competition, environmental or climatic changes) can modulate its expression (Berthold 1999). The ‘environmental threshold model’ – originally formulated by Wright (1934) – well explains the evolution and inheritance of bird migratory behaviour and predicts the incidence of residents or migrators in a population. Under this framework, the propensity to migrate is considered a continuous trait, and a threshold determines whether the genetic predisposition to migrate is expressed or not: an individual (or population, or species) migrates (or is resident) only if it is above (or below) the threshold. Any shift of the threshold due e.g. to environmental effects increases or reduces the manifestation of the trait. This theoretical mechanism was inspected and described accurately by several experimental studies that demonstrated that some partial migratory populations can became resident in a few generations (Berthold et al. 1990, Able and Belthoff 1998, Berthold 1999, Nilsson et al. 2006).

In the light of these considerations, partial migratory species are good models for investigating the consequences of rapid climate changes on migratory species because they are expected to quickly modify their migration strategy according to new conditions (Fiedler 2003). For these reasons, one study reported in this thesis will focus on a partial migrant, the European robin (see Chapter 4).

1.2 Migratory connectivity and seasonal interactions

One of the most fascinating aspect of migration regards the long journeys travelled by birds. Since a relevant part of my Ph.D. thesis focused on this topic, I will outline some common features of bird migration on large spatial scales.

The Afro-Palearctic migratory system represents probably the largest and most impressive migration system on earth as it includes about two billion birds migrating from Europe to sub-Saharan Africa and backward each year (Hahn et al. 2009). During these journeys, individuals cross two main geographical barriers, the Mediterranean Sea and the Sahara Desert, where harsh environmental and climatic conditions threat their survival. The migration between Europe and Africa can take two forms: a) birds can fly directly toward the non-breeding quarters with a quick journeys with no long-stopovers (single-stage migration) – it is the case of the Barn swallow and the Common cuckoo *Cuculus canorus*, or b) make detours and stopover at the edges of the desert (i.e. North Africa or Sahel) for resting or, in some cases, moulting, and then move further south to their final wintering destinations (two-stage migrations) – it is the case, for instance, of the Sand martin *Riparia riparia* (see Newton 2008 for further examples).

In most cases, the eco-physiological mechanisms adopted by long-distance migrators for crossing ecological barriers are still unclear. Possible alternatives are: a single sustained flight; intermittent migration with diurnal or nocturnal rests (Schmaljohann et al. 2007); or a mixed strategy chosen in accordance with current conditions (Biebach et al. 2000; see also Bairlein et al. 1983; Bairlein 1985, 1991; Biebach et al. 1986; Biebach 1990; Pearson et al. 1988; Schmaljohann et al. 2006 for a review on these studies).

Migration between distinct biogeographical regions also implies that birds cope with seasonal variability of the ecological conditions in both the breeding and the non-breeding ranges. Hence, it requires a fine synchronization of all life-cycle phases with seasonality, and the ability to tune the migration schedule according to environmental conditions *en route* or at destination (Berthold 2001). Considering the difficulties and constrains imposed by long migrations, all the biological traits distinctive of migratory lifestyle are expected to be more expressed in long than in short-distance migrants (for more details see Piersma et al. 2005).

For several long-distance migrants (i.e. trans-Saharan species), migratory routes and locations of wintering grounds of different geographical populations are still largely unknown, especially among small-sized passerines (e.g. the Barn swallow) (Newton 2008). General descriptions of migration routes and destinations are available for a minority of them, and are based mainly on ringing data or isotopes analyses. In contrast, tracking devices, which provide detailed descriptions of non-breeding areas and migration routes, were applied only on few passerine species. In fact, despite device miniaturization, small sized species still cannot be equipped with satellite or radio tracking devices and hardly even with the smallest devices (light-level geo-locators) currently available (Boulet et al. 2006, Saino et al. 2007, Ambrosini et al. 2011, Ryder et al. 2011). In addition, the cost of these devices often limits their application to few individuals from few geographical populations and in a few years, which further limits our understanding of migration strategies. In contrast, large databanks of ring recaptures are available also for those species that are too small to be equipped even to the smallest tracking devices currently available (e.g. the EURING databank) (du Feu et al. 2016). These data can be quickly put in use for assessing distribution and movements of several bird species, but they are still largely underexploited mainly because the analyses of these data is hampered by several difficulties, the main of which is the large spatial and temporal heterogeneity in sampling effort (Korner-Nievergelt et al. 2010, see below). In chapter 2 I propose a novel and automatizes method for representing migratory routes though the analysis of ring recoveries or sight-resight data. The method was applied to ring recoveries of the Barn swallow in western and central Europe and was able to accurately represent routes followed by birds during both spring and autumn migration. The aim of this work was to allow the identification of migratory movements of birds by a procedure that can be easily applied to a large number of species by analysing data that are already available.

Individuals belonging to distinct geographical populations can follow different routes, schedule the migration journey differently and mix at different extent during winter season. In addition, individuals from the same breeding population seem able to largely shift their wintering ranges from year to year (Liechti et al. 2015). Similar processes occur also in short-distance migrators, but with further complexity. Indeed, the simultaneous occurrence of both wintering and resident individuals in the same area is a common feature in species that show facultative or partial migration. It is quite common that, at a particular date of the year, the same place hosts sedentary, wintering or migrating individuals (Newton 2008).

Although hampered by intrinsic difficulties due to the vast spatial scale at which these processes occur, an accurate knowledge of migration patterns is pivotal for assessing the environmental conditions that birds face during the staging phases of their non-breeding periods (‘wintering’) or during their journeys, because they are supposed to have strong influence on the demographic trends of several European migrant populations (Szép and Møller 2005, Holmes 2007). Indeed, several empirical evidences have revealed that the quality of wintering habitats (e.g. vegetation cover, rainfall occurrence, primary productivity) are the main forces driving bird wintering survival and timing of spring departure, which, in turn, affect recruitment and reproduction in the following breeding season (Peach et al. 1991, Szép 1995, Saino et al. 2004b, 2007). Moreover, the migratory journey is an energy-demanding process and entails the highest risk of mortality within the annual cycle of a bird (Sillett and Holmes 2002, Newton 2008). Hence, the physiological condition of migrants at departure and the food availability at stopover sites are crucial, especially for species that do not feed in flight and cross wide ecological barriers (i.e. oceans and deserts). In chapter 3, I present a study investigating the phases of the annual life-cycle of the Barn swallow that critically affect the dynamic of one breeding population of the species.

The journeys of migrators physically connect geographically separated ecosystems, and the events occurring in one area may reverberate on populations in another distant area days or months later. ‘Migratory connectivity’ refers to the spatial dimension of these connections, as it refers to link between breeding and non-breeding ranges of migratory animals due to migration (Webster et al. 2002, Marra et al. 2006). More specifically, it describes at which extent individuals from the same breeding population overwinter together or, equivalently, how they are geographically arranged in two or more periods of their annual cycle (Webster et al. 2002). A ‘weak’ (or diffuse) connectivity occurs when individuals from the same breeding population move to several non-breeding locations and vice versa. In contrast, a ‘strong’ connectivity occurs when most individuals from the same breeding population move to the same non-breeding location, while a small proportion of individuals spread to other locations (Marra et al. 2006). In chapter 4, I present a study where the analysis of migratory connectivity was a necessary preliminary step for identifying geographical population of the partial migratory

European robin which differ in both migration propensity and migration distance.

Determining the magnitude and patterns of the connections between breeding and non-breeding populations is critical for improving our understanding of the ecology, behaviour and evolution of bird migration (Webster et al. 2002, Webster and Marra 2005, Marra et al. 2006). Indeed, studies on migratory connectivity can contribute to clarify the mechanisms determining population dynamics, life-history strategies, reproductive success and recruitment, evolution of different patterns of migration, genetic flows among sub-populations and, consequently, the adaptability/susceptibility of birds to selective pressures (Webster et al. 2002, Webster and Marra 2005, Marra et al. 2006). More generally, migratory connectivity helps to elucidate the profound implications of seasonal interactions on animals that exhibit migratory behaviour. The concept of ‘seasonal interactions’ indicates *how* and *in what extent* environmental factors and events (i.e. climate and weather patterns, habitat alterations, etc.) occurring at non-breeding grounds have carry-over effects on individuals or populations at breeding grounds (Fretwell 1972). This information, in turn, helps understanding the ecological consequences of rapid environmental changes due to global warming or habitat alterations. Finally, migratory connectivity investigations offer a precious support to conservation and management plans, driving the allocation of resources and the selection of the most important areas of protection (Marra et al. 2006).

1.3 “Winged sentinels”: impact of environmental conditions on avian migrators

Birds are considered so excellent bio-indicators of the ongoing global environmental change (Berthold et al. 1998, Crick 2004, Møller et al. 2004) that they have been nicknamed “winged sentinels” of climate change (Wormworth and Şekercioğlu 2011). This is for several reasons. Birds are present in quite all habitats, and, due to their diurnal habits, they are easy to observe, monitor or capture if compared with other organisms. Centuries of ornithological studies – including ringing activity – have increased the knowledge of their biology, behaviour, distribution and evolution. Often birds occupy high levels in the trophic chain and their distribution therefore mirrors the presence of other more cryptic and difficult to study species. Migrants have a key role in ecological processes by connecting distant geographical ecosystems across the globe. In addition, migrants, particularly long-distance ones, are very sensitive to environmental changes probably because during their life-cycle they experience different patterns of change in ecological conditions (Both et al. 2009, Lehikoinen and Sparks 2010). The phenomenon of global warming is occurring at different rates across the globe, and at higher rate at higher latitudes (Pachauri et al. 2014). This spatial variability further limits the adaptive responses of migrants to environmental changes because populations moving between distant geographical areas are exposed to divergent rates of climate change (Lehikoinen and Sparks 2010).

Some general features of the ongoing climate change and habitat alterations are now discussed, particularly their negative impacts on the biology and behaviour of migratory birds.

1.3.1. Climate change

Climate warming is an unequivocal phenomenon carefully observed and monitored by the scientific community. The Intergovernmental Panel on Climate Change (IPCC) reports a mean increase in the surface temperature by 0.72°C since the second half of the last century (IPCC 2014). This alarming trend shows no sign of decline, and extreme weather events (such as heavy rainfalls, cyclones or droughts) are supposed to become more frequent and severe in the near future (IPCC 2014).

In the northern hemisphere, increasing temperatures have led to a generalized advancement of spring events, such as the leafing or flowering of plants, and to milder winters with significant alterations of the phenology (i.e. the timing of life-cycle events or phases of animals or plants) of several taxa (Tank et al. 2002). Ecologically linked species need to match the timing of their life-cycle events with spring onset by consistently adapting their phenology to the new ecological and weather conditions of their habitats. If the plasticity of birds response is not sufficient to compensate the changes of seasonality, they will suffer an ‘ecological mismatch’ (Saino et al. 2011). A failure to face climate changes may cause large demographical declines of populations and even drive species to extinction (Saether et al. 2004, Møller et al. 2008).

Variability in the responses of long and short-distance migrants

Adaptive adjustments of migration phenology of birds can be a combination of phenotypic responses and evolutionary changes. Several empirical studies suggest that the variability of bird migrations in response to climate changes is largely due to phenotypic plasticity which is supposed to be faster than adaptive evolution (Charmantier et al. 2008; Pulido and Berthold 2004 but see Pulido 2007; Møller 2007). However, a central role of evolution and hereditary cannot be excluded, but these mechanisms are difficult to examine in wild populations and require a lot of data over long periods of times (Pulido and Berthold 2003, Knudsen et al. 2011).

In general, phenological responses to climate changes are more limited and weaker in long-distance migrators than they are in short-distance ones or in sedentary species (Lehikoinen et al. 2004, Pulido and Widmer 2005, Rubolini et al. 2010). This difference may be due to the fact that long-distance migrators are thought to show a low plasticity in their propensity to migrate, since it relies upon a rigid endogenous control (Both and Visser 2001, Coppack and Both 2002). During their entire life-cycle, they undergo different and divergent patterns of changes in ecological and climate conditions and are exposed to different selective pressures that can limit their adaptive responses. Moreover, the observed low genetic variance of migratory behaviour among birds that migrate long-distances may also reduce their micro-evolutionary responses to climate change (Pulido and Widmer 2005).

In contrast, short-distance migrators are considered more flexible in their migration propensity and able to adjust their phenology to current climatic conditions more easily and faster than long-distance migrators. During their annual life-cycle, they are exposed to ecological and climatic conditions that are more similar or, at least, are changing at more similar rates, than those experienced by long-distance migrators, and this may allow short distance migrants to react more rapidly to global warming.

The most common responses of birds to climate change can be schematically divided into an earlier spring migration and breeding, a variation in the timing of autumn migration, a shift in the non-breeding ranges, and a lower propensity to migrate. Below, I briefly examine these responses, which will be investigated in details in the studies on the Barn swallow and European robin proposed in the following chapters.

Earlier spring migration and breeding

A growing amount of evidence from long-term studies revealed that birds are advancing their arrival to breeding grounds and the laying date (Crick and Sparks 1999) in response to the advancement of spring phenology (Sparks et al. 1999, Lehikoinen et al. 2004, Møller et al. 2008). According to Lehikoinen, Sparks, and Zalakevicius (2004), the first arrival date of several Eurasian migrators was estimated to occur 2-4 days earlier in each decade since the half of the 20th century. An optimal synchronization of arrival date with seasonality is fundamental for the breeding success of migrators. Indeed, an early arrival entails the risk of incurring in bad weather conditions and food shortage, while a late arrival entails stronger mate competitions and can reduce the breeding success. Moreover, the timing and progression of reproductive events should guarantee that the peak of food availability coincides with the maximum request of food by the offspring. There are three main strategies that birds can pursue in order to arrive early in the breeding ranges: 1) leaving early from wintering ranges; 2) migrating faster; 3) shortening migratory flights by wintering closer to breeding grounds (Lehikoinen et al. 2004, Newton 2008, Møller et al. 2010).

The year-to-year variation of spring arrival dates seems influenced by several factors. The arrivals of migrators, especially short-distance ones, are mainly correlated with the average spring temperatures in the breeding grounds (Tryjanowski et al. 2013). In additions, weather, climatic and ecological conditions in the wintering grounds and during the migratory flight or at stop-over sites are likely to influence the progression and timing of autumn journey and, ultimately, determined arrivals at breeding quarters. These latter effects are more evident in long-distance migrators, like the Barn swallow whose migratory behaviour has been object of several investigations since a long times.

Variation in the timing of autumn migration

Climate warming can extend the reproductive season of several bird species, shifting the timing of autumn migration and subsequent life-cycle events. The departure toward African wintering ranges seems to coincide with heavy rainfalls and declining temperatures during the late summer (Alerstam and Lindström 1990, Liechti et al. 2015). As observed by Jenni and Kéry (2003), the peak of passage of avian migrators in autumn through western Europe has significantly changed together with the increase of mean autumn temperatures. However, the shift of the departure date from European breeding grounds seem to occur in opposite directions for long- and short-distance migrators (Lehikoinen et al. 2004, Sokolov 2006, Newton 2008). Species that winter to the south of the Sahara generally advanced the autumn migration and, often, the entire breeding season with respect to the past, probably because they take advantage from earlier autumn departures and avoid the dry season during the passage across the desert. In contrast, a general delay of autumn migration and, consequently, a longer breeding season has been observed for short-distance migrants that winter in southern Europe and North Africa, probably because they do not cross large geographical barriers and may thus benefit from a longer stay in the breeding grounds.

The studies conducted so far thus revealed a considerable asymmetry in the impacts of climate changes on long and short-distance migrants, especially while focusing on the migration phenology. Some studies also revealed that short-distance migrants may benefit from a warmer climate because higher mean temperature and milder winters extend their reproductive seasons, conferring them an evolutionary advantage over other species (Both and Visser 2001, Wormworth and Şekercioğlu 2011). The global warming may thus be a serious threat particularly for birds migrating long distances and probably represents one of the main reason for their decline in Europe (Møller et al. 2008, 2010, Saino et al. 2011, Morrison et al. 2013).

Shift of wintering ranges

Bird species can respond to the advancement of spring onset also by shortening the migratory routes and wintering closer to breeding ranges. Higher mean temperatures during spring and milder winters create new suitable grounds at more northern latitudes where individual can spend their non-breeding season. This tendency is evident in several short-distance migrants, while it occurs less frequently in long-distance ones (Newton 2008). Species migrating between two biogeographical regions need to cope with the global polarization of biomes, and the consequent southward shift and contraction of suitable wintering habitats (Barbet-Massin et al. 2012). This phenomenon − another well-documented effect of global change − further increases the distance between suitable staging grounds. In response to this, some trans-Saharan migrators shortened the distance migrated by overwintering in southern Europe since the half of the last century (Fiedler 2003, Newton 2008), or shifted their wintering grounds northwards, sometimes in drier and less suitable habitats (Newton 2008, Ambrosini et al. 2011). An example of this phenological response is provided in the study on chapter 4 on the European robin.

1.3.2. Environmental changes

Beside climate change, alteration of habitats used by migratory birds, and changes in ecological conditions experienced at any stage of their annual life-cycle can severely affect the demographical trends and the population dynamics of several species. Temperatures, rainfalls and duration of daylight are some of those abiotic factors birds are more sensitive to because they affect environment productivity, food abundance (e.g. invertebrates for insectivores and seeds for granivores) and the length of breeding seasons (Grüebler et al. 2008, Newton 2008, Pillar et al. 2015).

In the breeding grounds, ecological conditions strongly affect the survival probability and reproductive performances of individuals as they determine availability of food and nestling sites and suitable rearing conditions for offspring. Mean spring temperatures and, more generally, weather conditions are expected to influence the overall number of clutches laid by breeding adults, the brood sizes, the phenotypic quality of nestlings and, ultimately, the total reproductive output at population level (Verhulst et al. 1995, Hansson et al. 2000, Ambrosini and Saino 2010). A favourable environment during early spring allows migrants to restore their physiological conditions at arrivals from migration journey, and, in on a longer term, to allocate sufficient resources for the next autumn migration (Pilastro and Magnani 1997, Newton 2008).

Several studies (Siriwardena et al. 1998, Chamberlain et al. 2000, Donald et al. 2001, 2006) have highlighted that European populations of migratory farmland birds breeding in intensively cultivated agro-ecosystems have declined in recent decades due to the agricultural intensifications that started in the 50's of the last century. The extensive mechanizations and use of agrochemicals (Campbell and Cooke 1997), the abandonment of traditional farming management (Ambrosini et al. 2002a, 2012a), the loss of hedgerows and hayfields (Evans et al. 2003a, Sicurella et al. 2014) have determined a homogenization and simplification of rural habitats, with a consequent loss of biodiversity, a depletion of food resources and suitable nesting sites (Campbell and Cooke 1997, Wilson et al. 1997, Chamberlain et al. 1999). All these processes negatively affected the breeding and foraging biology of birds (Chamberlain et al. 2000, Bruun and Smith 2003, Fuller et al. 2004), in particular farmland birds that will be the topic of discussion of the review in chapter 5.

Similar mechanisms are supposed to occur in non-breeding grounds and stopover sites, but they have been less investigated because they are still poorly known. In particular, the degradation and loss of habitats within the drylands of the Sahel − which are a main staging area for migrants in the European-African migration system, − seems to concur to the decline of several open-dry avian species migrating to the south of the Sahara (Sanderson et al. 2006, Zwarts et al. 2009). These environmental conditions may thus affect the date of departure from migration, which, in turn, affects arrival date in the breeding grounds, with carry-over effects on reproductive success, fitness and recruitment of individual in the next generation of a migratory population (Berthold 2001, Saino et al. 2004a, b, Newton 2008, Balbontín et al. 2009). In chapter 3, I present a study whereby I investigated the consequences of variation in ecological conditions at the wintering grounds and along migration routes on the dynamic of one Barn swallow population breeding in northern Italy.

1.4 Ringing data in the study of bird migration

In 1899 Hans Christian Cornelius Mortensen equipped some starlings with the first rings (Jespersen and Tåning 1950). Before that date, the knowledge of the movements of the avian species was restricted to some anecdotal evidence and to mere speculations about their migratory routes and destinations, and this kind of study was the prerogative of a few ornithologists (Bairlein 2001). More than one hundred years has passed from that day, and nowadays researchers can rely on huge databases holding millions of ringing data on the majority of the avian species.

Ringing data have unravelled fascinating aspects of bird migrations, such as: spatiotemporal patterns of bird movements, breeding and wintering ranges, stopover sites, migratory routes and directions, and several other characteristics of migratory behaviour (du Feu et al. 2016). Moreover, bird ringing contributes to our understanding of other aspects of avian ecology and evolution, like annual population trends and the adaptation and responses to variability of climate, environment and habitats. Furthermore, retrospective analyses on population dynamics occurring over time are feasible thank to the improvement of statistical methods and analytical tools (du Feu et al. 2016).

The traditional aim of bird ringing is the description of migratory routes made possible by multiple observations of the same individual. The re-encounter data, such as ring recoveries (recoveries comprise live recaptures of birds by ringers, live re-sightings or dead encounters) give the location of an individual at two or more points in time. These data are extensively used in atlases and papers (see BTO 2017 for a recent review on these studies) to describe bird movements, directions of migration, species and populations distribution (Clark et al. 2009). Recently, beside mere qualitative representations, there has been an increased interest in quantitative analyses of movement patterns inferred from ring recoveries. Unfortunately, almost every study based on ring data is affected by the problem of the large heterogeneity of recovery rate ('re-encounter probability' as defined by Thomson et al. 2009) in space and time. In fact, the re-encounter probability is the product of the chance of a marked bird to be found ('finding probability') and to be reported in ringing databases ('reporting probability') (du Feu et al. 2016). The outcomes of the analyses may thus reflect the unequal distribution of observes more than the real presence of the species in an area. For example, biases can arise when dead or live re-encounters are not discerned. If a migrant is more likely to be found dead, a great amount of records is expected to be found for example during the most critical life-phases, such as the arrival from spring migration or during the hunting seasons. In such cases, the large number of recoveries does not reflect the real density of individuals. This 'unbalanced' set of data may provide biased estimate of the phenomena under study.

The assessment of spatial-temporal biases in recovery rate is a subject of current and intense debates among biologists and statisticians worldwide (Lokki and Saurola 2004, Korner-Nievergelt et al. 2010, Thorup et al. 2014). So far, several studies tried to account for this potential source of bias, and several solutions have been proposed using complex and complementary statistical models. Re-running the analyses on different subsets of data or including additional information other than ringing data are some of the most common attempts (some exhaustive examples are reported in Korner-Nievergelt et al. 2010 and Thorup et al. 2014). A recent review (Korner-Nievergelt et al. 2010) suggested four approaches to control for heterogeneity of ringing data. These approaches are: 1) directly measuring re-encounter probability by means of estimates from others source of data (radio or satellite telemetry); 2) modelling the spatial variation of re-encounter probability using environmental covariates, such as human population density or extent of suitable habitats for birds; 3) approximating re-encounter probability to the distribution of observers or ringing stations; 4) comparing groups characterized by an equal re-encounter probability (i.e. geographical populations, age classes). Unfortunately, these approaches cannot be applied in all the cases and a preliminary evaluation of the available datasets and *ad hoc* solutions are often necessary.

Beside bird ringing, new technologies for monitoring bird movements have been developed. These technologies can track the movements of an individual, using radio signals or satellite transmission, and record its positions. This information can then be stored in an internal memory (data logger) of sent to a receiver through radio, GSM network or satellite transmission (Fiedler 2009). These new devices have both advantages and limitations (see above), and still cannot replace ringing activities, but rather allow comparing and integrating different kinds of information concerning one species. In conclusion, bird ringing and bird-tracking technologies are equal partner in ornithological studies to disclose patterns of avian migration (Fiedler 2009).

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1.5 Avian microbiomes

Bateria are everywhere, and colonize the most diparate environments, including other organisms. Any surface and cavity of any metazoan is colonized by bacteria, which often are not simple commensal or parasites, but are mutualist organisms that play fundamental roles in host phisiology (Ley et al. 2008, Archie and Theis 2011, Muegge et al. 2011). The amount of evidence for a role of microbiual communities in shaping the life-history of their hosts is growing quickly in recent years. Most studies focused on species used as models for studies on human health, but evidence of the importance of microbiome in affecting also birds ecology and behaviour is increasing (McFall-Ngai et al. 2013, Hird 2017).

Among the several functions of the microbiota (the ensamble of bacteria that live on all internal or external surfaces of an organism), the gut microbiota is well-known to regulate physiological processes and henance the immune responses, while plumage bacteria can affect the espression of secondary sexual traits, thus indirectly affecting mating choices (Kokko et al. 2002, Grande et al. 2004, Shawkey et al. 2007). Up to date, the majority of investigations have been done on a restricted number of species, often reared in captivity, but investigation on wild populations are increasing (Xenoulis et al. 2010, Wienemann et al. 2011). The structure of bird-hosted microbiotas can be shaped by both endogenous traits − such as sexes or age classes, foraging habits and health status of individuals − and external factors − such as environmental conditions typical of habitats and nest sites, and mating or parent-offspring interactions (Lewis et al. 2016). In migratory birds, the compositions of gut microbiota seems to affect fat deposition rates during stopover and, ultimately, the rate of migration (Lewis et al. 2016).

Recent works by Hirds and colleagues (2014, 2015) stress the importance of the ‘physical space’ – meant as geographical areas − among other abiotic variables, in shaping bacterial communities hosted by birds. Hence, they hypothesize that similarity in the structure of microbiotas among individuals may be a geographic signal of the same origin and localisation. A first empirical evidence to support this hypothesis comes from studies of Bisson and colleagues (2007, 2009) on American migrants. They proved that acquisition of feather bacteria is progressive throughout an annual life-cycle: it begins in the first year at natal breeding locations and reaches equilibrium at the Neotropical wintering sites of these species. They conclude that migration may play a fundamental role in shaping the microbial community of the plumage and may reflect bird distribution. Moreover, differences in the plumage microbial community from samples collected in different area of the wintering range of these species. In conclusion, microbiotas could be useful in clarify patterns of distribution and migration of avian species. In chapters 6 and 7, I investigated this topic by characterization the feather bacteria of Common swift and the gut microbiome of the Barn swallow by high-throughput DNA sequencing, which is considered the best technology available to date for microbiome investigation.

1.6 Begging behaviour

The Barn swallow is not only a model species for studying avian migration, but also a model species for behavioural ecological researches. Besides investigating the migration ecology of this fascinating migrant, during my Ph.D. I also investigate an aspect of the breeding biology of this species, particularly the social interaction among nestlings mediated by the behaviour of producing begging even when parents are absent (‘parent-absent begging’ or PAB).

In recent years increasing attention has been devoted to the effects of the conditions experienced during early phases of life because they can have long-term consequences on the fitness and reproductive success of individuals (Ricklefs 1982, Lindström 1999). The intense social interactions among siblings in the nest, particularly those mediated by ‘begging’ signals (Kilner and Johnstone 1997, Wright and Leonard 2007), play a fundamental role in driving the allocation of resources by parents to offspring in altricial species, with consequences on the quality of nestlings (Saino et al. 2000, Roulin 2004). The begging behaviour typically includes a number of acoustic, behavioural, morphological and chemical displays addressed by young to their parents to solicit the provision of food or other critical resources (Kilner and Johnstone 1997, Cotton et al. 1999), and is considered an honest and reliable signal of need (Godfray 1991).

In some species, like the Barn swallow, the begging is often displayed also when parents are not in the proximity of the nest (i.e. they do PAB), and, for this reasons, it is considered as a misdirected behaviour, caused by low sensitivity in detecting parental visits (Leonard and Horn 2001, Leonard et al. 2005). However, some studies (Roulin et al. 2000, 2009, Dreiss et al. 2010) support the ‘sibling negotiation hypothesis’ according to which PAB displays promote cooperation among siblings. Through PAB displays, the nestlings may communicate to each other their status and willingness to compete for the nest food item delivered by parents and, then, modulate their begging solicitations to reduce the payoff of a sibling competition. This hypothesis was firstly observed, and largely proved in the Barn owl (*Tyto alba*) (see Roulin et al. 2000, 2009), and recently also in the Barn swallow (Romano et al. 2012).

In chapter 8, I report an experimental study on the PAB of Barn swallow nestlings. The aim of this work is to identify proximate factors (such as brood size, age, sex and body mass) that determine the intensity of PAB in a brood under conditions of normal and experimentally reduced food intake, and a possible relation with frequency of parental arrival at the nest and food intake.

1.7 Thesis structures

The first part of the thesis includes three chapters that deal with different traits of bird migration and the adaptive responses of birds to climate changes. In chapter 2, a novel analytical method aiming at representing migration routes of the Barn swallow from ring data is proposed. This work improves the knowledge of the patterns of spring and autumn migration of this species in the part of its range where ring-recoveries are more abundant (i.e. western and central Europe). This analysis aims at contributing to elucidate the migratory patterns of this species across its breeding ranges, whose knowledge is preliminary to further investigations of its migratory behaviour. Moreover, the procedure – whose methodological details are clearly described – can be applied on several other species for which recoveries data are available over large geographical areas.

Chapters 3 and 4 focus more specifically on the influence of climate change on bird migration. The study in chapter 3 aims at assessing the critical phase(s) in the life-cycle of the Barn swallow that may explain the demographic fluctuations of a population breeding in northern Italy. In particular, the relative importance of the ecological conditions at non-breeding, breeding and stopover sites in determining the year-to-year fluctuations of population size is evaluated. In the chapter 4, a study investigating the effect of winter temperatures on the migration of the European robin is reported. The objectives of the study were to assess whether migratory propensity and distances increase in cold winters. The study also investigates whether this species is shifting its breeding or wintering grounds are occurring and if these shifts are occurring to the same extent in all geographical population. These two works, therefore, deal with different aspects of avian migration through the statistical analysis of ringing data and reveal the large potential of ornithological datasets to elucidate complex migratory behaviours on large spatial scales.

The second part of the thesis includes studies aiming at increasing our knowledge of the biology and ecology of some avian migrant. The first study in chapter 5 is a meta-analysis of the effect of livestock farming on the presence of farmland birds, among which several migratory species are included. Changes in zootechnical practices and intensification of agriculture in Europe deeply modified the rural habitats where several migratory birds breed (Donald et al. 2001, 2006). A special focus is given to the effect of livestock farming on the Barn swallow due to the large number of studies published on this species.

Chapters 6 and 7 report the description of bacterial communities associated to two long-distance migratory birds. The first describes the cloacal microbiome of the Barn swallow, while the second the feather microbiome of the Commons swift (*Apus apus*). These two studies were conducted in two breeding colonies in northern Italy, and bacterial communities were determined by high-throughput DNA sequencing of the 16S rRNA gene. Studies about avian microbiomes are still in their infancy, and this thesis can provide only basic and preliminary descriptions of bacterial associated to birds. However, links between avian microbiomes and bird migration ecology can be clearly envisaged by the effect that bacteria can exert on host condition and fitness (Waite and Taylor 2015, Colston and Jackson 2016). In addition, recent investigations have revealed that bacteria may differ among distinct populations and hosts, which are supposed to have a peculiar microbial fingerprint (Bisson et al. 2007, 2009, Hird et al. 2015). Thus, it can be hypothesized that the observed geographical variation in avian microbiomes may be used as an intrinsic mark for assessing the origin of migrants and thus estimating the level of migratory connectivity between breeding and non-breeding quarters. Despite, this hypothesis is currently a mere speculation, one of the study presented here shows some evidences in this direction.

Finally, the study in the chapter 8 regards the begging behaviour and intra-brood competition of Barn swallow nestlings. This study thus focuses on a behavioural trait of a long-distance migratory species.

I close the present dissertation with some general remarkable conclusions arising from all the studies (chapter 9).

The scientific works reported in each chapter have been conducted during my Ph.D. Some papers have already been published (chapter 3, 4, 5, 6 and 8) while other are currently submitted to international journals for publication (chapter 2 and 7). The papers have been reported so to reflect the original published or submitted version as closely as possible, with minor editorial changes concerning the position of figures and tables. At the end of each article, the relative references to the main manuscript are reported. Supplementary materials are included as text in the relative "Supplementary materials" part, while for other types of electronic materials an external link to the journal pages of published articles or to open-directory in google drive is provided.

The references of the "General introduction" and "General discussion and conclusion" are at the end of the thesis.

1.8 Study species

A comprehensive investigation of the consequences of ongoing climate change and habitat alterations on avian migrators would be very difficult to achieve, as it would require enormous amount of time and resources. Therefore, in ornithological researches on these topics some target birds are chosen as *model species*, because they embody general features of migratory birds, thus the outcomes of the studies can be generalized to a wide category of other similar species.

The works reported in this thesis focuses on: the Barn swallow and the Common swift two obligate trans-Saharan migrators, and the European robin a facultative partial short-distance migrator. These birds have been chosen for several reasons. First, they are suitable species for studying bird migration, and several researchers focusing on them are currently present in literature. In particular, the first two migrate on long-distances between Europe and south of Sahara every years, hence are good models for observing the phenological responses of birds to climate change and habitat loss at broad spatial-scales. The third is a short-distance partial migrator that shows a cline in migratory behaviour with the number of migrators increasing with latitudes from north-eastern to south-western Europe

(Cramp 1988, Adriaensen and Dhondt 1990). This species is therefore suitable for studying among-population variations at continental scale. Second, all three species are insectivorous. Since insect availability is strictly linked to seasonality (Wilson et al. 1999), insectivores are supposed to be more sensitive to changes in environmental and meteorological conditions both in the breeding and in the non-breeding ranges and at stop-over sites, than non-insectivorous species (see e.g. Thomas et al. 2004, Conrad et al. 2006). Moreover, the species are closely associated with farmland or urban habitats whose alteration is increasing.

1.8.1. The Barn swallow (*Hirundo rustica*, Linnaeus 1758)

The Barn swallow is a small (17-19 cm; wing-span 32-34 cm) migratory passerine that breeds throughout the Holarctic region from northern subarctic latitudes to the Mediterranean area, with the exception of the arctic tundra and deserts (Cramp 1988, Turner 2006). Six subspecies have been describe: *rustica*, *tytleri*, *gutturalis*, *eryhtrogaster* are obligate migrants breeding respectively in Europe and West Asia, in South Siberia and Mongolia, in East and South Asia, and in North America; *transiva* and *savignii* are resident respectively in Lebanon, Syria, Israel and Jordan, and in north Egypt. The westerly Palearctic populations of the subspecies *rustica* migrate from European and western-Asian breeding quarters to sub-Saharan African wintering quarters.

Individuals are semicolonial: during the breeding season inhabit rural habitats and at a less extend urban areas for foraging and nesting, while for the rest of the year form communal roosts of hundred birds to spend the night. The species, like others hirundines, is an aerially insectivorous and feeds exclusively on preys caught on the wings (Cramp 1988).

Barn swallows is easily recognized by its streamlined body shape, long pointed wings and forked tails due to the elongated outermost feathers. The plumage is steely blue on the dorsal side and rufous to tawny on underparts. The blue crown and face contrast with the chestnut foreheads, throat and chin. The species is characterized by a slight sexual dimorphism with the males having longer tails, larger body size and a more intense colouration of the ventral plumage than females. Juveniles at their first autumn are heavier and paler coloured than adults, and the sexes cannot be identifiable from the plumage until the first complete moult, which occurs in African wintering ranges.

It is a socially monogamous bird, and adults create stable pairs for all the reproductive season from April to August. Females lay one to three clutches of 2-7 eggs each, and the first brood is often larger than the following ones. The incubation of eggs is prerogative of females, while both parents provision offspring with food throughout the nestling period. Adults can occupy pre-existing nests or build new ones in human-made structures such as barns, cowsheds and old houses. Nestlings hatch in 1-3 days, after an incubation of approximately three weeks. It was demonstrated that the hatching asynchrony usually leads to a clear body-size hierarchy among nestlings, and was proved to affect the food allocation by parents and to drive the sibling competition incisively (Cotton et al. 1999). The fledging occurs when nestling have 20 days, but for a few days, they are fed by parents and can return at the nest to spend the night.

The Barn swallow has been chosen as model species in several ecological and behavioural studies because its wide distribution and synanthropic live-style ease the observation and capture of individuals (see Turner 2006). Much concern has been expressed from the ornithologists regarding the population status of the Barn swallows in Europe, where long-term analyses revealed a decline of 34% (1980-2014) that slowed down in the decade 2005-2014 when a decline of 16% was observed (BirdLife International 2017). Although some discrepancy in local trends among countries, researchers agree to attribute the cause of this decline to the climate change and deterioration of rural habitats in breeding ground. As several long-distance migrants highly susceptible to climate change, the Barn swallow populations have recently modified their migration phenology with advancements in spring arrival and laying dates (Rubolini et al. 2007a), earlier autumn migrations (Ambrosini et al. 2014), and northward shift of African wintering ranges (Ambrosini et al. 2012b).

Similarly to other farmland birds, the Barn swallow has been greatly affected by the intensification of farming practises and new-land management (Evans et al. 2003a, b, Ambrosini et al. 2012a, Sicurella et al. 2014) that have occurred since the second half of the last century (Donald et al. 2001). The shift from a traditional agriculture mosaic to large-scale homogeneous and intensively cultivated agro-ecosystems, the remodelling of rural buildings and abandonment of mix-system of agro-zootechniques led to a generalized loss of farmland biodiversity. In particular, the Barn swallow suffers for the reduce in suitable foraging habitats and nesting sites (Møller 2001, Ambrosini et al. 2002a).

The number of studies on species is increasing constantly, also thanks to the EURING Swallow Project. Thanks to the detailed knowledge of its ecology and its popularity, the Barn swallow is now, more than ever before, a global symbol of bird migration and a flagship species for farmland bird conservation.

The Barn swallow

1.8.2. The Common swift (*Apus apus*, Linnaeus 1758)

The Common swift is a medium-sized (16–17 cm; wing-span 42–48 cm), long-lived migratory species that breed in western Palearctic (Cramp 1988). The distribution and consistency of European populations are extremely large, and demographic trends throughout the continent are stable (BirdLife International 2014).

Common swifts are monomorphic, with a black-brown plumage except for white and pale gray chin and throat. Like in others Apodiformes, the legs are small with limited functions, and used primarily for clinging to vertical surfaces. The wings are very long and swept-back, and the tail is short and forked. Because of their crescent-shaped wings, the flying swifts are often confused with Barn swallows and Common house martins (*Delichon urbicum*). This resemblance comes from convergent evolution that reflects similar ecological and behavioral traits, such as their habit to feed on insects caught in flight. The Common swift is a strict aerial insectivorous, and feeds almost exclusively on air-born flying insects (Cramp 1988).

Females lay only one clutch of 2-3 eggs per year with a laying interval of 2 days, and the incubation is of 20-22 days. Both parents share parental duties, including incubation, and feed the nestlings until their fledging, which occurs at approximately 40 days. No post-fledging parental cares have observed (Lack and Lack 1951, Cramp 1988, Carere and Alleva 1998). The Common swifts nest preferentially in artificial cavities of buildings or towers, but natural holes in trees or cliffs may be used.

The Common swifts are well known for being highly aerial, and spend up to ten months airborne also at night (Hedenström et al. 2016). Rarely, they settle on the ground, with the exception of breeding adults that reach the nest sites or some juveniles that occasionally roost in building during bad weather (Holmgren 2004). Because of the aerial lifestyle, the Common swifts are particularly sensitive to meteorological conditions, and can survive adverse weather entering a kind of torpor, which involves considerably slowing their metabolism for a few days (Lack 1956, Lyman 2013). Due to these features, this species has been chosen as a model to investigate the effects of meteorological conditions on nestlings' survival and growth patterns (Lack and Lack 1951, Lack 1956, Martins and Wright 1993) (Lack & Lack 1951, Lack 1956, Martins & Wright 1993, Cucco & Malacarne 1996, Martins 1997).

The knowledge of Common swift migratory behaviour is limited and mainly based on recent information provided by small light-level geolocators. Recent works of Åkesson and colleagues (2012, 2016) on Swedish populations revealed that during the autumn birds cross the western Sahara on a broad front, making longer detours with several stopovers. The spring journey is faster with three alternative routes to cross the desert: western and central ones across the Sahara, and an eastern one on the Arabic peninsula. The majority of Common swifts prefer to migrate along the western routes in order to exploit more fuelling condition (high insect abundance) and tailwind assistance durin spring.

Chapter 1 - General introduction

Two Common swifts in the nest hole: an adult (on the back) and a nestling (on the front).

1.8.3. European robin (*Erithacus rubecula*, Linnaeus 1758)

The European robin is a small (14 cm; wing-span 20–22 cm) passerine bird that breeds in upper and middle latitudes of west Palearctic, from boreal to Mediterranean zones (Cramp 1988).

The adults are monomorphic with grey-brown dorsal and whitish belly plumage and a distinctive orange-red breast, that lack in juveniles. It is a socially monogamous species and fiercely territorial, especially males. The breeding pairs lay two-three clutches of 4-6 eggs. The incubation of eggs is prerogative of females, while both parents provision offspring with food throughout the nestling period until the fledgling occurs at about 14 days after hatching (Cramp 1988). The European robin is insectivorous and takes its prey on the ground, although during winter it can also feed on seeds and small fruits (Cramp 1988).

The European robin is a partial migrator. The populations breeding in north-eastern Europe are completely migratory, with females migrating farther than males, while in the southern of the continent they are largely sedentary (Cramp 1988, Adriaensen and Dhondt 1990, Remisiewicz 2002). This characteristic allowed the investigation of phenotypical differences among populations in migration patterns, and of the effects of climate change, which was hypothesized to be a major driver of such differences in migration behaviour.

The widespread presence of European robins across the continent has allowed the collection of about 150.000 ring recoveries over the last 70 years, which are stored in the EURING Data Bank. The population are not suffering demographic declines, as the consistency of its populations appeared to be increasing throughout its distribution range in the last decades (IUCN 2014).

The European robin

PART I

Chapter 2

Representing migration routes from re-encounter data: a new method applied to ring recoveries of Barn swallows (*Hirundo rustica*) in Europe

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2.1 Abstract

Bird ringing was established more than a century ago to gather information on bird movements. Since then, ornithologists have systematically collected huge databases of records of birds ringed and subsequently re-encountered, but, to date, there have been few attempts to identify migratory routes from ringing data. Here, we develop a novel, quantitative method for describing migration routes using ringing data and we applied it to a dataset of 72,827 ring recoveries of Barn swallow *Hirundo rustica* through western and central Europe from EURING and SAFRING databanks spanning 1908-2011. We considered movements of 332 individuals during spring migration and 1509 during autumn migration. The results indicate that, in spring, Barn swallows enter western Europe through Gibraltar or by crossing the Mediterranean Sea through the Balearic Islands, Sardinia, and the Italian peninsula. They then spread over a wide front. In northern France, Belgium' and the Netherlands routes diverge, pointing either toward the British Isles or Denmark and Scandinavia. Autumn migration routes are similar to those in spring. The general migration pattern that emerged from the analyses was consistent with previous descriptions of migratory movements of this well-known species. However, this analysis also revealed some previously undocumented migration patterns. For instance, in spring, some migrants moved from the Balearic Islands to Corsica and Italy, thus making a rather long eastward crossing of the Mediterranean Sea. In autumn, some migrants moved from the Balkan Peninsula westwards toward Italy.

Analyses restricted to recoveries within the same spring or the same autumn and to birds found dead showed similar patterns. Our procedure was therefore able to identify migration patterns over a large geographical area, and may be extended to those species for which large datasets of ring recoveries or sight-resight data are available.

2.2 Introduction

Every year, about 2.1 billion birds migrate between Europe and Africa (Hahn et al. 2009). Migratory birds connect ecosystems and communities across continents, and play fundamental roles in ecosystem functioning (Bauer and Hoye 2014). They are therefore important components of the animal communities of entire continents and detailed knowledge of their migration routes is fundamental to understand their ecology and population dynamics and to plan conservation actions, particularly for those migratory species that are suffering sharp declines worldwide (Robbins, Chandler et al. 1989, Baillie 2001, Morrison et al. 2013). Despite much work has been devoted to migration research (see e.g. Weimerskirch et al. 2006, Combreau et al. 2011, Åkesson et al. 2012, Sergio et al. 2014, Hallworth et al. 2015, Stanley et al. 2015), detailed knowledge of migration route is scant for the vast majority of species, particularly small-sized ones that cannot carry even the smallest tracking devices that are currently available (Robinson et al. 2010, Bridge et al. 2011). In addition, most tracking studies focus on relatively small samples of individuals, often from one or a few populations (Fiedler 2009) because of the cost of these devices and the impact they can have on tagged individuals (Costantini and Møller 2013, Scandolara et al. 2014, Morganti et al. 2017, but see Peterson et al. 2015, Wijk et al. 2016). Finally, tracking devices cannot yet provide information on long-term changes in migration patterns, which is a fundamental information to understand current responses to climate and other environmental changes. Ringing data therefore remain a valuable and powerful tool in the study of migration ecology of many bird species (Rubolini et al. 2002, Baillie et al. 2009, Ambrosini et al. 2011, 2016).

In Europe, the EURING databank hosts over 10 million records (www.euring.org/data-and-codes) (du Feu et al. 2016). Such large data sets provide information on the spatial and temporal distribution of species and populations and have proven useful in analyzing, for instance, the degree of clustering of breeding and wintering populations (i.e. migratory connectivity) (Ambrosini et al. 2009, Korner-Nievergelt et al. 2012), dispersal patterns (Paradis et al. 1998), shifts in breeding and wintering ranges (Visser et al. 2009, Ambrosini et al. 2011, Lehikoinen et al. 2013), and timing of migration (Ambrosini et al. 2014).

The original aim of bird ringing was to gather information on bird movements (Mortensen 1901, Greenwood 2009), but this method does not generate information on long-distance movements efficiently, as the low percentage of individuals recovered compared to the those ringed show (Baillie 2001, Anderson and Green 2009, Thorup et al. 2014). Moreover, the analysis of ring recovery data is complex because of large spatial and temporal variability of both ringing effort and recovery probability (Thorup et al. 2014). For this reason, the interpretation of ring recoveries to assess migration directions and movements has been mostly qualitative. Von Lucanus (1919) first reviewed recoveries of ringed birds, and, based on their spatial distribution, identified several major migratory routes across Europe (Bairlein 2001). Since then, several analytical tools have been developed for the analysis of movements based on ring recoveries (Kania and Busse 1987, Pilastro et al. 1993, Fiedler 2009). For instance, van Noordwijk (1995) suggested accounting for the spatial and temporal distribution of observers in order to obtain unbiased estimates of dispersal distances. More recently, capture-mark-recapture models at state/national scale have been applied to assess transition probabilities among specific locations (e.g. Korner-Nievergelt et al. 2014, Thorup et al. 2014), while a Europe-wide analysis of timing of migration has allowed inferring the main migration pathways of the Barn swallow (*Hirundo rustica*) (Ambrosini et al. 2014).

To date, most studies and recent national atlases that used ringing data to describe bird migration routes have relied almost entirely on visual interpretation of maps of ring recoveries (e.g. Spina and Volponi 2008a, b, Saurola et al. 2013, Bairlein et al. 2014, Thorup et al. 2014, Valkama et al. 2015, but see Wernham et al. 2002 for a more quantitative treatment). Although we are fully convinced that experienced ornithologists are able to provide reliable interpretations of ringing data, we argue that quantitative, fully reproducible procedures should provide a more objective way to assess migration routes from ring recoveries.

In this paper, we propose a new, automated and repeatable procedure for representing migration routes based on dead recoveries or live encounters of ringed birds, or any other type of information where the positions of the same individuals were recorded at different times during their movements (including resighting data). We apply the method to a large dataset of ring recoveries hosted at the EURING and SAFRING databanks for a long-distance migrant, the Barn swallow. The procedure provided quantitative and repeatable identification of the main migration directions during both spring and autumn migration through western and central Europe.

2.3 Methods

Study species

The Barn swallow is a small passerine breeding in the Northern hemisphere. European populations winter in Africa, south of the Sahara desert. Barn swallows exhibit high fidelity to their breeding quarters (Turner 2006), although the location of the wintering grounds of geographical populations breeding in different parts of Europe is less well known. The distribution of ringing data suggests the existence of distinct sub-Saharan wintering grounds for the different breeding populations: those from Great Britain, and northern Europe mostly winter in southern Africa, while those from Italy, Switzerland, southern France and Spain migrate to central and western Africa (Saino et al. 2004, Turner 2006, Ambrosini et al. 2009, Hobson et al. 2012). Finally, ring recoveries from Fenno-scandia indicate a migratory divide, with birds breeding in Sweden and Norway migrating through France, Italy and Spain, while those breeding in Finland move through Eastern Europe and the Balkan peninsula (Thorup et al. 2014).

Datasets

Ringing data for Barn swallows were obtained from the EURING databank (du Feu et al. 2016) and from SAFRING (Underhill and Oately 1994). Overall, our dataset included 72,827 records of birds ringed and re-captured or found dead (in any circumstance, including natural and man-induced causes e.g. trapped or hunted birds) (‘ring recoveries’ hereafter), gathered between 1908 and 2011. Records include information on date and locality at ringing and at any subsequent encounter of the birds, but only for those that have been re-encountered. Information on birds that were ringed but not re-encountered was generally not available because most of these data are not computerized. Most ring recoveries were from western and central Europe (i.e. from the Iberian Peninsula to Finland, Baltic States, Poland, Czech Republic, Austria and Hungary; Figs 1 and 2).

The data required for the analyses using the method described in this paper are individual identification code (e.g. ring number), coordinates (latitude and longitude in decimal degrees) and date when each individual was recorded. In addition, records should relate to one period of migration. For instance, data used for analysing spring migration should only refer to the months when the study species performs its pre-nuptial migration. However, records from stationary periods before and after migration can be included as they can represent the origin or the final destination of birds. An individual can be included in the analysis if it is recorded twice during one period of migration (e.g. twice during the months of spring migration or during migration and the stationary periods preceding or following spring migration) even if it has been re-encountered in different years. For our analyses of Barn swallow migration, all records collected during January to July were used for modelling pre-nuptial (‘spring’) migration routes (‘spring dataset’ hereafter, n = 20,363 records), while records in August-December were used for modelling post-nuptial (‘autumn’) migration routes (‘autumn dataset’ hereafter, n = 21,644 records). Thus, the dataset included data collected in months when Barn swallows are largely stationary on the breeding and wintering grounds (Turner 2006).

When multiple recoveries of the same individual at the same coordinates are available, only one is considered. Multiple criteria for selection are possible, but we emphasise that this arbitrary choice should have little effect on the outcome of the procedure since the most relevant information for the analysis is bird identity and locality of ringing and of recovery. For this analysis, we selected the most recent record. An alternative criterion applicable to datasets of species with more than one re-sighting per individual could be to prefer records in those year for which other records of the same individuals at different coordinates are available, or to prefer records during non-stationary periods.

Large variation in ringing effort could potentially bias the spatial and temporal distributions of ring recoveries (Thorup et al. 2014) and, consequently, also the outcome of our analyses. We thus calculated weights to account for variation in ringing effort. In addition, we applied our method to birds recaptured alive and found dead separately, because the second dataset should be less sensitive to variation in ringing effort.

Comparison of the results should therefore provide an indication of how sensitive our conclusions are to ring reporting bias. Finally, we re-ran the analyses by selecting only recoveries of the same individuals that occurred during the same 'migration event', i.e. either during the months of spring or of autumn migration in a given year. Although this selection reduced our sample size to 202 records for spring and 1,942 for autumn migration, it has the advantage that patterns of migration inferred from recoveries during the same migration event are insensitive to potential biases that may have arisen if individuals vary their migration routes between years (see also the Discussion).

Data analysis

Below we provide an outline of the new method we devised for representing migration directions and migration routes of Barn swallows across western and central Europe from ringing data. An extended description of the procedure with full technical details and an annotated script implemented in R 3.2.1 (R Core Team 2013) are provided in Supplementary materials Appendices 1 and 2, respectively. The procedure can be schematically divided into three parts: 1) identification of individual movements; 2) creation of a geographical grid and identification of migration direction(s) at each cell of the grid; 3) flyway simulation.

Identification of individual movements

The movements of each individual ('individual movements' hereafter) are first reconstructed by connecting the positions occupied by an individual during one migration period (i.e. during spring or autumn migration separately). Positions of birds recaptured within the same year are connected chronologically. In contrast, records of the same bird in different years are connected by a clustering algorithm which assumes that individuals move northwards during spring migration and southwards during autumn migration, independently of the date when a bird was recovered (Fig. 3; see Supplementary material Appendix 1 for full details). In addition, the procedure allows further options (Appendix 1). In particular, movements between positions at approximately the same latitude can be retained or discarded, because their direction is unclear if recaptures are not within the same migration event. Movements in a direction opposite to that expected for the migration period under study can also be discarded because they may represent vagrancy or pre-migratory movements rather than 'true' migration. For the analyses of the Barn swallows, we discarded movements between positions that differed in latitude less than 0.2° (4.1% of movements for spring and 1.9% for autumn migration) and movements in a direction opposite to that expected (e.g. we discarded southwards movements during spring migration, 5.5% and northwards movements during autumn migration 7.4%). Although some individuals may have actually moved in a direction opposite to the main seasonal migration direction (e.g. to avoid adverse weather conditions), such movements should not be relevant to the general patterns of Barn swallow migration across Europe. The procedure also allows setting a lower and an upper limit to the length of movement to be retained, which is calculated as the loxodromic distance between the starting and ending position of each movement. For instance, in the analysis of the Barn swallow, we discarded encounters of the same individual at less than 100 km because they may reflect dispersal or vagrant movements (Ormerod 1991, Turner 2006) rather than true migration, and encounters at more than 800 km, a distance slightly longer than a direct crossing of the Mediterranean Sea (e.g. from Northern Algeria to Southern France), because we considered that movement between them could not be reasonably approximated by a straight line connecting these positions (Ambrosini et al. 2014). Obviously, all the above options can be set to different values depending on the specific assumptions, goals and species concerned by the study.

All Barn swallow movements satisfying these criteria, and falling within the geographical limits 19° W - 44° E longitude, 20° N - 68° N latitude (‘study area’), were included in the analyses. Only two movements within South Africa were outside this area and were excluded because they were too few for providing any information on migration routes in this part of Africa. The final dataset thus included movements of 332 individuals during spring migration and 1,509 during autumn migration. Table A1 in Supplementary material Appendix 1 summarizes the values of all the parameters used for this analysis.

Geographical grid and migration directions

The study area is divided into cells and all movements intersecting each cell are associated to the cell. Hence, each movement can be associated to more than one cell, and each cell can have a set of associated movements. Cell size is chosen arbitrarily by the investigator. For the analyses of the Barn swallow, we used cells of 0.25° × 0.25° latitude × longitude because this cell size allowed: 1) a detailed description of migration patterns of this species; 2) maximization of the extent of the geographical area covered by the analysis; 3) a reasonable computation time (which increases non-linearly with cell number; see Supplementary material Appendix 1, Table A2). We note that analyses re-run with cells of different size (0.5° × 0.5° and 1° × 1°) gave similar results (see Results).

Azimuth direction of each movement (i.e. the clockwise angle between the North and the direction of the movement in degrees) is then calculated. Then, the directions of all movements crossing a given cell are combined to provide a mean cell-specific migration direction. To reduce the effect of spatial heterogeneity of recoveries and to evaluate the contribution of each movement to the mean cell direction, three weights are estimated. The first is the 'effort' weight, and is equal to the total number of records in the cells where the individual was observed during a given migration period (i.e. including those records that did not produce a movement). This weight thus improves the contribution of movements connecting cells with a large number of records because they should represent several Barn swallows moving between the cells. Admittedly, this weight only partially corrects for spatial heterogeneity in ringing or recovery effort, but we stress that a proper measure of ringing or recovery effort is simply unavailable for all western and central Europe. The second is the ‘length’ weight, which is inversely proportional to the length of the movement. Indeed, the direction actually followed by a bird moving between two locations is estimated with greater accuracy for shorter than for longer movements (see Supplementary material Appendix 1 for details). This weight was developed by considering that we cannot be sure that a bird actually moved straight from the starting to the ending position of a movement. However, it is more likely that it really crossed a cell close to positions where it was actually observed, than one half-way. This ‘distance’ weight thus assumes maximum value at the cells where a movement begins and ends, and minimum values at the cells at the middle of a movement (see Supplementary material Appendix 1 for details). Thus, the effort and the length weights have a constant value for each movement, while the distance weight changes its value for each cell crossed by a movement. The investigator can choose whether to include or exclude each of these weights as well as how they are combined to calculate the final weight for each movement at each cell. Options for combining weights are to sum or to multiply them. Summing allows weights to “compensate” each other, and was the option chosen for the analyses of the bardn swallow, while multiplying emphasizes the effect of small values (see Supplementary material Appendix 1 for details). Including weights can be computationally demanding for large datasets (see Supplementary material Appendix 1, Table A2).

Migration direction at each cell is then calculated as the (weighted) mean circular direction of all movements associated with the cell, if a cell has fewer movements than an arbitrary chosen threshold (five for the Barn swallow). In contrast, if a cell has more associated movements, a cluster analysis is run by the *pam* procedure in the cluster library (Maechler et al. 2016) in R to identify whether movements are all in a same direction or not (see Supplementary material Appendix 1 for details). If the clustering procedure reveals ‘weak’ or no clustering structure in the data (*oasw* value < 0.5), or the mean circular directions of the two clusters do not diverge much ($< 45°$ in the analyses of the Barn swallow), the cluster analysis is discarded and the circular mean direction is calculated for all the movements associated with the cell. In contrast, if there is substantial or strong clustering structure in the data (*oasw* value ≥ 0.5) and the mean circular directions of the two clusters diverge ($\geq 45°$ for Barn swallows), both directions are associated with the cell. Hence, either one or two mean migration directions can be obtained for each cell. Cells with double directions represent areas where flyways diverge (i.e. migratory divides), cross, or converge.

The procedure finally produces a map (‘direction map’), a table with estimated mean migration direction(s), its variance and number of movement at each cell, which can be used for further analyses, and one with summary statistics about the dataset and the number of discarded positions or movements. These are the main results from the analysis.

Scripts for exporting these maps to shapefiles or Google Earth™ files are also provided in the Supplementary material Appendix 2.

Route simulation

The last step of the procedure produces maps of expected pathways followed by a bird during migration (‘flight routes’ hereafter) to facilitate the visual interpretation of direction maps. This step can be useful, for instance, for assessing flyways followed by birds breeding or wintering in different geographical areas. In the case of the Barn swallow, we simulated the flight routes of (hypothetical) individuals breeding in 1) the United Kingdom and Ireland; 2) Scandinavia; 3) Germany, Belgium and the Netherlands; 4) Italy, Austria, Switzerland and Slovenia. In addition, the number of simulated birds from each country can be set. For instance, we set the number of simulations from each country proportional to the estimated size of the Barn swallow population breeding there (BirdLife International 2017) (see also Supplementary material Appendix 3). Google Earth™ maps of simulations extended to all countries in western and Central Europe are available in Supplementary material Appendix 4.

Each simulation starts from a randomly chosen position within the area and moves through the study area according to the direction(s) represented in direction maps until the simulated bird exits the study area or moves across a given number of empty cells (64 for the Barn swallows, corresponding approximately to the length of a direct crossing of the Mediterranean). Simulations can be run by considering a random direction at each cell with double directions or by considering either the easternmost or the westernmost direction at each cell with double directions. Random choice of direction at each double-direction cell produces simulated flight routes with potentially unnatural zigzags, while fixed choice of direction does not, and appears to be biologically realistic for bird species where migration behaviour is controlled genetically. Flight routes can also be smoothed to further reduce changes in direction (see Supporting material Appendix 1). Finally, simulations can be run “backward” while starting from positions in the final destination of birds and then moving according to a direction opposite to the mean direction at each cell (see Supplementary material Appendix 1 for further details). Backward simulations allow the production of reasonable representations of flight routes when information on the species distribution, abundance and movements at the starting location of migration is poor. For instance, we opted for the backward procedure for simulating spring light routes of European Barn swallows because information on the actual distribution of Barn swallows in Africa is very coarse due to lack of ring recoveries.

2.4 Results

Spring migration

Ring recoveries identified 332 movements during spring migration (one movement per individual), with most movements in the UK and central Europe, where the density of ring recoveries is highest (Fig 1). Google Earth™ maps of Barn swallow movements and of migration directions are available in Supplementary material Appendix 4, while Fig. 4 shows migration directions for representative geographical areas. Overall, 4,388 cells were intersected by at least one movement during spring migration. Cluster analyses on the 213 cells crossed by five or more movements during spring migration indicated the presence of two clusters in 112 (2.6%) cells, which were therefore considered double-direction cells, while the remaining 4,276 cells (97.4%) were considered single-direction cells. Double-direction cells were concentrated in northern Corsica, central and northern Italy, England and Wales, northern France, and Belgium (Fig. 4a-d). In addition, migration directions seem to converge in Denmark (Appendix 4). Some individuals crossed the Mediterranean from Balearic Islands to Corsica or Italy (Fig. 1).

Flight route maps suggest that Barn swallow populations breeding in different parts of Europe migrate following different routes. Birds breeding in the UK and Ireland (green lines in Fig. 5) seem to enter Europe through Gibraltar and then move toward the Atlantic Ocean and western France. From there, some individuals appear to head directly towards the southern coast of the British Isles by crossing the Channel, while others reach northern France, Belgium and the Netherlands and then turn west towards England. From south-central England, birds move northward to Scotland on a wide front, while they reach Ireland by leaving the coast of Wales and southwest England. Barn swallow populations breeding in Belgium, the Netherlands and Germany (red lines in Fig. 5) enter Europe from the north-western African coast, especially from Algeria. They then cross the Iberian Peninsula by moving along the eastern coast or through the Balearic Isles to reach France and central Europe. France and Germany are crossed over a wide front, as suggested by the several parallel routes. Some simulated flight routes suggest that other individuals pass through the Italian peninsula from Corsica, Sardinia or Sicily. In central Italy, some birds head eastwards toward Slovenia to reach their breeding grounds in eastern Germany, while others cross the Alpine chain (Fig. 5), apparently along different routes. Probably birds take advantage of large valleys (e.g. the Ticino valley) as indicated by a cluster of cells with double arrows in this area (Fig. 4d). Unfortunately, the resolution of our analysis does not allow detailed identification of other passage areas across the Alps.

Simulated flight routes of Barn swallows breeding in Scandinavia (orange lines, Fig. 5) show that several routes converge in Denmark, suggesting that Barn swallows tend to avoid a long sea crossing. One spring simulation suggests that some individuals may follow a more easterly route (Fig. 5).

The simulated flight routes of Barn swallows breeding in Italy, Switzerland, Austria and Slovenia (pink lines Fig. 5) also partially overlap with those of the German populations (red lines). The maps highlight Italy as another favoured entry point into Europe. In central Italy, routes diverge with individuals pointing east toward the Balkans or north toward the Alps.

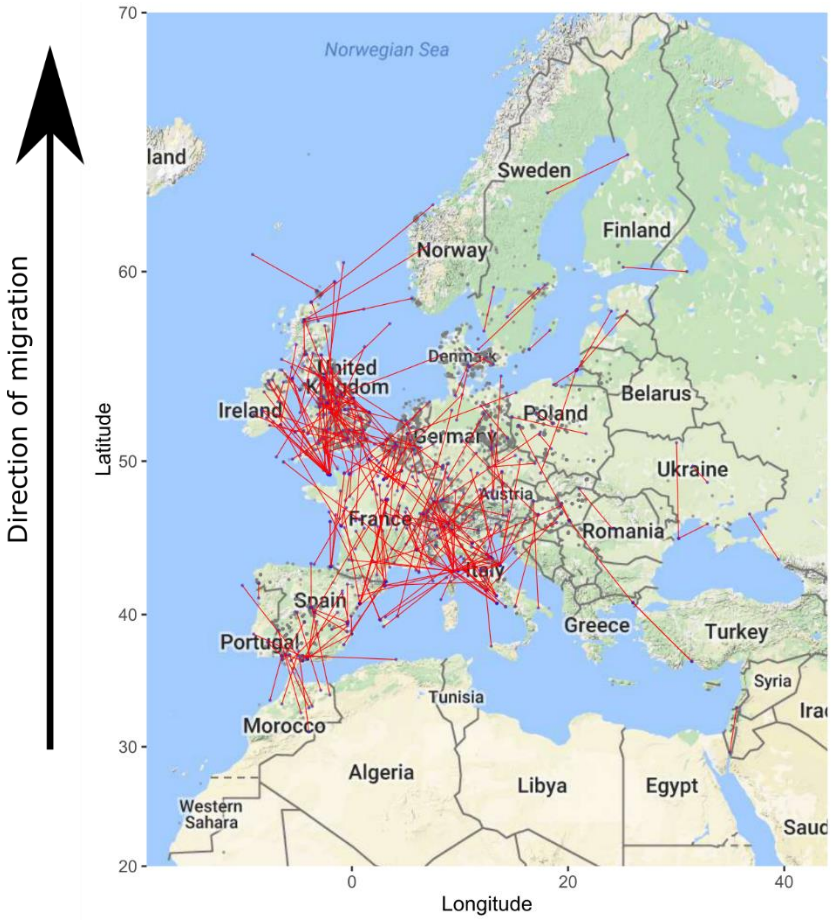

Figure 1. Spring movement map. Red lines represent movements connecting recovery positions of the same individual Barn swallows. Grey points represent all ring recoveries during January-July while blue points indicate ring recoveries connected by movements.

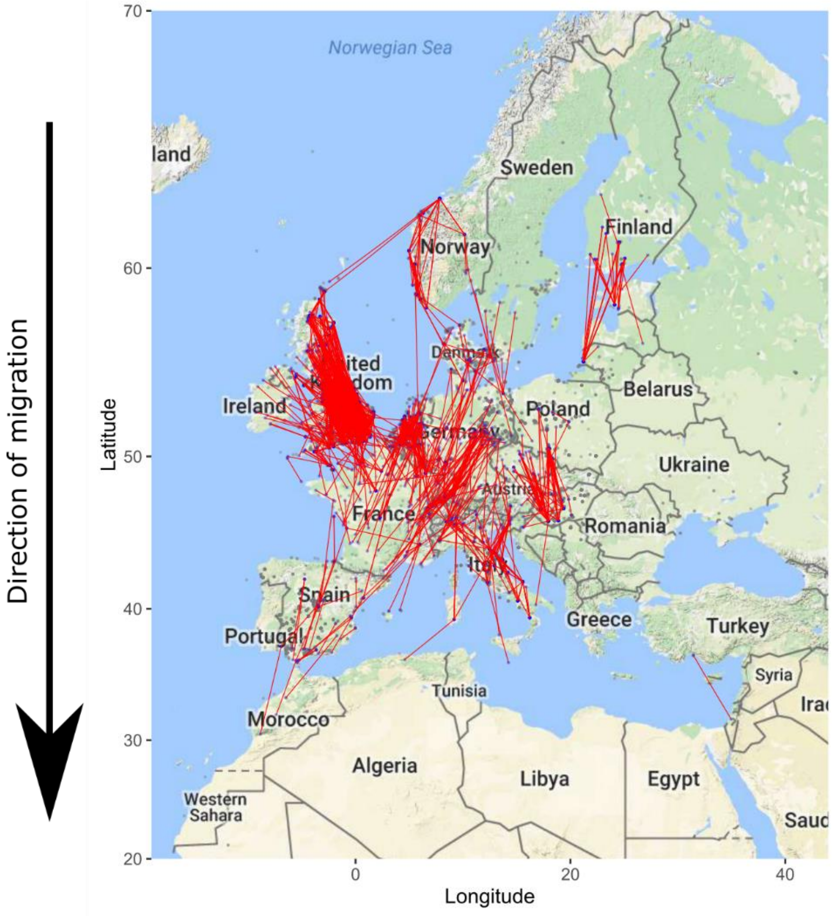

Figure 2. Autumn movement map. Red lines represent movements connecting recovery positions of the same individual Barn swallows. Grey points represent all ring recoveries during August-December while blue points indicate ring recoveries connected by movements.

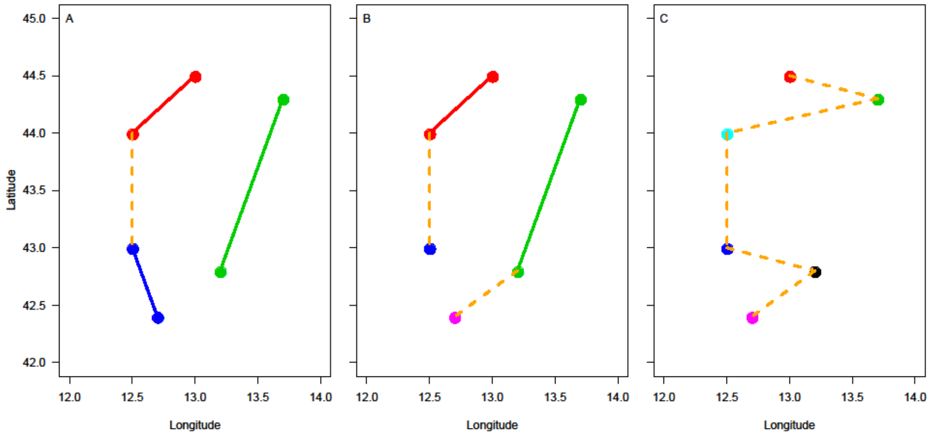

Figure 3. Schematic representation of the output of the clustering procedure used to connect individual positions. Dots represent the positions where an individual was observed. Dots of different colours represent observation of the same individuals in different years. Solid lines connect positions where an individual was observed during the same year. Dashed lines represent movements reconstructed by the clustering algorithm. In A) the individual was observed twice in each year. In B) it was observed twice in two years and once in other two years. In C) it was observed once in each year. Note that, despite positions are the same in all panels, they are differently connected by the procedure, and that multiple observations in the same year are always connected by a movement.

Figure 4. Details of direction maps. Detailed maps of spring migration focus on British Isles (A), France (B), Italy (C), central Alps (Ticino valley) (D), while details for autumn migration focus on British Isles (E), Italy, Switzerland, Austria and Hungary (F) and Germany, France, Netherlands, Belgium and Denmark (G). Arrows represent the mean direction of movements crossing a cell. The blue arrows are drawn in cells with one direction; red and orange arrows are respectively the easternmost and the westernmost direction in cells with double directions.

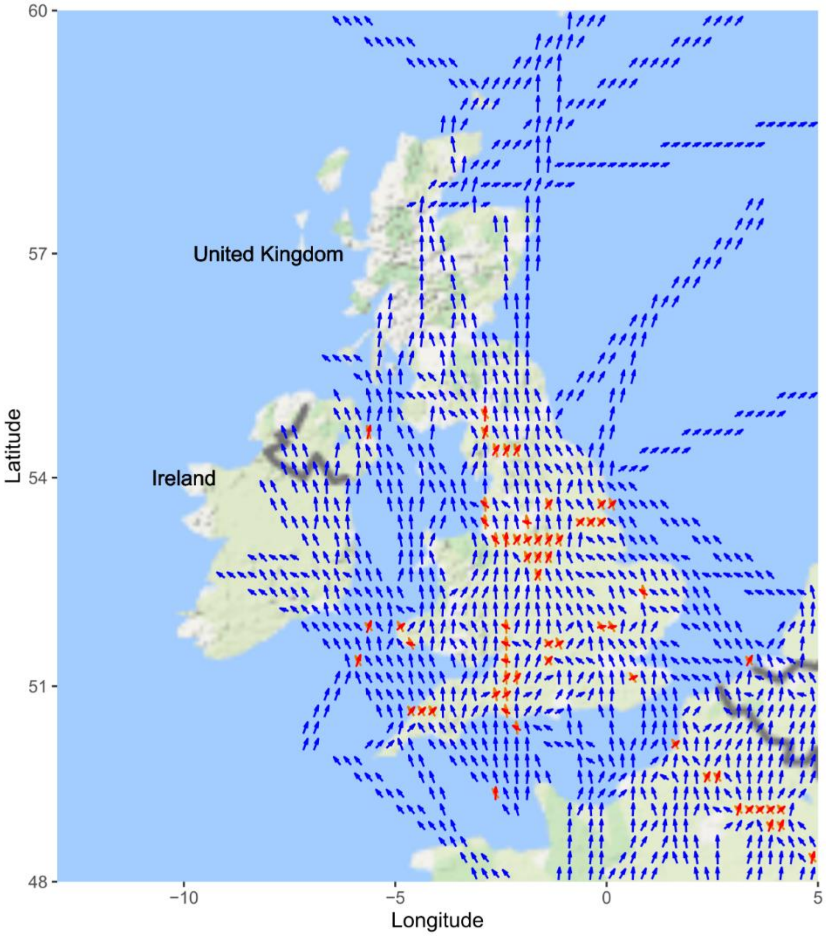

Figure 4 A

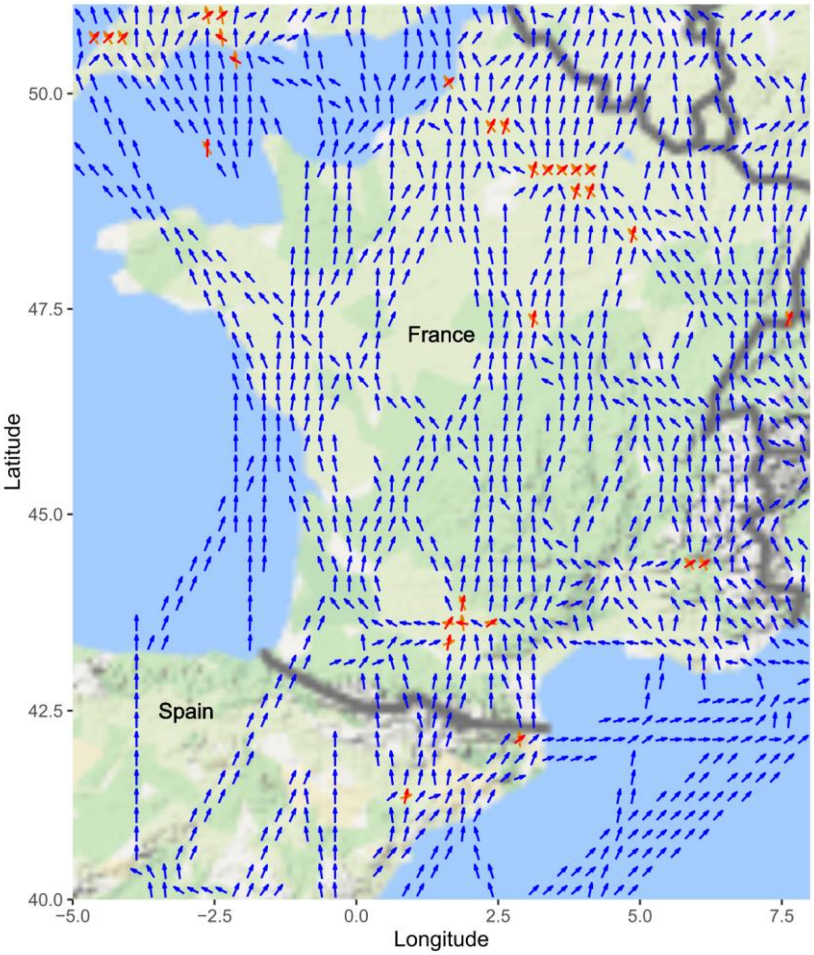
50.0
47.5
45.0
42.5
40.0
Latitude
−5.0
−2.5
0.0
2.5
5.0
7.5
Longitude
France
Spain

Figure 4 B

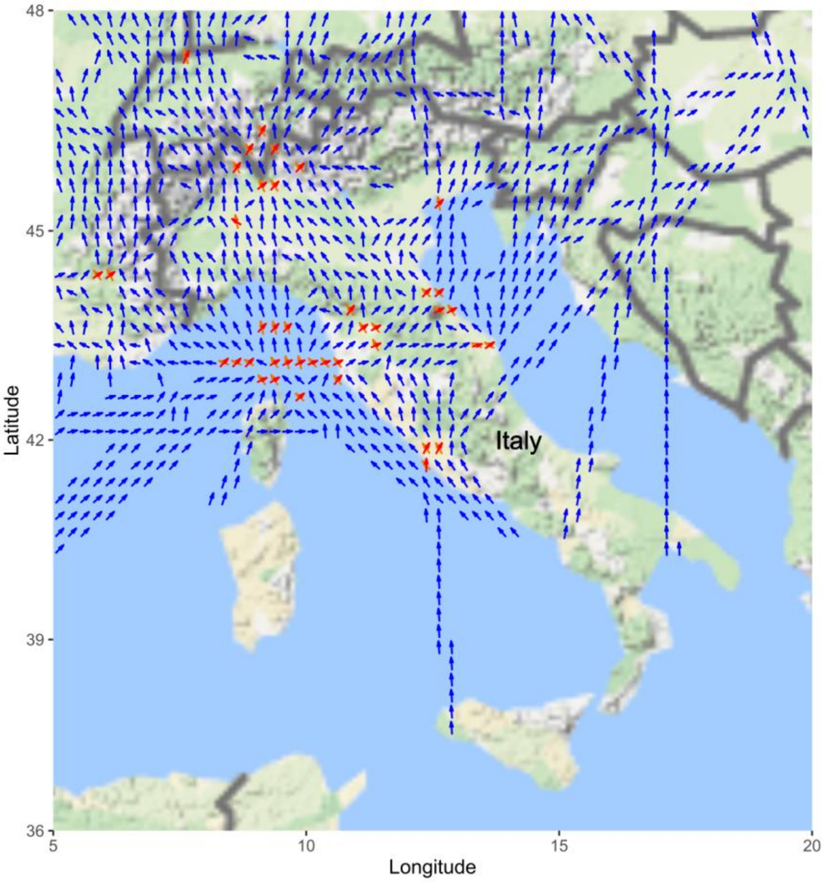

Figure 4 C

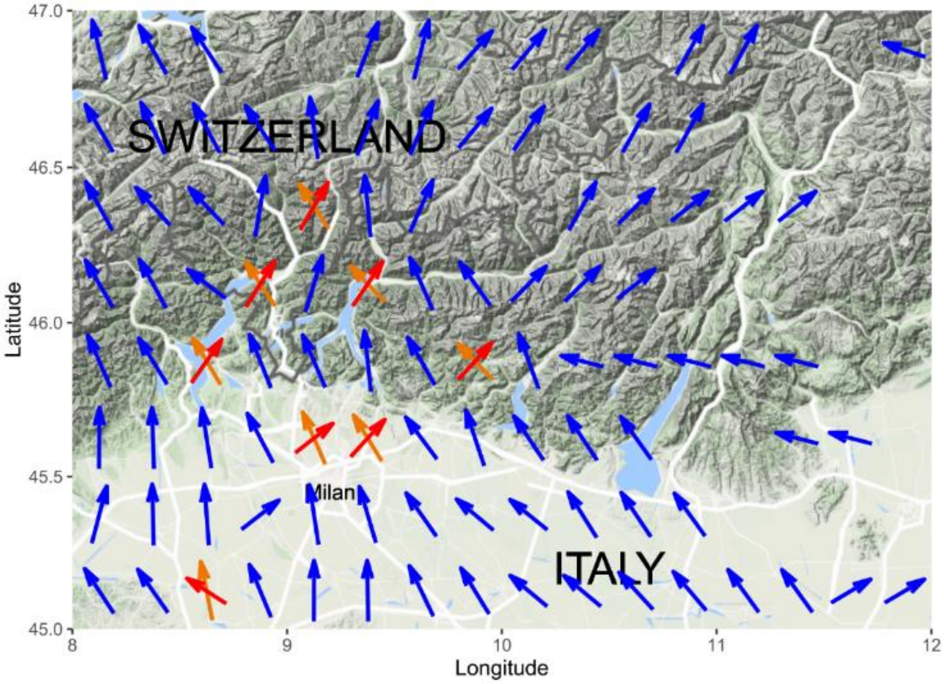
47.0
46.5
46.0
45.5
45.0
Latitude
8
9
10
11
12
Longitude
SWITZERLAND
ITALY
Milan

Figure 4 D

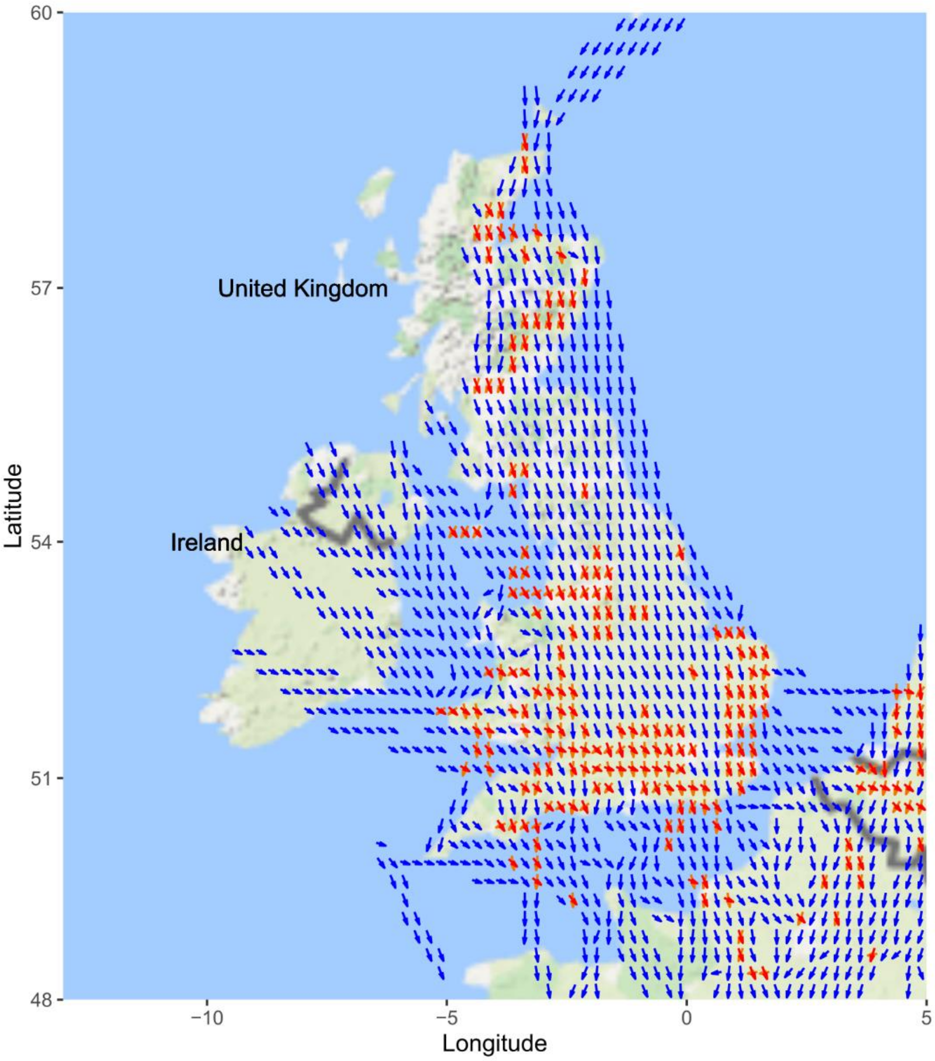
60
57
54
51
48
Latitude
−10
−5
0
5
Longitude
United Kingdom
Ireland

Figure 4 E

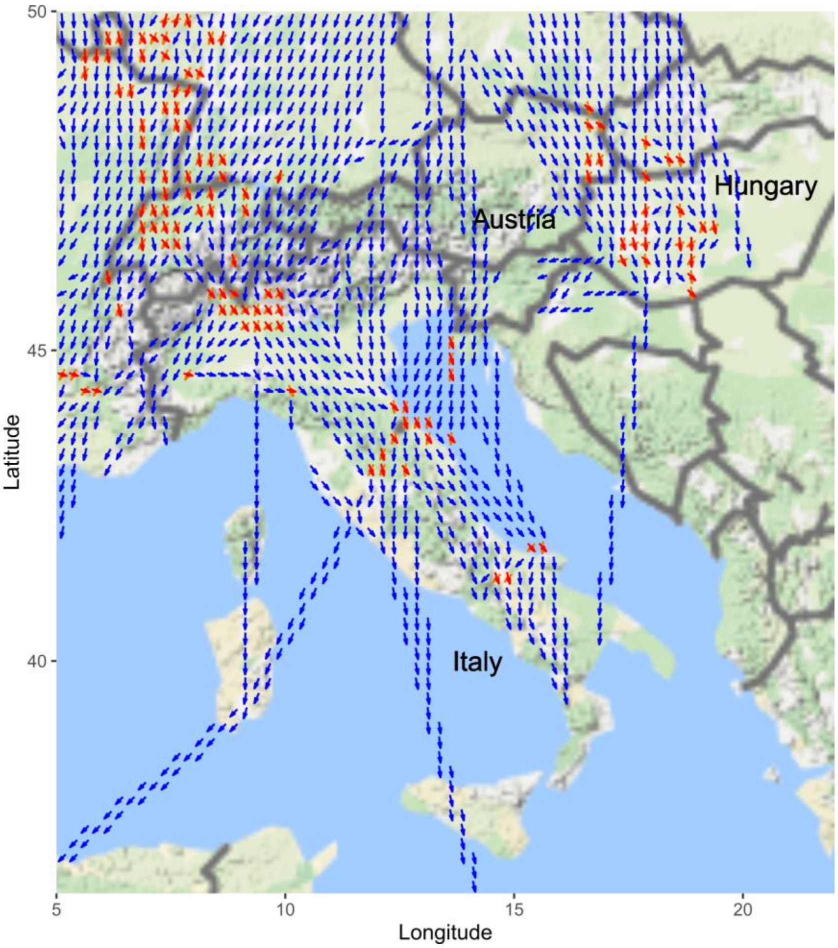

Figure 4 F

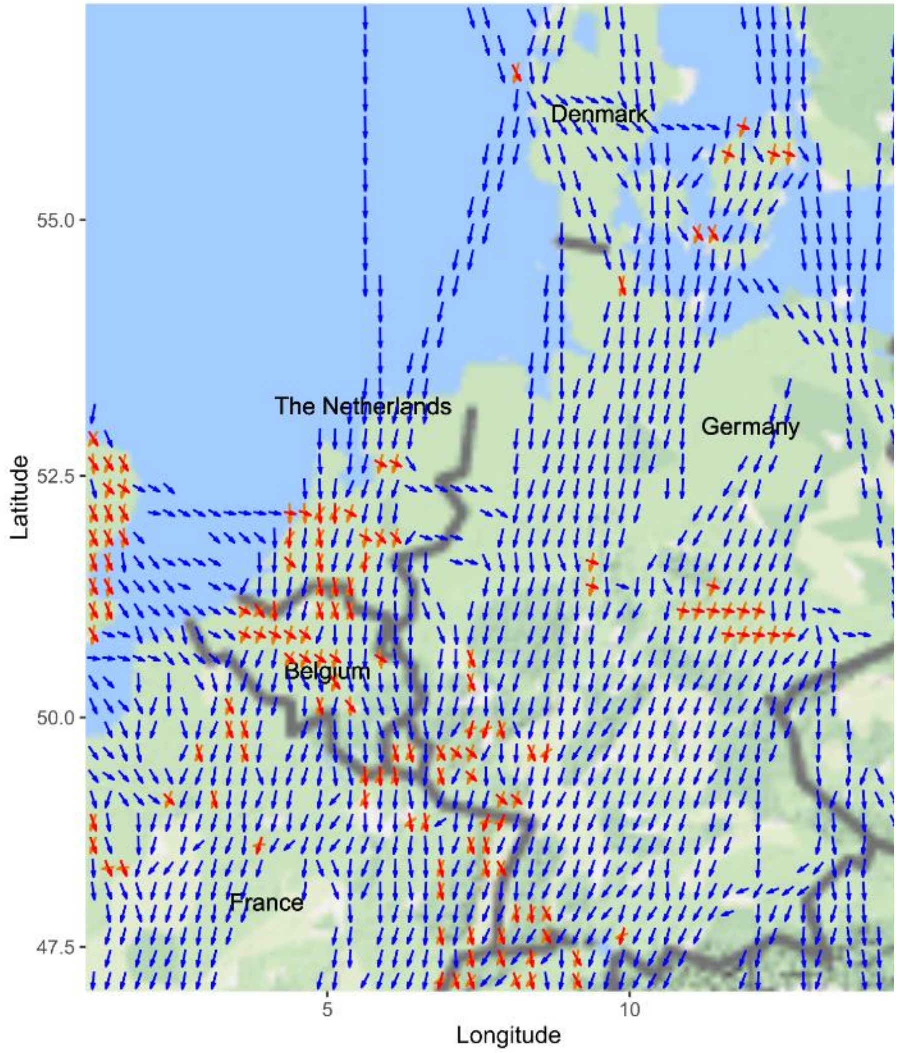
Denmark
The Netherlands
Germany
Belgium
France
55.0
52.5
50.0
47.5
Latitude
5
10
Longitude

Figure 4 G

Autumn migration

We identified 1,510 movements during autumn migration (one movement per individual with the only exception of one individual for which three records were available, thus it was associated with two movements). Overall, 4,589 cells were intersected by at least one movement during autumn migration and 1,223 by five or more movements. Cluster analysis indicated the presence of two clusters in 391 (8.5%) of these cells. Cells with double directions are clustered in central and northern Italy, Switzerland, Belgium and the Netherlands, Britain on the border between France and Germany, Hungary and Denmark (Fig. 4e-g). In addition, directions seem to converge around Gibraltar (Supplementary material Appendix 4). Areas where migration routes diverge, cross or converge are similar for spring and autumn migration, as suggested by the number of cells with double directions both in the map of spring and in that of autumn migration (observed = 27, expected = 11.7, $\chi^2_1 = 22.05$, $P < 0.001$).

Flight route maps suggest that migration routes are quite similar for spring and autumn migrations. Birds breeding in the British Isles seem to leave Europe through Gibraltar following routes similar to those described for spring migration but in the opposite direction (green lines in Fig. 6). Similarly, Barn swallow populations breeding in Belgium, the Netherlands and Germany (red lines in Fig. 6) mainly cross the Iberian Peninsula and then leave Europe toward the north-western African coast. However, some simulated flight routes go through the Italian peninsula to Corsica, Sardinia or Sicily, as in spring. Simulated routes of Barn swallows breeding in Denmark, Sweden and Norway (orange lines, Fig. 6) diverge in Denmark, showing a pattern similar to that of spring migration. Simulations also suggest that in autumn, birds from Norway can migrate either along the coast or across the country toward southern Sweden and then Denmark, while some others attempt a direct crossing from the coast of western Norway to the Netherlands. The simulated flight routes of Barn swallows breeding in Italy, Switzerland, Austria and Slovenia (pink lines Fig. 6) partially overlap with those of the populations from Germany (red lines), with routes running along Italy probably to avoid a long direct crossing of the Mediterranean.

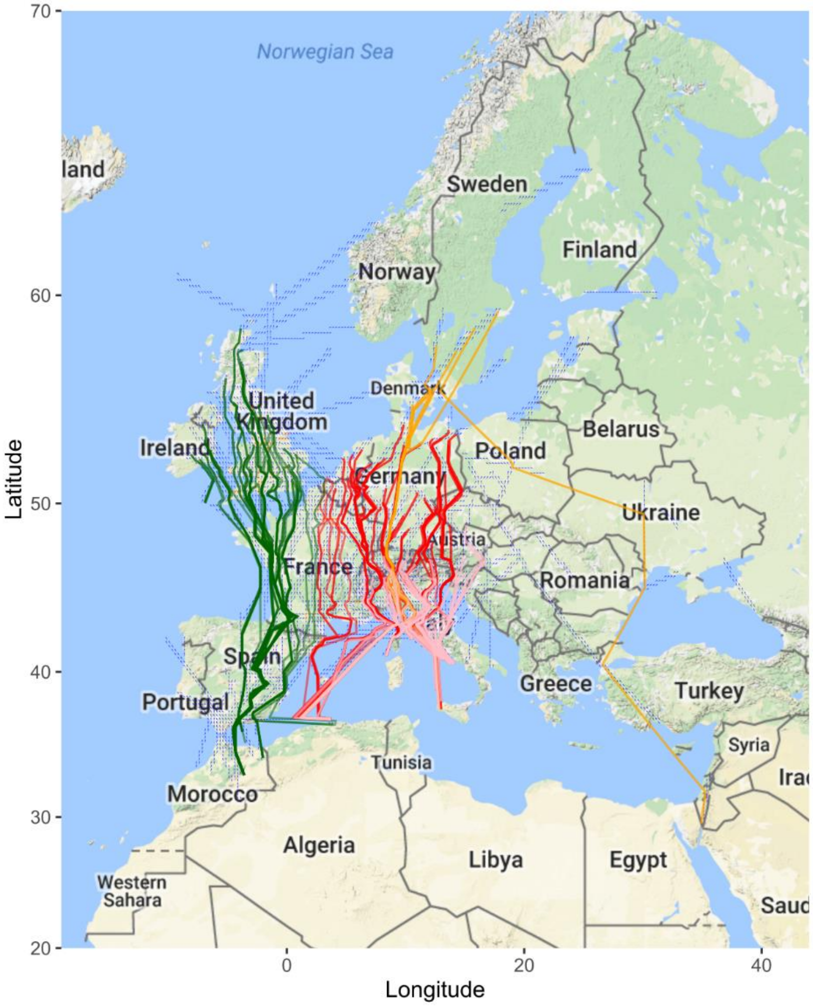

Figure 5. Spring flight route map. The map shows the routes of Barn swallows simulated by generating random positions within United Kingdom and Ireland (n 60 routes in green), Denmark, Norway, Sweden (n 18 routes in orange), Germany, Belgium, the Netherlands (n 94 routes in red), Italy, Austria, Switzerland and Slovenia (n 80 routes in pink) and moving points northwards according to directions reported in the spring directions map (Supplementary material Appendix 4; see the main text for details on the simulations).

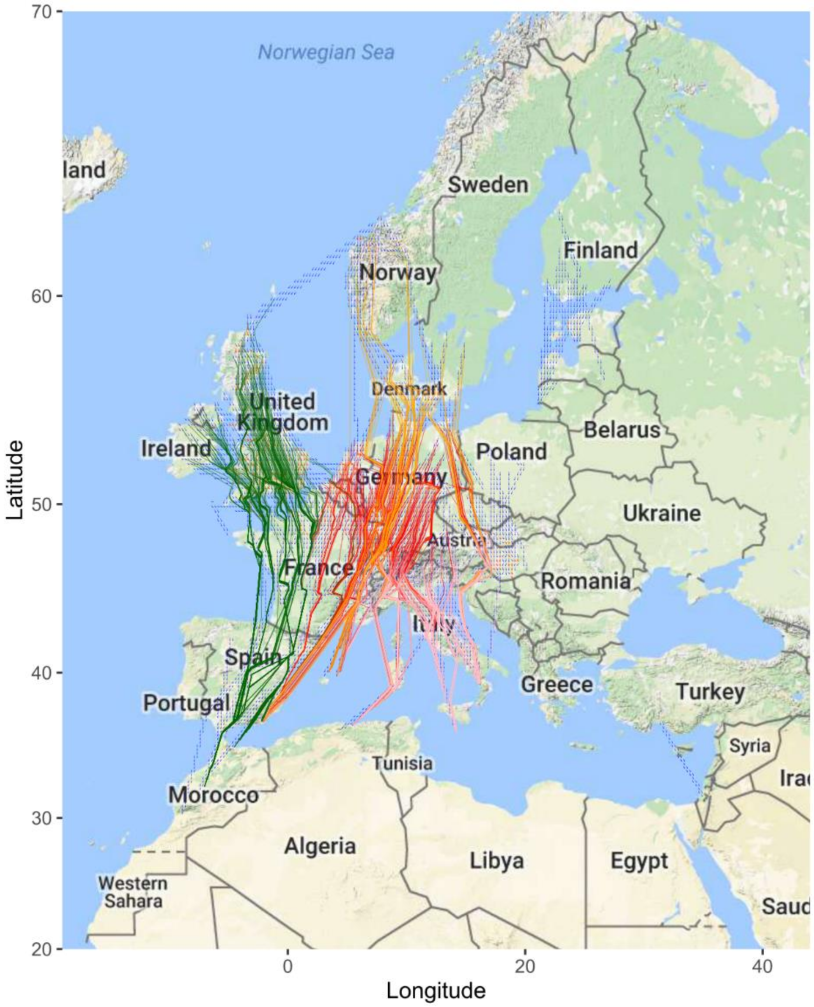

Figure 6. Autumn flight route map. The map shows the routes of Barn swallows simulated by generating random positions within United Kingdom and Ireland (n 420 routes in green), Denmark, Norway, Sweden (n 154 routes in orange), Germany, Belgium, Netherlands (n 436 routes in red), Italy, Austria, Switzerland and Slovenia (n 476 routes in pink) and moving points southwards according to directions reported in the autumn directions map (Supplementary material Appendix 4; see the main text for details on the simulations).

Multiple recoveries within the same migration event

Double recoveries of the same individuals within the same spring migration event were available for 101 Barn swallows, generating one movement per individual within the same spring (30.4% of the spring movements in the whole dataset), and for 971 Barn swallows during autumn migration (64.3% of the autumn movements in the whole dataset). No individual was recaptured more than twice during the same migration event. Analyses on these restricted datasets showed patterns of migration generally consistent with those of maps based on the whole dataset (Supplementary material Appendix 1, Figs A1 and A2; see also Appendix 5 for Google Earth™ maps). To test for such consistency, we used a Mann-Whitney U-test for paired samples and Spearman correlation to compare mean migration directions at each cell from the analyses on the whole dataset and from those on the restricted dataset. These tests are tenable because migration directions all head northwards for spring migration and southwards during autumn migration and directions heading north-westwards were expressed as negative angles. For cells with double directions in both analyses, we entered both the easternmost and the westernmost directions, while we discarded those cells that had one direction in one analysis and double direction in the other. These analyses showed that migration directions estimated by either analysis both for spring and autumn migration did not significantly differ ($z \leq 1.739$, $P \geq 0.082$; $n = 1581$ direction for spring and $n = 2944$ directions for autumn migration) and were significantly and positively correlated (Spearman's rho ≥ 0.741, $P < 0.001$).

Movement and direction maps from individuals recaptured during the same spring or the same autumn also confirmed that some individuals crossed the Mediterranean from the Balearic Islands to Corsica or Italy during spring migration. Indeed, one individual was captured in Mallorca in April 1984 and recaptured in Corsica four days later and another was captured in Mallorca in May 1978 and recaptured in Italy two days later. However, analyses on this restricted dataset did not show some of the migration patterns that appeared on the whole dataset, for instance migration through Gibraltar in spring and the crossing of the North Sea between Scotland and Norway during both spring and autumn (Supplementary material Appendix 1, Figs A1 and A2 and Appendix 5).

Comparing movement patterns from dead recoveries and live recaptures

For spring migration, 166 movements (50%) were available for birds that were recovered dead and 166 (50%) from birds that were recaptured alive. Direction maps generated on these two datasets separately depict similar patterns of migration (Supplementary material Appendix 5). Indeed, spring migration directions based on dead recoveries and live recaptures did not differ significantly (Mann-Witney U-test: $z = 0.631$, $P = 0.528$, $n = 975$ directions) and were positively correlated (Spearman's rho = 0.783, $P < 0.001$).

During the autumn migration, the number of records of birds found dead was only 14% ($n = 216$) of the data set, while 86% ($n = 1294$) of records were live recaptures. Several details of the autumn migration routes were consequently lost in the map from dead recoveries, for instance migration through Norway and Spain and along the Italian peninsula. (Supplementary material Appendix 5). In addition, direction from autumn dead recoveries differed by 1.836° ± 0.017° SE westwards from those on live re-encounters (Mann-Witney U-test: $z = 2.132$, $P = 0.033$, $n = 1122$ directions) but were significantly and positively correlated (Spearman's rho = 0.672, $P < 0.001$).

Analyses with cells of different size

Analyses run with cells of 0.5° × 0.5° and 1° × 1° show qualitatively similar patterns of migration to those depicted by the analyses at 0.25° × 0.25° (compare direction and flight route maps in Supplementary material Appendix 4 and 5).

The number of double direction cells increased when larger cells were used. Indeed, in the analyses of spring migration, 2.6% of cells had double direction in the analyses run with cells of 0.25° × 0.25° latitude × longitude (see also above), and this proportion increased to 10.3% with cells of 0.5° × 0.5° and 20.8% with cells of 1° × 1°. For autumn migration, double direction cells were 8.4%, 14.1% and 18.8%, respectively.

2.5 Discussion

In this paper, we propose a new method for describing migration routes and seasonal movements of birds using information provided by ring recoveries, or by any other source of data on positions of the same individual during its movements. We applied this procedure to ring recoveries of Barn swallows collected between 1908 and 2011 to model migratory movements of Barn swallows Unfortunately, large heterogeneity in the distribution of ringing data, which reflects geographical variation in sampling effort and encounter probability of marked individuals, limited the geographical extent of the present investigation to western and central Europe, and prevented us from providing a complete picture of the migratory movements of the European populations of this species. Flight route maps aid the visualization of migration movements showing, for instance, that Barn swallows breeding in the British Isles follow different routes than those from Belgium, the Netherlands and Germany, a pattern that is more difficult to assess by looking at direction maps only.

The overall pattern of migration disclosed by our analyses is consistent with previous descriptions of Barn swallow migration through Europe (Ormerod 1991, Turner 2006). On the one hand, such consistency in the results is not surprising, since ornithologists based their description of migration routes, in large part, on the same ringing data we used for our analysis. On the other, such consistency indicates that our method is able to show, through a standard and repeatable procedure, the same migration patterns identified by experienced ornithologists, supporting the reliability of our procedure. Our analyses were also able to highlight some migration patterns that, to the best of our knowledge, have been previously neglected, such as the existence of direct movements from the Balearic Islands to Corsica or Italy during spring (Fig. 1), between Norway and Scotland by direct crossing of the North Sea (Fig. 4a and 4e) (Wernham et al. 2002), and from southern Italy to Central Balkans (Fig. 4c and 4f) (Dontschev 1976). Clearly, these routes were depicted based on rather few recoveries, and their importance should be better investigated by future studies.

Direction maps highlighted areas where cells with double directions, which indicate where migration routes diverge, cross or converge, concentrate, for instance, in central Italy and southern England (Fig. 4). Clearly, double direction cells occur more often in areas where movements are abundant, and increase when large cells are used. Using cells of small size gives a more detailed spatial representation of migration directions, yet we acknowledge that cell size and other parameters (i.e. the minimum number of moves crossing a cell for running cluster analysis) strongly depends on the species, the aim or the extent of the geographic area under study. Geographical areas where routes cross, converge or diverge are also consistent between spring and autumn migration, as suggested by the excess of cells with the double direction in both migration periods, confirming that Barn swallow follow similar routes during both spring and autumn migration (Turner 2006).

Our method of analysis seems therefore able to capture the general patterns of Barn swallow migration though western and central Europe and even to disclose some novel patterns of migration. This indicates that this method can provide new insights into migration movements even of one of the passerine species for which the largest amount of information on migration is available. Importantly, the general patterns of migration described so far also clearly appeared in the analyses run at different spatial resolutions (Supplementary material Appendix 5), thus indicating that the arbitrary choice of cell size did not largely affect the outcome of the analyses. Another main advantage of our analysis is that it is based on an automated and repeatable procedure, while previous descriptions of migration routes based on ringing data were almost entirely based on subjective description and interpretation of maps of ring recoveries.

We emphasize that this analysis only uses information provided by ring recoveries, i.e. only date and position where an individual was observed. No information on topography (e.g. altitude or coastlines) or environment (e.g. climate or land use) was entered in the analyses. The model only considered actual movements of individuals as if they were occurring in an isotropic space, thus limiting the amount of information necessary to run the analyses. Interestingly, maps showed that migration directions point toward sites where Barn swallows were expected to cross the geographical barriers they encounter during migration despite the fact that geographical barriers were not considered in the procedure. We consider this evidence to further support the robustness of our approach.

The method of analysis we used is not without limits, however. For instance, large shifts in migration routes of the same individual in different years may affect the results. Importantly, most patterns were confirmed by analysing movements occurring during the same migration event (Figs A1 and A2), which thus confirms the robustness of the method. Large differences in sampling effort are another major problem that affects all the analyses of ringing data and may bias the results. When estimating migration directions, we tried to account for different uncertainty in the identification of migration directions, by using three different weights (see Methods and Supplementary material Appendix 1 for details). We emphasize that detailed information on sampling effort at each geographical area would provide key information for correcting one of the main sources of bias in any analysis of ringing data, although this information is rarely available (Thorup et al. 2014). In such circumstances, comparison of results obtained on different subsets of data may allow assessment of the existence and the extent of the possible biases (Fiedler et al. 2004). With this aim, we compared direction maps produced from movements of birds either recaptured alive or recovered dead and found that they were qualitatively similar, particularly the maps of spring migration, which were made on a similar number of movements. In contrast, we found a slight but significant difference in direction estimated by live re-encounters and dead recoveries, which can be due to the rather small number of movements from dead recoveries in the autumn dataset or to geographical differences in the sampling effort of live recaptures and dead recoveries. Another limit of the present method of analysis is that a number of arbitrary choices have to be made, for instance the choice of cell size, or whether to include only movements within the same year. However, when we repeated the analyses with different values of arbitrary parameters and by varying sample size, we found very consistent results. This further confirms that this method of analysis is generally robust.

In conclusion, the procedure we developed is a simple and versatile method to represent patterns of migration from ringing data or other similar datasets (e.g. sight-resighting data of humpback whales *Megaptera novaeangliae*; Barlow et al. 2011), provided that individuals can be identified and observed at least twice in different positions. Although current technologies are greatly improving our knowledge of animal movements, new methods for analysing large and already available datasets of ring recoveries or sight-resight data are still very important. Here, we described the main migration routes of Barn swallows through western and central Europe using a standard, repeatable and robust procedure, which disclosed novel insights into migration movements and highlighted areas of route convergence, crossing, and divide. This analysis can thus complement previous information based on investigation of ring recoveries (Ambrosini et al. 2009, 2011, 2014, Thorup et al. 2014), miniaturized tracking devices (Arizaga et al. 2015, Liechti et al. 2015), stable isotopes (Szép et al. 2009, Evans et al. 2010, Hobson et al. 2012) and analysis of population fluctuations (Szép et al. 2006) in elucidating the migration ecology of Barn swallows. The method of analysis described and applied here for the first time can therefore provide much needed information for planning conservation strategies for migrants because it allows inferring migration pathways from data so far exploited only to a fraction of their potential (Bairlein 2001, Ambrosini et al. 2016).

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Chapter 3

Environmental conditions at arrival to the wintering grounds and during spring migration affect population dynamics of Barn swallows *Hirundo rustica* breeding in Northern Italy

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Chapter 3

3.1 Abstract

Several populations of long-distance migratory birds are currently suffering steep demographic declines. The identification of the causes of such declines is difficult because population changes may be driven by events occurring in distant geographical areas during different phases of the annual life-cycle of migrants. Furthermore, wintering areas and migration routes of populations of small-sized species are still largely unknown, with few exceptions. In this paper we identified the critical phases of the annual life-cycle that most influence the population dynamics of a small passerine, the Barn swallow *Hirundo rustica*. We used information on temporal dynamics of a population breeding in Northern Italy, whose wintering range and timing of migration have been recently described by miniaturised tracking dataloggers. Our results indicated that primary productivity in the wintering grounds in the month when most individuals arrive from autumn migration and primary productivity in an area that is probably a stopover site during spring migration, influenced population dynamics more than habitat conditions at the breeding grounds. By using annual variation in primary productivity at the wintering grounds and stopover sites as predictors, we replicated the observed interannual population changes with great accuracy. However, the steep decline recently suffered by the population could be replicated only by including a constant annual decline in the model, suggesting that changes in primary productivity only predicted the interannual variation around the long-term trend. Our study therefore suggests the existence of critical periods during

wintering and migration that may have large impact on population fluctuations of migrant birds.

3.2 Introduction

Populations of several migratory bird species breeding in Europe are suffering sharp declines (Berthold et al. 1998; Sanderson et al. 2006), whose causes have been often attributed to changes in land use in the breeding areas and to unevenly distributed climate change (Pimm and Raven 2000; Sala et al. 2000; Both et al. 2006; Jones and Cresswell 2010). Despite long-term studies have collected large amount of information on population size in several geographical areas where migratory bird populations breed, in many cases the actual ecological determinants of the observed demographic trends are still uncertain. Indeed, the complexity of migrants' life-cycle often hampers evaluating the actual causes of population changes, because such an assessment would require information on variation of ecological conditions faced by individuals at breeding and wintering grounds and along migration routes (Newton 2004; Holmes 2007).

Furthermore, for the vast majority of migratory species, which are typically small-sized, detailed information on the timing of different phases of the life-cycle, on the precise location of wintering grounds, and on migration routes is lacking. Indeed, such pieces of information should ideally be available for any single population whose dynamics is to be modelled, while rarely they are actually available at such level of detail. Recently, novel technological devices are bridging this gap of knowledge, by allowing ornithologists for the first time to track small sized birds during migration and wintering (Fiedler 2009; Bächler et al. 2010). For example, miniaturized light-level dataloggers (geolocators) are revealing information on migration timing and routes and on the spatial distribution of wintering grounds of several small sized species (see e.g., Liechti et al. 2015).

In this paper we aimed at assessing the critical stages in Barn swallow *Hirundo rustica* (Linnaeus 1758) life-cycle that affect population size. Detailed information on size and reproductive success of a geographical population of this species was collected during a long-term monitoring project that is ongoing in Northern Italy since 1999. We have previously shown that this population has declined by about 7 % per year since then (Ambrosini et al. 2012; Sicurella et al. 2014). However, changes in habitat conditions that occurred in the breeding area during the same period accounted for only 5% of the observed decline (Sicurella et al. 2014). Hence, this population seems to be mainly regulated by the ecological conditions faced during periods of the annual life-cycle other than reproduction. The deployment of miniaturized geolocators on a large sample of birds from this and other two nearby geographical populations has provided a precise identification of the wintering areas and a detailed description of the phenological events of their annual life-cycle, including time of departure from and arrival to both wintering and breeding quarters (Liechti et al. 2015). Hence, for the first time, detailed information on crucial details of the lifecycle of this population out of the breeding period is available.

A precise description of migration routes of Barn swallows is difficult to obtain even with the use of geolocators, because these instruments do not allow for reliable estimates of latitude for periods close to equinoxes, which, unfortunately, largely coincide with the migration periods of this species. However, routes and timing of Barn swallow migration can also be investigated by analysing long term ringing data. Indeed, the analysis of ringing data collected over one century throughout Europe and North Africa and stored in the EURING Databank (http://www.euring.org) allowed the identification of main migration routes of Barn swallows breeding in Western Europe (Ambrosini et al. 2014).

Hence, by combining information from geolocators with the analyses of ring recoveries, it is now possible to obtain novel insights into the timing of important stages of the life-cycle, and on the geographical position of the wintering grounds and main migration routes of this Barn swallow population.

The assessment of ecological conditions faced by birds during wintering and migration often involves large geographical areas of the globe and is typically made by satellite-assisted remote sensing of indices of primary productivity (Tucker 1979; Tucker et al. 1985). In particular, the Normalized Difference Vegetation Index (NDVI), which measures photosynthetic activity and reflects the effect of rainfall on the distribution and biomass of vegetation (Boone et al. 2000; Schmidt and Karnieli 2010), is widely used, and several studies indicate that it is related to bird population changes (see for example Maurer 1994; Osborne et al. 2001;

Bailey et al. 2004; Saino et al. 2004a; Szép et al. 2006; Giralt and Valera 2007; Balbontín et al. 2009). In particular, NDVI can indicate ecological conditions experienced by Barn swallows during migration and wintering, because this species feeds on flying insects, whose abundance is strictly dependent on rainfall and vegetation dynamics (Saino et al. 2004a, b; Gordo and Sanz 2008; Balbontín et al. 2009, 2012; Pillar et al. 2015). We therefore used NDVI to investigate the effects of ecological conditions faced by swallows during migration and wintering on year-to-year variation in population size.

We hypothesized that inter-annual variation in population size is influenced by processes acting in different phases of the Barn swallow life-cycle. First, population size can be obviously affected by the annual reproductive output of the preceding year, as returning young Barn swallows mostly disperse within a few kilometres from their natal site (Scandolara et al. 2014). Secondly, population size is affected by year-to-year survival of both adult and young individuals, which is mainly determined by conditions encountered en-route during autumn and spring migration, and in the wintering quarters (Saino et al. 2004a; Szép and Møller 2005; Turner 2006).

We also hypothesized that ecological conditions at different times of the wintering period may have different effects on survival. For example, survival rate of white stork *Ciconia ciconia* (Linnaeus 1758) is mainly due to variation in primary productivity at one staging area in the eastern Sahel that these birds visit from October to November, just at the end of their crossing of the Sahara, but not to variation in primary productivity at their wintering grounds (Schaub et al. 2005). Hence, ecological conditions encountered at arrival from migration may have an impact on annual survival of migrant birds. Similarly, ecological condition just before departure for spring migration may affect population levels observed at the breeding grounds because only individuals that are able to accumulate sufficient fat resources can survive migration (Gordo and Sanz 2008). We therefore investigated whether ecological conditions during specific periods of wintering, and in particular at arrival to and at departure from the wintering grounds, have an impact on population fluctuations.

3.3 Methods

Study organism and field methods

The Barn swallow is a small (about 18 g) passerine bird breeding in vast areas of the northern hemisphere. Populations breeding in Europe winter in Africa to the south of the Sahara desert (Turner 2006). The population of Barn swallows breeding in the Parco Regionale Adda Sud, a wide (24,260 ha) protected area in Northern Italy (coordinates of the approximate centre: 45°19’N, 9°40’E, see Fig. 1), has been intensively studied since 1999 (Ambrosini et al. 2002). Censuses took place annually from April to June and were conducted according to a standardized protocol (Ambrosini et al. 2002; Sicurella et al. 2014). In 1999 a sample of 121 farms was randomly chosen within the 340 farms in the whole study area. Since then, the number of farms censused at each year varied depending on our opportunities to perform field work and on farm owners' willingness to let us visit their properties. For the present study, we selected 83 farms censused every year from 1999 to 2014. Briefly, all farms were visited every second week, all nests at each farm were inspected and their content recorded. The maximum number of nests simultaneously active at each farm was used to estimate the mean number of breeding pairs at each farm during April– June (i.e., during the period of first clutches) of each year (Ambrosini et al. 2002). In the present study we used the mean number of breeding pairs per farm as an estimate of the size of the Barn swallow population in each year. Overall 385 to 1098 pairs bred in these 83 farms in each year. To estimate breeding success we used the mean number of nestlings surviving until 10 or more days after hatching (mortality after age 10 days is less than 5% of total nestling mortality, N. Saino, unpublished data) observed in all active nests (i.e., all nests where eggs were laid).

In 2008 and in 2010–2014 37–48 farms were censused also in July–August following the same protocol to assess number of breeding pairs and breeding success during the period of second clutches. We preliminary investigated these data to assess whether any environmental variable may account for year-to-year variation in the relative importance of reproductive output during the period of first (April–June) and second broods (July–August) (see Electronic Supplementary Material (ESM) for full details on these analyses). Since no variable seemed to affect the relative importance of reproductive output during first and second broods, we considered the number of chicks produced during first broods to be a good proxy of the total annual reproductive output of the population.

In 2011–2013 a study with miniaturized light-level geolocators provided information on wintering and migration of a total of 94 individuals from this and other two Barn swallow populations breeding in Northern Italy and Southern Switzerland (Liechti et al. 2015). Most of the studied individuals wintered in an area of about 1000 km in radius centred in southern Cameroon (coordinates of the median point 5°47’N 13°30’E), with no significant differences in wintering areas between populations (Liechti et al. 2015). In addition, geolocator data showed that the majority of individuals reached their wintering grounds in October, and departed for spring migration in March (Liechti et al. 2015).

The analysis of ringing data has allowed the identification of two main migration routes in Western Europe followed by Barn swallows during both spring and autumn migration, one through Gibraltar, and one across the Mediterranean, over Tunisia and Algeria (hereafter Tunisia, for brevity) (Ambrosini et al. 2014). Importantly, exploratory analyses of geolocator data based on longitude data only (which are available from geolocators even close to the equinoxes) suggested that individuals breeding in Northern Italy follow both these migration routes during spring migration, while during autumn migration they follow only the route through Tunisia. In addition, this preliminary investigation of geolocator data suggested that Barn swallows from this population mainly cross Tunisia in September during autumn migration and both Tunisia and Gibraltar in March during spring migration (R. Ambrosini, unpublished data).

NDVI data

We calculated monthly mean NDVI values from September to March in an area of 1000 km in radius from the median position of the wintering area identified by the geolocator study (05°48'N 13°30'E, see Fig. 1). Indeed 88% of individuals from the study populations wintered in this area (Liechti et al. 2015).

We also considered monthly NDVI data recorded in Tunisia (in a squared area spanning 35°00'–38°00'N and 01°30'–11°00'E, Fig. 1) and Gibraltar (in a squared area spanning 34°36'–37°24'N, and 03°30'–06°30'W, Fig. 1). These areas were selected on the basis of main migration routes identified by analyzing ring recoveries (Ambrosini et al. 2014).

Monthly data on NDVI were obtained from the Moderate Resolution Imaging Spectroradiometer (MODIS, http://neo.sci.gsfc.nasa.gov/). We used MODIS-NDVI dataset because recent analyses showed that these data performed better than other NDVI datasets (e.g., AVHRR-NDVI) in the African continent (Beck et al. 2011). Unfortunately, MODIS-NDVI data are not available before 2000 and this led to the exclusion from the analysis of data on Barn swallows collected in 1999 and 2000.

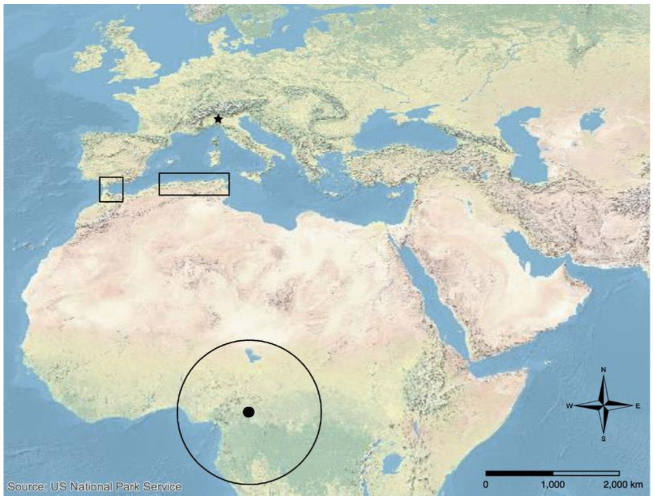

Fig. 1 Geographical position of the breeding area (star), staging areas ring spring and autumn migration (squared areas) and wintering quarters (circle, the dot represents its centre at 05°48’N 13°30’E) of Barn swallows from our study population

Statistical methods

The main aim of this paper was to model the mean number of breeding pairs recorded in a sample of farms in the Parco Regionale Adda Sud in each year. We hypothesized that year to year variation in the mean number of breeding pairs per farm can be affected by different factors acting at different phases of the annual life-cycle of this species. Schematically, these factors are:

1. Reproductive output of the previous year;
2. Ecological conditions encountered en-route during autumn migration;
3. Ecological conditions in the wintering quarters;
4. Ecological conditions encountered en-route during spring migration.

In the first part of the analyses we aimed at identifying the determinants of year-to-year variation in the mean number of breeding pairs per farm. This variable showed clear temporal trend (coefficient ± SE: -0.007 ± 0.001 pairs $year^{-1}$, t_{14} = -7.921, $P \leq 0.001$, according to a linear regression model) and strong temporal autocorrelation (auto-correlation function (*acf*), estimated by the *acf* procedure in R, equalled 0.676 with lag = 1). With the aim of simplifying the statistical analyses by removing both temporal trend and autocorrelation in the dependent variable, we calculated the difference in the mean number of breeding pairs in our sample of farms between one year and the preceding one. This procedure de-trended the time series of data, as indicated by the fact that the difference in the mean number of breeding pairs showed no temporal trend (0.017 ± 0.134 pairs $year^{-1}$, t_{13} = 0.130, P = 0.899), and weak temporal autocorrelation (*acf* = -0.238 with lag = 1). All the following analyses were therefore based on differences in the mean number of breeding pairs in our sample of farms between consecutive years. This procedure was preferred over the calculation of the intrinsic growth rate of the population ($r = \log_e\lambda$, where λ is the ratio between mean number of breeding pairs per farm in year t and year t - 1) because growth rate values calculated for our population did not follow a normal distribution (Kolmogorov–Smirnov test: *D* = 0.463, P = 0.002), while the difference in the mean number of breeding pairs per farm between year t and year t - 1 did (Kolmogorov–Smirnov test: *D* = 0.167, P = 0.735).

Given the relatively small number of data available and the large number of variables potentially affecting population dynamics, we tried to avoid model over-fitting in two ways: first, we selected the predictors according to previous knowledge on the biology of Barn swallows; secondly, we followed an Information Theory-based approach in the analyses.

We selected the following predictors:

1. Mean breeding success of first broods in the previous year, which is a good proxy of the total reproductive output in the preceding year (see ESM);

2. NDVI in the areas of Tunisia in September, i.e., in the month when most of our swallows migrate southwards (Ambrosini et al. 2014; Liechti et al. 2015);

3. NDVI in the wintering area in October and March, because they are respectively the months when our population reaches its wintering grounds and when it departs for spring migration (Liechti et al. 2015). The effects of these variables were also further investigated in additional analyses (see below);

4. NDVI in the areas of Gibraltar and Tunisia in March, i.e., in the month when most of the swallows of our population should reach this geographical area during spring migration (Liechti et al. 2015).

In addition, since our analyses were based on the difference in the mean number of breeding pairs between consecutive years, we entered as predictors also the differences between one year and the preceding one for all of the above mentioned variables (hereafter named adding 'Δ' to variable name for brevity, e.g., ΔNDVI). Positive values of ΔNDVI therefore indicate a higher NDVI value in year i than in year i - 1. The logic behind this approach is that year-to-year difference in the number of breeding pairs observed in our study area may be determined by year-to-year variation of predictors between consecutive years. For example, a decline in NDVI values in the wintering grounds is expected to determine a decline in the number of breeding pairs. However, if very low NDVI values occurred in consecutive years in the wintering quarters, and these low NDVI values determined very low winter survival of Barn swallows, then we will observe a decline in the population between the two years even if NDVI values were exactly the same (i.e., ΔNDVI = 0). Our approach aimed at investigating both possibilities.

As a further predictor we included also calendar year (not included as a difference) in the analyses in order to account for potential variation in the year-to-year differences in the mean number of breeding pairs through time. Hence, an effect of year would imply that difference in the mean number of breeding pairs between consecutive years changed through time and therefore the mean number of breeding pairs changed non-linearly. Finally, we note that the intercept of a model based on year-to-year differences indicates a constant year-to-year variation (increase or decrease depending on its sign) in the mean number of breeding pairs. In particular, the intercept of such a model indicates a constant variation not accounted for by the other predictors entered in the model.

As mentioned before, given the few data available (only 13 year-to-year differences) and the large number of predictors (13 variables), the analyses were performed following an Information Theory-based approach. We produced all the Linear Models (LMs) assuming a Gaussian error distribution, including the difference in the number of breeding pairs between consecutive years as dependent variable and all the possible combinations of the above mentioned variables as predictors, by limiting to three the maximum number of predictors simultaneously included in each model (for a total of 378 models, see Table S1 in ESM). In this way we aimed at avoiding model over-fitting. We then selected the LMs with $\Delta AIC_c < 2$ with respect to the LM with the lowest AIC_c and finally averaged model coefficients (Johnson and Omland 2004). Models with $\Delta AIC_c < 2$ are considered to have similar support from the data (Burnham and Anderson 2002).

Entering into the same model the actual value of a predictor in each year and its difference between consecutive years rises collinearity issues. Indeed, Pearson correlation between actual values of predictors and year-to-year differences of the same variables ranged between 0.484 and 0.915. However, we are confident that collinearity among predictors should not have biased our results for two reasons. First, collinearity may have affected only models that included both the actual value and the year-to-year difference of the same predictor because Pearson correlation between any other pair of variables was \0.6 (other details not shown), and none of these models was selected in the model averaging procedure (see

‘‘Results’’ and Table S1 in ESM). Second, we further checked whether collinearity may have biased model selection, by re-running the analyses while including in the set of predictors both the mean value of predictors between consecutive years and their differences between consecutive years. This procedure is tenable because no information is lost if one enters in a model e.g., NDVI value in years t and ΔNDVI between years t and t - 1, or the mean NDVI value between years t and t - 1 and ΔNDVI between years t and t - 1. In addition, the mean values and the differences between consecutive years were only weakly correlated (r_P ranged between -0.075 and 0.060). In any case, the set of models providing model-averaged parameter estimates never included actual values (details not shown).

In a second set of analyses we aimed at checking for the consistency of our results with respect to a possible incorrect selection of the predictors. Indeed, ΔNDVI values in the wintering grounds in October, and ΔNDVI values in Tunisia in March seemed to affect Barn swallows population dynamics (see ‘‘Results’’), but ΔNDVI values in other months may have similar effects. We therefore ran some additional analyses to confirm that ΔNDVI values in these months have the largest effect on variation in breeding pairs. To this aim we ran all LMs by including ΔNDVI values in the wintering ground in each month from September to March, while keeping ΔNDVI values in Tunisia in March as the second predictor, and noted their AIC_c values. Similarly, we ran all LMs by including ΔNDVI values in Tunisia in all months between February and April, while keeping

ΔNDVI values in the wintering grounds in October as the second predictor, and compared their AIC_c values.

In the last part of the analyses, we used the final LM based on differences in the mean number of breeding pairs between consecutive years to reproduce the population dynamics observed during annual censuses in the Parco Regionale Adda Sud. We used the number of breeding pairs observed in 2001 as the only input for this simulation. This year was chosen because the model based on year-toyear differences included the difference between 2002 and 2001 as the earliest datum. We then predicted the mean number of breeding pairs in the Parco Regionale Adda Sud in 2002 by summing to the mean number of breeding pairs observed in 2001 the difference in mean breeding pairs between 2002 and 2001 predicted by the model. Mean number of breeding pairs in 2003 was then estimated by adding the difference in mean breeding pairs estimated by the model to the number estimated for 2002 as above, and so on until 2014. As a measure of the ability of this procedure to correctly reproduce the observed variation in the mean number of breeding pairs per farm we calculated a pseudo-R^2 equal to the squared correlation coefficient between the observed mean number of pairs in each year and that estimated by this procedure (Efron pseudo-R^2, Efron 1978).

To estimate standard errors for these predicted values, we had to account for uncertainty both in the estimate of the starting value (i.e., the mean number of breeding pairs observed in 2001) due to sampling and to uncertainty in the estimate of year-to-year variation from the model due to variance in model coefficients. To this end, we re-ran the procedure exposed above by:

1. Using as starting value a random number drawn from a Gaussian distribution with mean equal to the mean number of breeding pairs observed in 2001 and variance equal to its squared standard error;

2. Estimating year-to-year variation in the mean number of breeding pairs while taking into account model uncertainty by drawing a random number from a Gaussian distribution with mean equal to the difference between one year and the preceding one estimated by the model and variance equal to the variance of this estimate.

This procedure was repeated 1000 times and the standard error of the mean number of breeding pairs estimated at each year by these 1000 simulations was used as an estimate of the standard error of the mean number of breeding pairs predicted by our model. The analyses were performed with the lm and predict procedures in the stats package and the *dredge* and *model.avg* procedures in the *MuMIn* package (Barton 2014) in R 3.0.3 (R Core Team 2014).

3.4 Results

Year to year variation in the number of breeding pairs

Two models were selected based on our procedure. The first included ΔNDVI in the wintering quarters in October and ΔNDVI in Tunisia in March ($AIC_c = 50.95$). The second model included only ΔNDVI in Tunisia in March ($AIC_c = 52.32$; Table S1 in ESM).

Averaged coefficients from these models indicated that a decrease in NDVI values between one year and the preceding one both in the wintering grounds in October and along migration routes in March were linked to a decrease in the number of breeding Barn swallows in the Parco Regionale Adda Sud (Table 1). The 95% confidence interval of the intercept included zero (Table 1), thus suggesting that the year-to-year difference in the mean number of breeding pairs should have not changed in years when NDVI values did not change. In particular, since our population is declining (Ambrosini et al. 2012; Sicurella et al. 2014), and therefore the year-to-year difference in the mean number of breeding pairs was on average negative, if other variables not included in the model had affected population dynamics, we would have expected the intercept to be lower than zero. Indeed, a significant and negative intercept in a model based on year-to-year differences, indicates a constant unexplained decline in the population (see ‘‘Methods’’).

Checking for influential periods

Additional analyses whereby we entered ΔNDVI values in the wintering areas for different months clearly indicated that October was the most influential month, since ΔAIC_c values of models including ΔNDVI values for other months and that of the model including ΔNDVI values of October were always larger than 2 (Fig. 2a). Conversely, we had little evidence that models including ΔNDVI values in Tunisia in March performed better than models including ΔNDVI values in the same area in February, as ΔAIC_c value was lower than 2 (Fig. 2b). This small difference in AIC_c may be due to the strong positive correlation between ΔNDVI values in this area in March and in February ($r_P = 0.895$). We therefore retained ΔNDVI values of March as geolocator data suggested that Barn swallows from our population should mainly cross the Sahara and the Mediterranean Sea in this month (R. Ambrosini, unpublished data).

Modelling population dynamics

Variation in the mean number of breeding pairs reconstructed by our procedure matched the observed data with great accuracy (pseudo-$R^2 = 0.882$). Standard errors calculated by our procedure largely overlap with standard errors of the mean number of breeding pairs estimated at each year by annual censuses (Fig. 3).

Effect	Coef.	Adjusted SE	95% CI
Intercept	-0.620	0.403	-1.410, 0.170
ΔNDVI Tunisia March	24.993	9.621	6.136, 43.850
ΔNDVI Wintering October	41.542	20.118	2.110, 80.974

Standard errors and 95 % confidence interval of coefficients obtained from the model averaging are also shown ; Pseudo-R^2 = 0.606

Table 1 Coefficients from the model averaging of the two best models (ΔAICc < 2) of year-to-year differences in the mean number of breeding Barn swallows according to year-to-year differences in environmental conditions.

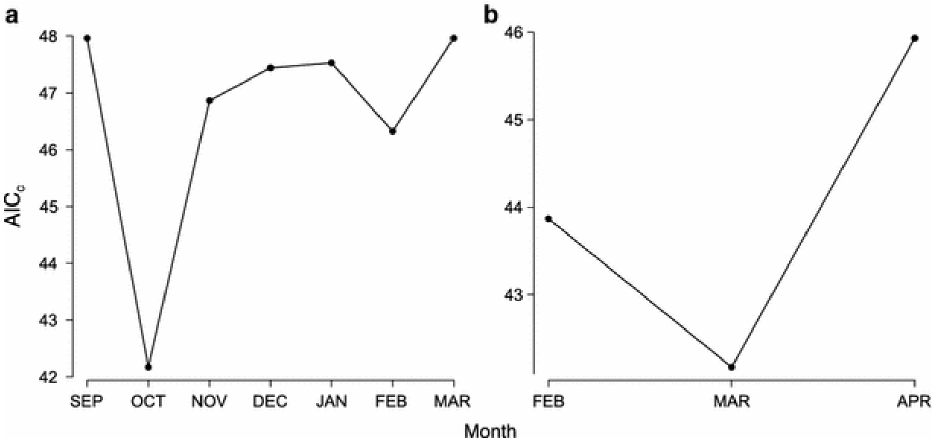

Fig. 2 Variation in AICc values of models including ΔNDVI values for different months in a the wintering areas of Barn swallows, and b the Tunisia area.

3.5 Discussion

In this work we aimed at assessing the critical stages of the annual life-cycle that influence the size of a geographical population of Barn swallow breeding in Northern Italy. We stress that in interpreting the results of our analysis, it must be taken in mind that our model is based on differences in population size, and not on population size per se. In particular, in a model based on differences in population size, the intercept models a constant variation (decline) in the population not accounted for by any predictor, the year effect models a variation in this constant decline (i.e., it indicates a non-linear decline), the ΔNDVI effects model variation in population size related to variation in ecological conditions (see also ‘‘Methods’’).

Our results suggest that ecological conditions encountered by individuals at their arrival at their wintering ground in sub-Saharan Africa and during spring migration in North Africa are the main determinants of year-to-year variation in the mean number of breeding pairs, which we consider an estimate of population size. These findings differ from those of Ockendon et al. (2014), which investigated the effect of NDVI on population dynamics of Barn swallows breeding in the United Kingdom, but did not find any effect of NDVI in the wintering quarters on annual population variation. However, the authors of this paper admit that this lack of any effect of NDVI may be consequence of the lack of information on the precise position of wintering quarters of their population, which forced them to perform the analyses at a very large spatial scale. Precise knowledge of the wintering areas of the geographical populations of long-distance migrants may therefore be necessary to correctly assess the relative importance of ecological conditions faced by populations in different phases of their annual life-cycle on population dynamics. Alternative explanations can however be put forward. Barn swallow populations breeding in Italy and UK are known to winter in different parts of Africa (Ambrosini et al. 2009, 2011), and therefore environmental conditions faced by UK populations during wintering may influence survival to a lesser extent than those faced by Italian populations. In addition, Ockendon and co-workers used average conditions during the wintering period to model winter survival, while our analysis disclosed that the effect of NDVI markedly varied between consecutive months, so that they may have neglected the effect of environmental conditions during critical periods within wintering. Indeed, one novel finding from our work is that NDVI values at wintering grounds at arrival of Barn swallows from autumn migration had a stronger influence on year-to-year variation in population size than NDVI values in other months (Fig. 2a). In addition, NDVI values in the wintering grounds in October were weakly correlated with those of other months ($|r_P| \leq 0.10$). These findings suggest that ecological condition encountered by Barn swallows at arrival in the wintering grounds from autumn migration may have a larger impact on annual survival than those faced during wintering. This may occur because at arrival birds are exhausted from the long migration journey, and may not recover under poor ecological conditions. The vast majority of the studies conducted so far on the impact of ecological conditions in the wintering grounds on population dynamics, took into account conditions during the entire winter (Boano et al. 2004; Szép and Møller 2005; Gordo and Sanz 2008; Grande et al. 2009) or condition immediately before departure for spring migration (Gordo and Sanz 2008). Conversely, the effect of ecological conditions at arrival to the wintering grounds has been mainly overlooked. Indeed, to the best of our knowledge, we are aware of only one previous study showing an effect of ecological conditions at arrival from crossing an ecological barrier on population dynamics of the white stork (Schaub et al. 2005, see ‘‘Introduction’’).

We also found that NDVI values of areas crossed by Barn swallows during migration influenced population size. This is coherent to the findings by Szép and Møller (2005), which found that NDVI data in northern Africa were related to annual survival rates of a Danish Barn swallow population. Similarly, Balbontín et al. (2009) found that arrival dates to the breeding grounds of a Spanish Barn swallow population, which in turn may influence reproductive performances and ultimately population size (Møller 1994), were significantly predicted by NDVI values at stopover sites in North Africa. During their migration from wintering to breeding grounds, Barn swallows must fly over two main ecological barriers, the Sahara desert and the Mediterranean Sea. Tunisia is along a migration route that implies direct crossing of both these barriers, and may function as a staging area whereby Barn swallows may refuel. Primary productivity during early spring in this area may therefore largely affect Barn swallow survival. In addition, primary productivity in Tunisia in

March may influence Barn swallow populations to a larger extent than that in Gibraltar, whose crossing implies following a route that mainly passes along the coast and over mainland. Gibraltar route may therefore be less challenging for Barn swallows, albeit longer (at least for the populations breeding in Northern Italy). In addition, a smaller proportion of individuals of the population we studied may migrate across Gibraltar than across Tunisia, thus reducing the impact of NDVI in this area on population dynamics.

Ecological conditions at the breeding grounds and during autumn migration seem not to affect population dynamics of Barn swallows. This is consistent with previous investigations of the relative effect of conditions during migration and wintering in determining annual survival of this and a closely related species (the Sand Martin *Riparia riparia* (Linnaeus 1758); Szép and Møller 2005; see also Gordo and Sanz 2008). Indeed, also in those studies, only condition during wintering and spring migration seemed to affect annual survival of adult individuals while those during autumn migration seemed to have no influence.

Admittedly, our study is based on a rather short time series of data, which was further reduced by the necessity to disregard 2 years of data, due to unavailability of MODIS-NDVI data before 2000. Nevertheless, the model averaging procedure clearly identified only two models as the best ones, and suggested that the others were by far less supported (Table S1 in ESM). In addition, our model had very good performance in interpolating the difference in the mean number of breeding pairs between years (pseudo-R^2 = 0.606; Table 1) and allowed us to reconstruct the observed population dynamics with great accuracy (pseudo-R^2 = 0.882; Fig. 3).

The analyses we ran also allowed us assessing the relative importance of NDVI data in different areas and months in determining population trend. Indeed, by rerunning the procedure used to model population dynamics while setting one or more of the model coefficients to zero, we could simulate how population dynamics would have appeared if no change had happened in ecological conditions in a given area. The results of this exercise showed that only models including the intercept were able to capture the decline that actually occurred in the populations, while all models not including the intercept predicted an increase, rather than a decrease of the population (Fig. 4). This occurred because NDVI in October in the wintering area of our Barn swallow population was almost stable during the study period, NDVI in Tunisia in March increased (Fig. 5), and coefficients of the final model for ΔNDVI values in both areas were positive (Table 1).

To correctly interpret the results from these simulations, it should be taken in mind that our final model was based on differences in ecological conditions between consecutive years, and predicted year-to-year changes in population size. Hence, if the ecological conditions considered in the final model had been the main drivers of population dynamics, a null variation in ecological conditions would have determined a null variation in population size (see also above and ‘‘Methods’’). These simulations clearly indicated that this was not the case, and that population declined independently from year-to-year variation in NDVI. However, models including actual NDVI values rather than differences were by far less supported by the data. In addition, simulation including ΔNDVI values had a better fit than those assuming a constant decline (i.e., including the intercept only), so that variation in NDVI seems to account for year-to-year variation around the general negative trend.

In summary, the actual determinants of the steep decline suffered by our Barn swallow population in recent years remain unclear, since the variations in NDVI values that occurred in the wintering grounds and along migration routes did not account for the observed sharp decline. Nonetheless, environmental conditions in this area seem to influence at least variation around the long-term decline at a larger extent than variation in ecological condition at the breeding grounds (Sicurella et al. 2014). Conditions during spring migration seem to affect population dynamics more than those during autumn migration, and there are critical periods during wintering that affect population dynamics. For the Barn swallow population we studied, this critical period coincides with the arrival at the wintering grounds, but further studies are needed to assess if this finding can be generalized to other populations of the same species and to other species. Hence, modelling population dynamics only according to average conditions faced by migrants during the whole wintering or just before departure for spring migration may prevent identifying the critical periods of the life-cycle that most influence population dynamics.

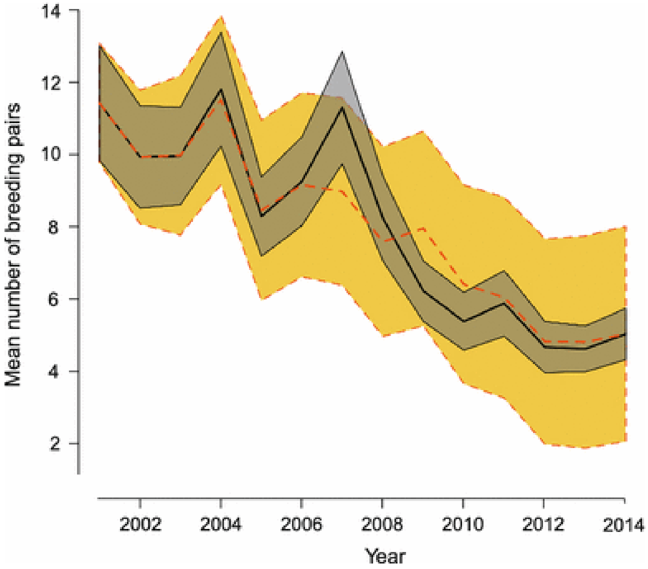

Fig. 3 Mean number of breeding pairs per farm observed during annual censuses (solid line) and estimated by the model (dashed line). The solid band around the solid line represents one standard error plus or minus the observed values. The dashed band around the dashed line represents one standard error (estimated by 1000 simulations) plus or minus the estimated values.

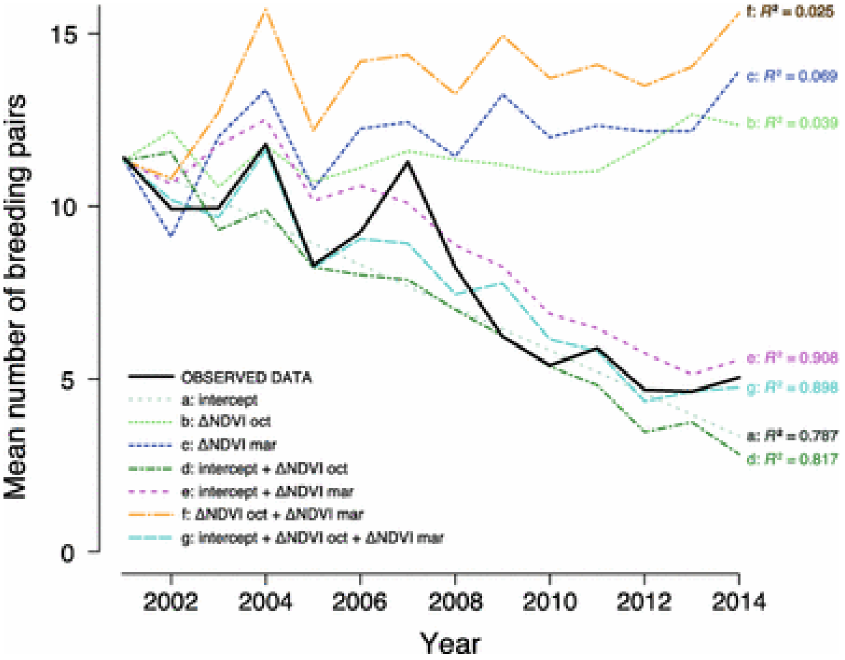

Fig. 4 Predicted annual variation in the mean number of breeding pairs from simulations produced by setting some coefficients of the model reported in Table 1 to zero. Pseudo-R^2 of each model was calculated as the squared correlation coefficient between observed and predicted number of pairs. The legend shows the predictors included in each model.

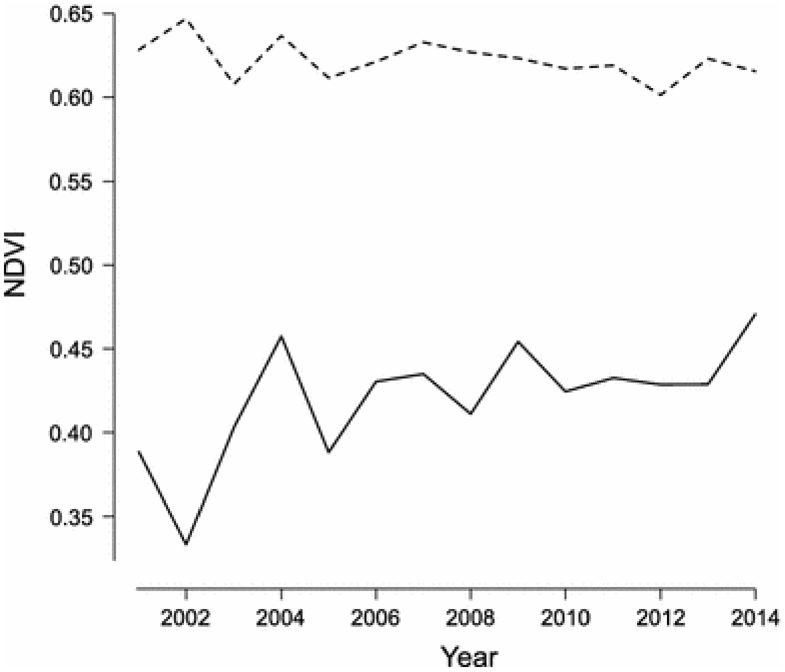

Fig. 5 Annual trend of NDVI values recorded in October in the wintering quarters (dashed line) and in March in the Tunisia area (solid line).

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Chapter 4

Migratory connectivity and effects of winter temperatures on migratory behaviour of the European robin *Erithacus rubecula*: a continent-wide analysis

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4.1 Abstract

1. Many partially migratory species show phenotypically divergent populations in terms of migratory behaviour, with climate hypothesized to be a major driver of such variability through its differential effects on sedentary and migratory individuals.
2. Based on long-term (1947-2011) bird ringing data, we analysed phenotypic differentiation of migratory behaviour among populations of the European robin *Erithacus rubecula* across Europe.
3. We showed that clusters of populations sharing breeding and wintering ranges varied from partial (British Isles and Western Europe, NW cluster) to completely migratory (Scandinavia and North-Eastern Europe, NE cluster).
4. Distance migrated by birds of the NE (but not of the NW) cluster decreased through time because of a north-eastwards shift in the wintering grounds. Moreover, when winter temperatures in the breeding areas were cold, individuals from the NE cluster also migrated longer distances, while those of the NW cluster moved over shorter distances.
5. Climatic conditions may therefore affect migratory behaviour of robins, although large geographical variation in response to climate seems to exist.

4.2 Introduction

Climate is changing rapidly with dramatic increases in temperature, in particular at high latitudes (IPCC 2013). Because the distribution of many species closely tracks isotherms during the coldest month (e.g. Root 1988), increasing temperatures have affected distribution, phenology and migration of birds and other organisms (Parmesan & Yohe 2003; Møller, Fiedler & Berthold 2010). Some of the most dramatic effects of climate change include shifts in the timing of migration by birds and other migratory organisms (review in Lehikoinen & Sparks 2010). However, the analysis of such phenotypic variation has rarely been extended to the behaviour of individuals.

Amateur and professional ornithologists have ringed millions of birds for more than 100 years providing a unique database of capture and recovery data. Surprisingly, these data have only been analysed to a small extent to investigate the effects and the consequences of climate change (but see Ambrosini *et al.* 2011, 2014). A few studies on Northern Hemisphere migrants have demonstrated reductions in migration distance over time, most notably in short-distance migratory birds (Fiedler, Bairlein & Köppen 2005; Maclean et al. 2008; Visser *et al.* 2009; Lehikoinen et al. 2013), but also in a long-distance migrant (Ambrosini *et al.* 2011), coherent with patterns of global warming. Moreover, Visser *et al.* (2009) provided a link between migration and climate change by showing that short-distance migrants wintered closer to their Dutch breeding grounds in years with milder winters. However, that study was limited to one breeding area only, while other studies lacked an explicit mechanistic link between migration and climate change.

Migration entails marked costs, mainly in terms of mortality, but so does residency during periods of severe winter weather (Newton 2008). However, several studies suggest that a climate warming scenario should result in fewer migrants (e.g. Pulido, Berthold & van Noordwijk 1996; Pulido & Berthold 2010), but also lower mortality of residents thanks to warmer winters (e.g. Sanz-Aguilar *et al.* 2012). Indeed, populations of birds can change from partially migratory to resident in a few generations, as shown by selection experiments (Pulido, Berthold & van Noordwijk 1996). Because polymorphic populations of migrants and residents are common in partial migrants (Chapman *et al.* 2011; Pulido 2011), rapid changes in current climatic conditions should result in rapid changes in migratory behaviour.

Here we analysed an existing database on migration of individuals in order to quantify the effects of climate change on the proportion of individuals that migrated ("migration propensity" hereafter) and on migration distance. To this end, we developed a novel framework for the statistical analyses of ring recoveries. We used a large database of ring recoveries of the European robin *Erithacus rubecula* L (hereafter robin), which is a model species for studies of partial migration (Adriaensen & Dhondt 1990) for which genetic variation in migratory behaviour has been demonstrated (Biebach 1983).

Winter temperatures are likely to be the most important ultimate selection pressure affecting migration propensity and distance in birds (Visser *et al.* 2009). However, winter temperatures in the breeding areas are not actually experienced by individuals that migrated from the study area in autumn, and are experienced by resident individuals only after they had decided not to migrate. We therefore aimed at assessing the potential mechanisms driving variation in migration propensity and distance according to winter temperatures. For instance, robins may use summer or autumn temperatures (i.e. the temperature actually perceived by birds soon before autumn migration; robin migration peaks in September-October; Cramp 2004) as proxies of winter temperatures and adjust their migration behaviour accordingly. Alternatively, variation in migratory behaviour according to winter temperatures may be the consequence of differential mortality of resident and migratory birds. Indeed, migratory behaviour is under genetic control in many bird species (Berthold 1996; Newton 2008), and, therefore, winter temperatures may affect the genetic structure of a population. For example, resident individuals or those migrating shorter distances could increase in the population in the winter following a mild winter season, resulting in a population-level decline of migration propensity and distance. Such changes may translate to time-lagged effects of winter temperatures on mean migration propensity and distance of a population (Dhondt 1983).

Based on these hypotheses, we analysed migration propensity and distance according to summer and autumn temperatures of year *i* at the breeding grounds, whose effect would suggest phenotypically plastic adjustment of migration behaviour. In addition, we analysed migration propensity and distance according to winter temperature in the breeding grounds in year *i*-1, whose effect would suggest that changes in migration behaviour are driven, at least partly, by differential mortality of resident and migratory individuals. Finally, to gain insight into the potential mechanisms driving a plastic adjustment of migratory behaviour according to winter temperatures, we investigated temporal autocorrelation in temperatures between years or seasons. Temporal autocorrelation of temperatures may in fact allow birds to forecast winter environmental conditions and to adjust their migration behaviour accordingly. We therefore investigated whether summer and autumn temperatures of year *i* in the breeding areas predicted winter temperatures of year *i* in the same areas, and whether winter temperatures in year *i*-1 predicted winter temperatures in year *i*.

Overall, the objectives of this study were to: 1) identify clusters of migrants with similar migratory behaviour (i.e. similar breeding and wintering ranges); 2) identify differences in migratory propensity and migration distance among clusters; 3) link migration propensity and distance to temperature in the breeding areas during the coldest winter month. Migration propensity and distance were expected to increase during severe winters compared to milder ones; 4) assess long-term shifts in breeding or wintering grounds of populations; and 5) identify potential mechanisms that may explain variation in migration propensity and distance of individuals according to winter temperatures in the breeding areas.

4.3 Materials and Methods

Dataset

For individually ringed birds, the EURING Data Bank (EDB) includes information on date and locality at ringing, as well as subsequent encounters ('ring recoveries') of birds ringed in Europe. Hence, ring recoveries include both ringing and finding information of any bird that has been re-encountered. In 2012, we obtained from the EDB all records for robins with at least one record in April-June and one record in November-February, i.e. in the focal months of reproduction and wintering for robins (Cramp *et al.* 2004). Before the analyses, we carefully checked for data consistency and excluded any dubious case (e.g. individuals recovered at sea). In addition, in order to reduce heterogeneity, we selected all records from individuals shot or found dead in winter and discarded the rest (see Supporting Information for further details). The final database used for the analyses consisted of two geographical locations (one for breeding and one for wintering) for 1111 robins in the period 1947-2011. Supporting Information provides additional details on the rationale behind this selection procedure as well as on the fields of EURING code used for data selection.

Migration distance and migratory connectivity

To identify geographical populations of robins with similar migration tactics, we applied the method proposed by Ambrosini, Møller & Saino (2009) for the analysis of migratory connectivity. Migratory connectivity is the degree to which individuals from the same breeding site migrate to the same wintering site (Webster *et al.* 2002; Trierweiler *et al.* 2014), and its intensity can be assessed by a Mantel test. Since the analyses showed evidence for connectivity (see Results), we identified the main clusters in which the population could be divided which represent groups of robins that both breed and winter close together. We emphasize that this analysis is based only on the reciprocal position of individuals both at breeding and wintering grounds, and it does not take into account the actual distance migrated by each individual. Supporting Information and Figure S1 provide further details on these analyses.

Migration distance was calculated as the great circle (orthodrome) distance between breeding and wintering locations of each individual. The radius of the home range of robins was estimated to be 0.571 km, corresponding to the geometric mean natal dispersal distance in the UK (Paradis *et al.* 1998). Robins that were found in winter within this distance from the breeding location were considered residents, while those found at longer distances were considered migratory. Although the geometric mean is an unbiased estimator of dispersal distance (Paradis *et al.* 1998), the choice of threshold distance may be considered arbitrary, so we checked for consistency of our results by re-running the analyses with different thresholds (namely 1, 2, 3, 5, 7, 10, 20, 30, 50, 70 and 100 km).

Climatic data

We used the R package *RNCEP* (Kemp *et al.* 2011) to retrieve temperature data from the NCEP/NCAR Reanalysis 1 model (parameter 'air.sig995' at level 'surface', representing air temperature at the surface of the Earth) for November-February in Europe. This model provides temperature data with a global scale at a spatial resolution of 2.5 × 2.5 ° latitude × longitude ('cells' hereafter) for the period 1948-2011 (robin records during winter spanned 1949-2011, so no data had to be discarded). For each cell, we calculated mean monthly temperatures for November-February, and for each winter (i.e. from November of year *i* to February of year *i* + 1) we selected the coldest of these months as predictor in the analyses. Temperatures were centred within cells in order to obtain temperature anomalies. We then assigned to each robin the temperature anomaly of the coldest month of the winter (November-February) when it was recaptured, recorded in the cell where it was found during the breeding season. For example, if a bird was found breeding in cell A in 1986, and wintering in 1987 in cell B, we determined for cell A the temperature anomaly of the coldest month of the winter 1987 (i.e. from November 1987 to February 1988). Hereafter, we refer to these temperature anomalies as 'winter temperatures'. The rationale behind this procedure is that individuals are expected to be faithful to their breeding grounds (at least at the coarse spatial scale of 2.5° × 2.5° latitude × longitude that we are considering), but would move longer distances from the breeding grounds according to the harshness of the climatic conditions of a given winter at the breeding grounds (Visser *et al.* 2009).

We also calculated monthly mean temperature anomalies at each cell for June-October and selected: 1) the warmest month in each summer (i.e. June-August of year *i*, 'summer temperatures') and 2) the coldest month in each autumn (September-October of year *i*, 'autumn temperatures'). Finally, we also associated winter temperature anomalies of winter *i*-1 to robin data in winter *i* to investigate the effect of harshness of the preceding winter on migration behaviour.

Population indices

We obtained breeding population indices for robins from Austria, Czech Republic, Denmark, Finland, France, Germany, Hungary, Netherlands, Norway, Poland, Spain, Sweden (Cuervo & Møller 2013), and from United Kingdom (data kindly provided by the British Trust for Ornithology) (Figure S2). We then associated to each individual the population index of the country where it was observed during the breeding season and for the year when it was found at the wintering grounds (winter *i*, see above). This information was available for 357 out of 1111 individuals (32.1%). Population indices were in all cases expressed as the proportional variation in population consistency with respect to a reference year, which however differed among countries. We rescaled these indices so that the population index in the reference year always equalled zero. In this way, marginal means for the other variables estimated the effect under scrutiny at the population level in the reference year for that country.

Regression models of migration propensity and distance

We first analysed whether an individual stayed during winter within its breeding home range (i.e. moved less than 0.571 km) or migrated (i.e. moved 0.571 km or more; 'migration propensity' hereafter). Migration propensity was modelled according to year (the winter when an individual was recovered in the wintering grounds, see above), winter temperatures and position (latitude and longitude) of the breeding grounds by using Generalized Linear Mixed Models (GLMMs) assuming a binomial error distribution. Cell identity was entered as a random effect accounting for residual spatial variability in migration propensity not accounted for by the latitudinal and longitudinal gradients. This analysis was run only on the North-West cluster because almost all individuals in the North-East cluster migrated (see Results).

We analysed the distance travelled by individuals that migrated (i.e. moved more than 0.571 km; individuals that moved less than 0.571 km were excluded) according to the same predictors listed above plus cluster identity and its interactions with the other predictors. For this analysis, we used a Linear Mixed Model (LMM) assuming a Gaussian error distribution, whereby cell identity was entered as a random effect and variances were estimated independently for each cluster to account for heterogeneity in variance among clusters (details not shown). The frequency distribution of migration distance had a large excess of very small values (Figure S3). However, plots of model residuals did not show marked deviations from normality (Figure S4), so no data transformation was applied.

Covariates included in all models were centred within cluster before analyses and were only weakly correlated ($|r| \leq 0.126$ in all cases). Hence this linear model was able to disentangle the independent effects of long-term trend in temperatures (or of long-term shift in any other variable not included in the analyses), which was accounted for by the year covariate, from the year-to-year variation in temperatures, which was accounted for by the winter temperature covariate.

Finally, we estimated effect size of each predictor as Pearson's correlation coefficient calculated from model coefficients and associated SE according to the formulae provided in Nakagawa and Cuthill (2007) and coefficients of determination of models (pseudo-R^2) based on the likelihood-ratio test and calculated according to Negelkerke (1991) by considering (conditional) or not considering (marginal) variance explained by random effects.

Potential mechanisms driving variation in migration propensity and distance according to winter temperatures

In order to assess the potential mechanisms driving changes in migration propensity and distance according to winter temperatures, we re-ran the analyses by including: 1) summer and autumn temperature of year i instead of winter temperatures in year i; and 2) winter temperatures of year i-1 instead of winter temperatures in year *i*. In addition, we investigated whether winter temperatures correlated with summer or autumn temperatures or with winter temperatures in the preceding year. To this end we mapped values of the partial correlation coefficients (while removing the effect of year) between winter temperature on one hand and summer and autumn temperatures and winter temperatures in the preceding year on the other hand.

Also in these analyses, temperatures included in all models as predictors were only weakly correlated with year ($|r| \leq 0.363$; all correlation coefficients calculated on data centred within cluster).

Accounting for other effects potentially influencing migration propensity and distance

Population size may alter the proportion of migrants and the distance they travel by affecting intraspecific competition for resources during winter. In addition, population size may bias the estimates of the variation in migration propensity and in distance travelled because e.g. reduced competition during winter may prompt a larger proportion of individuals to spend winter in their breeding grounds. To check for robustness of our results against this potential source of bias we re-ran models of migration propensity and distance with population indices as a covariate. Country was included as a further random grouping factor, beside cell identity, to account for repeated measures of the same population index for all individuals in a country. However, the random structure of each model was simplified by comparing AIC values of models including the two random terms or only one of them (see Zuur *et al.* 2009 for the rationale behind this procedure).

Juveniles may show higher migration propensity than adults because they are usually socially subordinate and therefore less likely to acquire the best territories, which in turn offer the best chances to survive winter (Newton 2008). Individuals were therefore classified as adults or juveniles according to the estimated age when they were found dead in winter (see Supporting Information for details on how birds were classified as adults or juveniles).

Information on potentially confounding effects may be unavailable for all individuals, and, consequently, analyses accounting for them may fail in detecting statistically significant effects because of low statistical power due to reduced sample size. Hence, we tested the power of analyses by re-running 499 times the same model fitted on the whole dataset (i.e. without the potentially confounding effect) on a subset of data randomly chosen from the whole dataset. At each run we randomly selected: 1) a number of individuals equal to that used in the model accounting for the confounding effect; and 2) a number of individuals *in each cluster* equal to that in the analysis accounting for confounding effects. Power was then calculated as the proportion of tests where an effect that was significant for the whole dataset was statistically significant also for the subset of data (with $\alpha = 0.05$).

Long-term temporal trends in migration distance and patterns of migratory connectivity

We investigated temporal shifts in breeding or wintering grounds of robins by multivariate regression models where latitude and longitude in the breeding or the wintering grounds were regressed on year. Multivariate regressions were also used to investigate patterns of individual migration within cluster by modelling position (latitude and longitude) in the wintering grounds according to latitude and longitude in the breeding grounds. These models therefore indicated how a shift by 1° in latitude or longitude at the breeding grounds translates into the position of individuals at the wintering grounds.

In all these analyses, qq-plots indicated that model residuals deviated from normality (details not shown). Significance of multivariate models was therefore assessed by a randomization approach whereby we randomly shuffled values of the year covariate 999 times and then assessed significance of the model by comparing the rank of the Pillai's lambda coefficient of the model fitted on original data with the distribution of values obtained from the randomization. All analyses were run in R 3.0.1 (R Core Team 2013) using packages *lme4* and *nlme*.

4.4 Results

Patterns of migratory connectivity and clustering of populations

The Mantel test disclosed statistically significant migratory connectivity ($r_M = 0.479$, $P < 0.001$, 999 permutations), and cluster analysis indicated that robins could be assigned to two clusters (*oasw* = 0.497, indicating "weak" cluster structure; Rousseeuw 1987; Figure 1). The first cluster included 515 birds, mainly from UK and Belgium (North-West cluster hereafter). This cluster consisted of 80.0% of birds breeding in the UK or Ireland, probably belonging to the *melophilus* subspecies, and of 20.0% of birds breeding on the continent, probably belonging to the *rubecula* subspecies. In addition, 43.7% of birds in this cluster were sedentary (when the migration distance threshold was set to 0.571 km; Table 1).

The second cluster included 596 birds, mainly from Germany, Czech Republic and Poland (North-East cluster hereafter), which were almost entirely migratory (99.3% moved more than 0.571 km; Table 1). Difference in migration propensity between clusters was statistically significant ($\chi^2_1 = 376.77$, $P < 0.001$). Records classified in either cluster spanned very similar ranges of time (Figure S5).

Variation in migration propensity

Migration propensity of birds in the NW cluster increased over time, but was unaffected by winter temperature anomalies (Table 2). The temporal increase in migratory propensity was confirmed in the analyses including

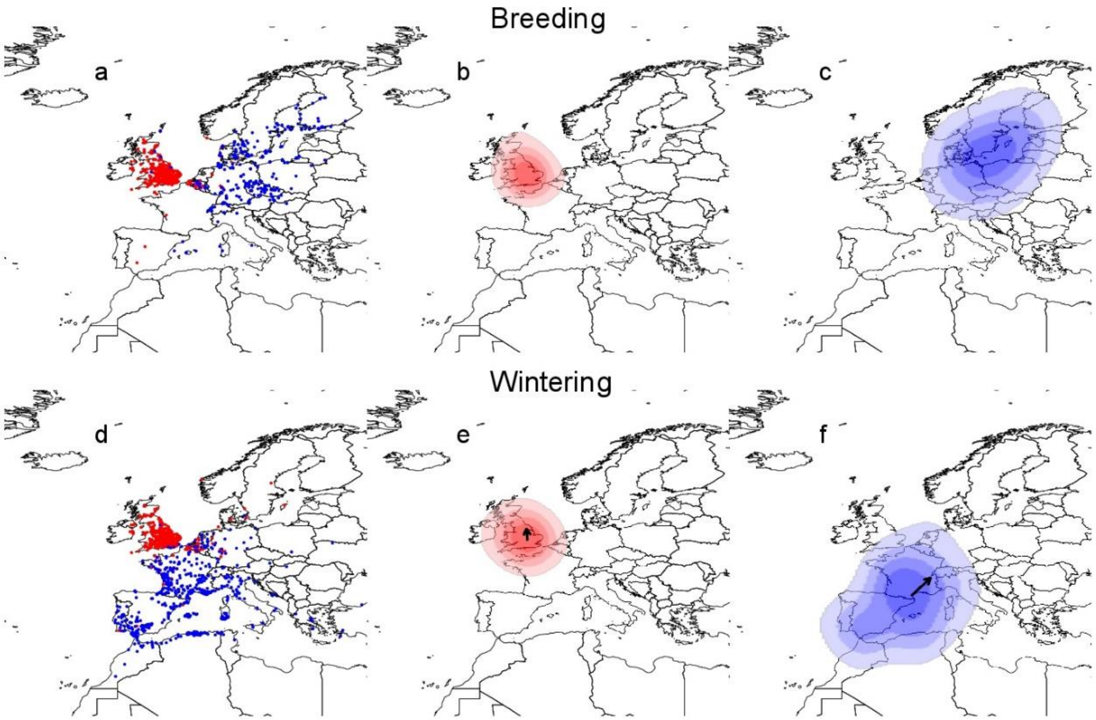

Figure 1. Position of individual robins in (a) their breeding and (d) their wintering grounds. Red = NW cluster (n = 560), blue = NE cluster (n = 702). Kernel density plots of the clusters identified by the migratory connectivity analysis during breeding (b-c) and wintering (e-f). Arrows indicate direction of the shifts in the wintering grounds.

Figure 2 Migration distance (km, excluding birds that moved less than 0.571 km, see Methods) as a function of winter temperature in the breeding areas (a-b), winter of recovery (c-d), breeding latitude (e-f) and breeding longitude (g-h). Regression lines are shown for ease of interpretation.

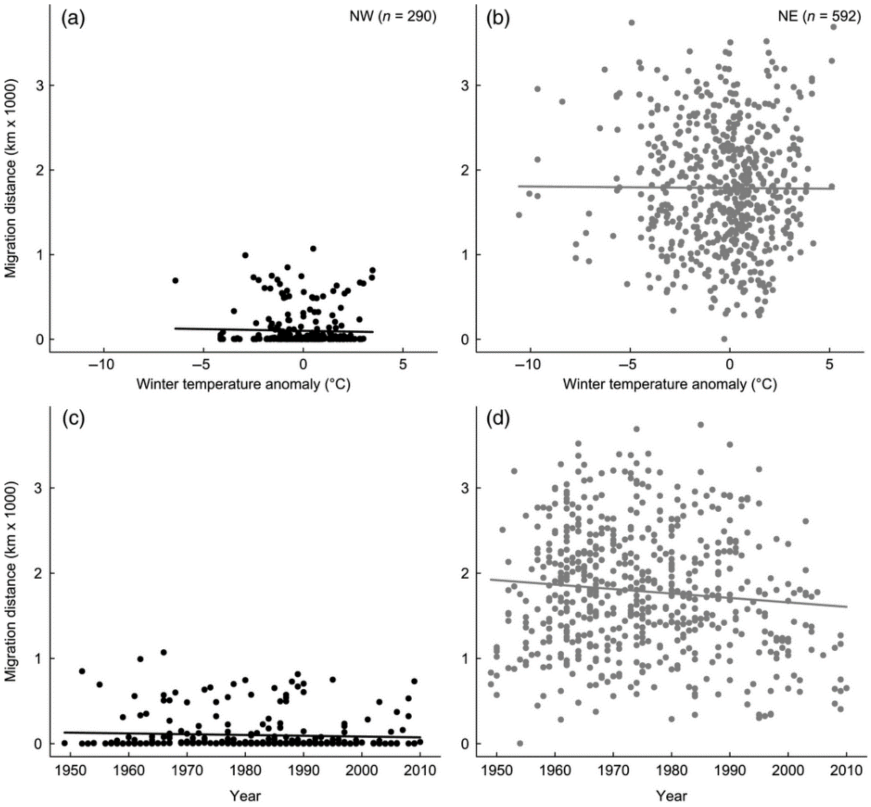

Figure 2 (A –D)

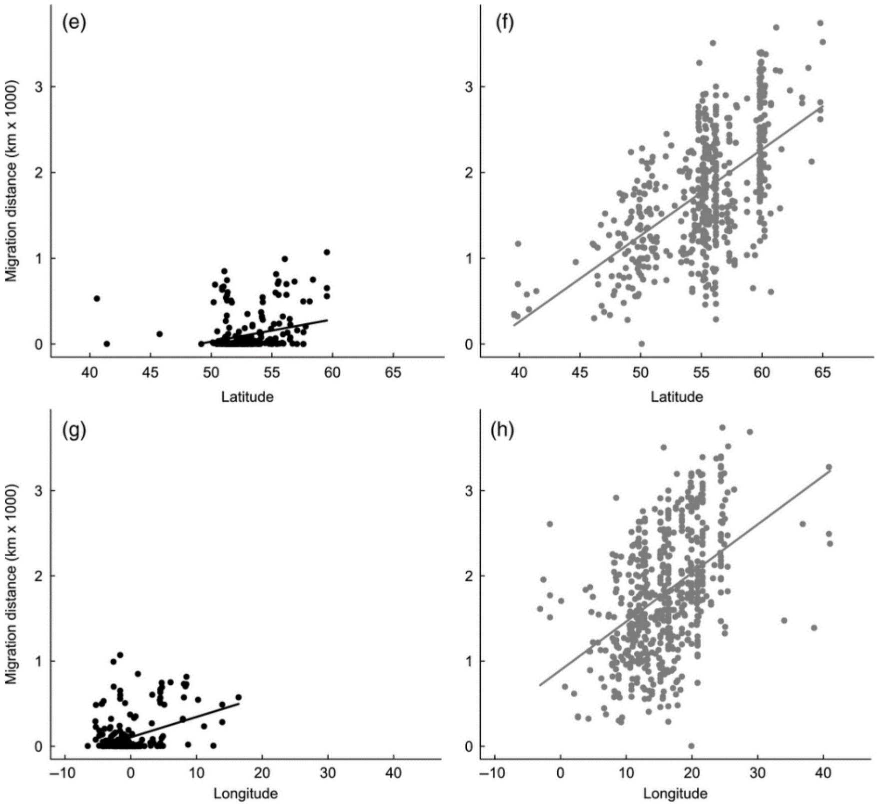
(e)
(f)
(g)
(h)
Migration distance (km x 1000)
0
1
2
3
Latitude
40
45
50
55
60
65
Longitude
−10
0
10
20
30
40

Figure 2 (E-H)

population indices and age of birds (Supporting Information). However, the effect of year was no longer statistically significant when the threshold used to separate sedentary from migratory birds was set to values ≥ 3 km (Supporting Information). Migration propensity also increased with latitude and longitude within this cluster (Table 2). Significance of the latitude and longitude effects was confirmed with threshold values up to 100 km and in the analysis including age of birds, but not in that including population indices (Supporting Information). However, in the latter analysis the effect of latitude was similar to that recorded for the whole sample (see Tables 2 and S1), and power analysis suggested that lack of statistical significance of these effects may be due to low power (≤ 0.595) of the tests run on a sample of reduced size. The model including population indices also showed that the proportion of migratory robins decreased at increasing breeding population indices (Table S1). Finally, including age of birds into the model did not affect the results, and showed that adult and juvenile robins of the NW cluster did not differ in migratory propensity (Supporting Information).

Table 1. Summary results of migration behaviour of individuals from each cluster.

Cluster	Sample size	Proportion of migrants (% of individuals that moved ≥ 0.571 m)	Mean migration distance (SE) (km)	Range of migration distances (min – max) (km)
NW	515	56.3	100.1(12.1)	1.0-1070.6
NE	596	99.3	1787.6(29.3)	3.0-3740.8

Table 2. Fixed effects of the binomial GLMM model of migration propensity (proportion of individuals that moved more than 0.571 km; see Methods) of robins in the NW cluster. All variables were centred to their mean values within the cluster before the analysis. Sample size is 515 individuals. Significance of each term was assessed by likelihood ratio tests (χ^2 values, associated *df* and *P*). Pseudo-R^2 values were calculated according to Negelkerke (1991) while effect sizes according to Nakagawa and Cuthill (2007). The random effect (grid identity) variance is 0.121.

Effect	χ^2	d.f.	*P*	Coef.	SE	Effect size
Intercept	4.70	1	0.030	0.421	0.152**	
Year	23.66	1	<0.001	0.034	0.007***	0.208
Winter temperature	1.58	1	0.209	0.077	0.061	0.055
Latitude	4.58	1	0.032	0.124	0.057	0.093
Longitude	5.45	1	0.020	0.102	0.043*	0.101

AIC = 672.4, Marginal Pseudo-R^2 = 0.098, Conditional Pseudo-R^2 = 0.113

Variation in migration distance

Migration distance differed between clusters, being longer in the NE than in the NW cluster (Tables 1 and 3). Winter temperature differentially affected migration distance depending on cluster (Table 3; Figure 2a-b). Indeed, milder winter temperatures determined a statistically significant decrease in migration distance of individuals from the NW cluster, but a statistically significant increase of those from the NE one (Table 3). The effect size of temperature on migration distance of individuals from the NE cluster was however smaller than that for individuals from the NW cluster (Table 3).

The statistical significance of the cluster by winter temperature interaction was not confirmed in the analysis including population indices (Table S2). However, this could be due to restriction of the dataset to recent (mainly post-eighties) years for most countries (Figure S2), as suggested by the fact that the same effect was not statistically significant in an analysis not including population index but restricted to the same dataset, and by the low power of the test run on a smaller sample (Supporting Information). Similarly, the cluster by winter temperature interaction became not statistically significant when the threshold was set to ≥ 30 km, probably due to a large reduction in sample size in the NW cluster which reduced the power of the test to 0.503 (see Supporting Information for details). In contrast, significance of the cluster by winter temperature interaction was confirmed by the analyses including age (Supporting Information).

Migration distance decreased through time in the NE, but not in the NW cluster (Table 2, Figure 2c-d). The effect of year on migration distance of individuals from the NE cluster was about 2.5 times larger than that of temperature. Conversely, the effect of temperature was about twice as large as that of year on individuals from the NW cluster. A reduction in migration distance was also confirmed in all analyses run on different data subsets (Supporting Information).

Migration distance increased with latitude in both clusters, but more so for the NE cluster than for the NW cluster (Table 2, Figure 2e-f). Finally, individuals of both clusters breeding more eastwards also migrated over longer distances (Table 2, Figure 2g-h).

Table 3. Linear Mixed Effect model of migration distance of robins (excluding birds that moved less than 0.571 km, see Methods). Sample size is 882 individuals (NW: n = 290, NE: n = 592). Covariates (year, winter temperature, latitude and longitude) were centred to their mean values before analyses. Significance of each term was assessed by likelihood ratio tests (χ^2 values, associated *df* and *P*). Asterisks denote coefficients that differ significantly (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) from zero, and different letters denote effects that differ significantly ($P < 0.05$) in post-hoc tests. Coefficients (marginal means and SEs) are reported for each cluster. Pseudo-R^2 values were calculated according to Negelkerke (1991) while effect sizes according to Nakagawa and Cuthill (2007). The random effect (grid identity) variance is 43318.0, and residual variance is 23072.0.

Effect	χ^2	d.f.	*P*	Cluster	Coef.	SE			Effect size
Cluster	1342.03	1	< 0.001	NW	179.588	31.203	***	a	
				NE	1800.708	44.252	***	b	
Year	1.22	1	0.269						
Winter temperature	5.14	1	0.023						
Latitude	10.78	1	0.001						
Longitude	12.54	1	< 0.001						
Cluster x Year	14.89	1	0.001	NW	-0.748	0.676		c	-0.029
				NE	-7.928	1.741	***	d	-0.094
Cluster x Winter temp.	8.66	1	0.003	NW	-13.642	6.017	*	e	-0.059
				NE	19.305	9.444	*	f	0.039
Cluster x Latitude	34.30	1	< 0.001	NW	22.714	6.917	***	g	0.086
				NE	81.516	7.664	***	h	0.269
Cluster x Longitude	2.33	1	0.127	NW	16.391	4.628	***		0.093
				NE	27.684	5.519	***		0.131

AIC = 12902.4, Marginal Pseudo-R^2 = 0.484, Conditional Pseudo-R^2 = 0.861

Analyses with summer and autumn temperatures and with winter temperatures in the preceding yearSummer or autumn temperatures or winter temperatures in the preceding year had no detectable effect on migration propensity of robins of the NW cluster ($\chi^2_1 \leq 2.22$, $P \geq 0.136$, |effect size| ≤ 0.066; other details not shown). Similarly, interaction effects between cluster and summer, autumn or winter temperatures in the preceding year had no statistically significant effect on migration distance ($\chi^2_1 \leq 2.27$, $P \geq 0.132$, |effect size| ≤ 0.043; effect sizes were here calculated based on slopes for either cluster; other details not shown). After removal of the cluster by temperature interaction, migration distance decreased at higher summer temperatures, (coef. ± SE: -16.019 ±8.292; $\chi^2_1 = 4.22$, $P = 0.040$, effect size = -0.054; other details not shown), with an effect size comparable to that of winter temperature in cluster NW (Table 3). This effect was not confirmed in the analysis including population indices and age of birds ($\chi^2_1 \leq 0.26$, $P \geq 0.612$), and its statistical significance varied from significant to marginally not significant (P-values between 0.013 and 0.069) at varying threshold levels, while effect size ranged between -0.053 and -0.085. Conversely, autumn or winter temperatures in the preceding year had no detectable effect even after removal of non-significant interactions ($\chi^2_1 \leq 0.16$, $P \geq 0.692$, |effect size| ≤ 0.010; other details not shown).

Shifts in breeding and wintering grounds and patterns of individual movements within clusters

We observed no shift in breeding grounds of NW or NE cluster (Pillai's $\lambda \leq 0.013$, $P_{\text{rand}} \geq 0.163$). In contrast, wintering grounds of both clusters shifted. In particular, those of the NW cluster shifted northwards (azimuth 355.6°) by 0.04° per year (Pillai's $\lambda = 0.046$, $P_{\text{rand}} = 0.001$), while those of the NE cluster shifted toward north-east (azimuth 47.7°) by 0.08° per year (Pillai's $\lambda = 0.035$, $P_{\text{rand}} < 0.001$) (Figure 1).

Wintering location of individuals of both clusters changed significantly with both latitude and longitude of the breeding grounds (Pillai's $\lambda \geq 0.021$, $P_{\text{rand}} < 0.001$). Coefficients of the model indicated that, for individuals of the NW cluster, a 1° increase in breeding latitude was associated with a 0.85° increase in wintering latitude (azimuth of the direction of shift 353.3°). In addition, an eastwards shift of 1° in breeding longitude translated into a 0.77° eastwards shift in wintering longitude (azimuth 91.5°). Hence, a unit increase in both breeding latitude and longitude was associated with a net shift by 1.20° towards northeast (azimuth 46.4°) of the wintering grounds of individuals of the NW cluster.

For individuals of the NE cluster, a 1° increase in breeding latitude translated into only a 0.42° shift towards west-northwest (azimuth 293.1°) and an eastward shift by 1° in breeding longitude was associated with an eastward shift of 0.47° (azimuth 94.7°) in wintering location. Hence, a unit increase in both breeding latitude and longitude determined a net shift of 0.15° towards northeast (azimuth 32.9°) in the wintering grounds of individuals of the NE cluster.

Hence, position in the wintering grounds of individuals from both clusters tends to mirror their position in the breeding grounds, but individuals breeding further northeast tend to migrate longer distance, and more so for individuals of the NE cluster than for those of the NW one.

Spatial variation of temperature anomalies

Winter temperatures showed generally increasing trends in Europe (Figure S6a). Detrended correlations between summer and winter temperatures indicated generally positive correlations in Northern Europe, and negative correlations in north-western Italy, Switzerland, southern France, Iberian Peninsula and the Mediterranean coast of Africa, from Morocco to Tunisia (Figure S6b). In contrast, detrended correlations between autumn and winter temperatures were generally negative across Europe (Figure S6c). Spatial pattern of variation in detrended correlations between winter temperature of consecutive years were correlated positively in northern Europe and negatively in the Iberian Peninsula and the western Mediterranean basin (Figure S6d).

4.5 Discussion

Spatial patterns of migratory connectivity and migratory behaviour

Many partially migratory species show phenotypic divergence in migratory behaviour among populations, with climate hypothesized to drive such divergence through effects on mortality (Newton 2008). Here we first found that European robins show migratory connectivity (see Ambrosini, Møller & Saino 2009 and Supporting Information), with two clusters of populations at continental scale, which show phenotypic differentiation in migration tactic ranging from partly to completely migratory. This result is consistent with previous knowledge of the movements of this species (Cramp et al. 2004) and with the results of Korner-Nievergelt, Liechti & Thorup (2014).

We observed that both migration propensity of individuals from the NW cluster and distance migrated by individuals of both clusters changed according to geographical position within the cluster, suggesting that individuals breeding to the north and the east were more likely to migrate (NW cluster) and moved longer distance (both clusters) than those breeding to the south and the west. This is expected based on the general pattern of variation in climatic conditions in Europe, with winter temperatures generally decreasing northwards and eastwards (Newton & Dale 1996). Interestingly, these patterns could be observed *within* the two clusters of individuals we identified, suggesting that geographical variation exists in migration behaviour of robins throughout Europe and occurs at different spatial scales.

While the increase in migration distance with longitude was similar in both clusters, an increase in latitude determined an increase of migration distance that was approximately eight times larger for individuals of the NE than for the NW cluster. This larger effect of latitude in the NE cluster is probably because robins breeding in eastern Scandinavia tended to winter further east than those from west (Cramp et al. 2004), and to the presence of the Mediterranean basin, which prevents individuals from the north-eastern part of this cluster to winter further northeast.

Temporal variation in migratory behaviour and the effects of population trends

We observed an increase in the proportion of migratory individuals in the NW cluster through time. This effect was confirmed in analyses including potentially confounding effects like population indices and age of birds, but disappeared when the threshold used to differentiate between sedentary and migratory robins was set to 3 km or more. This effect seems sensitive to the value of the threshold used to differentiate migratory and resident individuals, which, however, was chosen based on an estimate of home range of this species in the UK, i.e. in the area where the majority of individuals from the NW cluster breed. Therefore, caution is needed when considering this result.

We found a decrease in migration propensity in years when breeding population index was larger. This observation contrasts with the hypothesis that migratory individuals are mainly socially subordinates that could not find a suitable territory for spending the winter at their breeding grounds (Newton 2008). We can speculate that in years when general ecological conditions are better, not only populations increase, but also a larger fraction of birds may find suitable conditions for wintering at their breeding grounds.

Our continent-wide analysis also suggested that robins of the NE cluster tended to migrate a shorter distance in recent years, and that this long-term effect seemed due to an eastward shift in wintering grounds of this cluster during the last 60 years (Figure 1f). A northward shift of the wintering grounds of the NW cluster could also be detected (Figure 1e). However, this shift was much smaller, and probably did not determine a detectable reduction in the distance migrated by individuals of this cluster. The long-term reduction in migration distance observed in the NE cluster is consistent with the documented shift of the wintering ranges of migratory birds towards their breeding grounds due to climate change (Visser *et al.* 2009; Ambrosini *et al.* 2011). However, our analyses differentiated between long-term effects, potentially due to long-term variation in *any* of the ecologically relevant factors, including the generally increasing temperatures, and year-to-year variation in winter temperatures around the general trend, and identified a long-term effect determining a temporal reduction in migration distance for individuals of the NE cluster, and divergent short-term effects on individuals of either cluster.

Effects of climatic variability on migratory behaviour

We detected no effect of winter temperature on migration propensity of robins of the NW cluster, the only cluster for which this analysis could be run since the NE cluster included almost only migratory individuals. In addition, we observed differential effects of winter temperature on distance migrated by robins of different clusters (Table 3 and Figure 2a-b), since robins of the NW cluster migrated over longer distances in cold winters, while those of the NE cluster migrated over shorter distances. It is usually assumed that migratory birds should respond to favourable winter conditions by reducing migration distance or even by wintering at their breeding grounds in order to reduce the cost of long migrations (Newton 2009). However, for robins, the decision on whether to migrate or not may depend more on social interactions or resource availability in the local breeding territory than on general climatic conditions (Adriensen & Dohndt 1990). Admittedly, this hypothesis is speculative because local resource availability during winter should depend, among other factors, also on winter temperature. In addition, this hypothesis implies that socially dominant individuals should more likely become residents. However, we found no detectable effect of age on migration propensity, with adult robins migrating over longer, rather than shorter, distances than juveniles (see Supporting Information). Similarly, females should migrate more than males because they are socially subordinates (Adriensen & Dhondt 1990). Unfortunately, we could not test this latter hypothesis because the sex of individuals was unknown in our study. However, the fact that we could not distinguish male and female robins may have caused the unexpected age effect on migration distance. Indeed, since the analysis of migration distance is based only on individuals that migrated (i.e. moved ≥ 0.571 m), the adults may include a larger proportion of females than the juveniles, because most adult males are expected to show a lower migratory propensity and are therefore excluded from the analysis. Hence, the (unknown) sex ratio of adults included in the analysis of migration distance may be more female biased than that of juveniles, causing the unexpected result that, on average, old individuals migrate longer distances than young ones.

We observed a differential effect of winter temperature on migration distance in the two clusters, a discrepancy that is difficult to explain. On the one hand, the decrease in migration distance at increasing winter temperature observed for individuals from the NW cluster fits with the hypothesis of plastic migration behaviour of individuals, which should stay closer to their breeding grounds in favourable winters. On the other hand, individuals of the NE cluster showed an opposite behaviour. It may be speculated that individuals of the NW cluster, which are partial migrants and stay much closer to their breeding grounds than individuals of the NE cluster, show a greater plasticity in the response to variation in winter conditions than individuals from the NE cluster, which are almost entirely obligate migrants moving over long distances.

Why do populations differing in migratory behaviour respond differently to environmental cues?

Theoretical models of migration behaviour predict that the expression of migration or sedentariness should vary among populations living under markedly different environmental conditions and according to environmental variables (Pulido, Berthold & van Noordwijk 1996; Pulido 2011). The temperature of the coldest winter month in the breeding areas is a good proxy for the harshness of winter ecological conditions. However, our analyses seem to indicate that temperature influenced the distance travelled by individuals of different geographical populations in opposite ways, but did not influence their migration propensity. This may suggest that environmental conditions affect different aspects of migratory behaviour of individuals, and that different populations of robins showed different reaction norms to winter temperatures.

Short-distance migratory bird species are known to have advanced timing of spring migration more than long-distance ones in recent years, suggesting that they are better able to adjust their migration schedule to changing climatic conditions (Rubolini *et al.* 2007, Lehikoinen & Sparks 2010, Saino *et al.* 2011, Morganti 2015). Our findings suggest that similar patterns may occur at the within-species level. For instance, robin populations migrating over shorter distances (NW cluster) did not show long-term shifts in the geographical location of their wintering grounds, but reduced migration distance in cold winters. Conversely, populations that migrated longer distances and were almost completely migratory (NE cluster) showed a clear temporal shift in the location of their wintering grounds, and adjusted their migration behaviour to climate conditions oppositely to that of partially migratory populations. However, the relative size of these effects indicated that, in the partially migratory NW cluster, the effect of winter temperature on migration distance was twice as strong as the effect of the long-term shift in wintering grounds, while in the entirely migratory NE cluster the effect of the long term shift in wintering grounds was more than twice as strong as the effect of winter temperature. Hence, the adjustment of migration behaviour according to contingent climatic conditions may occur differentially also among different geographical populations of the same species and may depend on their relative degree of migratoriness.

Which Environmental cues affect migration behaviour?

The effect of winter temperature on migration behaviour of individuals may arise from phenotypically plastic responses or from differential selection in resident and migratory birds coupled with temporal autocorrelation of winter temperatures in consecutive years (see Introduction). In addition, winter temperatures at the breeding grounds are only experienced by residents, so that a phenotypic plastic response of robins may arise from their ability to anticipate overall winter harshness while they are still at the breeding grounds, i.e. in autumn or even earlier. Alternatively, some individuals may leave the breeding grounds during winter in direct response to harsh temperatures, as found in the European blackbird *Turdus merula* (Fudickar et al. 2013). To assess the mechanisms driving robin responses to winter climate, we re-ran the analyses by including summer and autumn temperatures, as well as winter temperature in the preceding year, and found that migration distance decreased at increasing summer temperature in both clusters. However, the summer temperature effect was not confirmed in analyses controlling for population index or age, and at different values of the threshold used to differentiate sedentary and migratory robins. Hence, the evidence that summer temperatures affect migration behaviour is weak. However, it may suggest that birds may use summer temperatures they experience at their breeding grounds to forecast future winter conditions and adjust their migration behaviour accordingly. Such long-term weather forecast may be based on correlations between summer and winter temperatures, which, albeit weak, are generally negative in Europe (Figure S6c). However, the analyses indicate that birds tend to migrate less after warm summers, and that warm summers are also associated with cold winters in most of Europe (Figure 6c). As a result, birds should migrate shorter distances in cold winters, as seems to occur for the NE but not for the NW cluster. Alternatively, we can speculate that winter temperatures show spatial autocorrelation throughout Europe, and that robins may decide to migrate farther if they experience harsh winter conditions in the place where they are. Hence, variation in migration distance may be the result of a direct response to contingent winter conditions, as has been recently demonstrated in the blackbird population (Fudickar *et al.* 2013). Unfortunately, the available data do not allow testing this hypothesis on robins because multiple recaptures of the same individuals within the same winter are very rare.

Methodological aspects

Analyses based on ring recoveries may be affected by several sources of bias, which ultimately derive from the large spatial and temporal heterogeneity in sampling effort (Fiedler 2003; Fiedler, Bairlein & Köppen

2005; Visser *et al.* 2009). Indeed, variation in ringing and recovery effort across Europe may blur the analyses because robin populations breeding in different parts of Europe segregate in the wintering quarters. Our analyses should however be robust with respect to this potential source of bias because: 1) we restricted our analyses only to birds shot or recovered dead, because they were always reported to the EDB by all national ringing schemes, and further selected the data to remove as much heterogeneity as possible (see Supporting Information); 2) clusters identified geographical populations with a connection between breeding and wintering ranges, and we incorporated this information in all analyses (see also Ambrosini *et al.* 2011 for a similar approach); 3) we accounted for possible spatial variation within clusters by including latitude and longitude as covariates and cell identity as a random factor in all analyses; and 4) temperature anomalies and dates were centred within cluster, so that analyses were unaffected by the differences in the time-span covered by ring recoveries in different clusters (Figure S3).

From a methodological point of view, the power analyses we applied to the tests run on different subsets of data demonstrated that a reduction of sample size dramatically lowered the power of the tests to detect effects. Hence, the analyses of ring recoveries should be based on the largest possible dataset, so that the attempt to remove as much heterogeneity as possible from the data should be carefully balanced with the necessity to use very large samples in order to detect true biological effects. In addition, the attempt to control for potentially confounding effects (e.g. population indices or age of bird) in the analysis should be carefully considered whenever inclusion of these effects determines a marked reduction in sample size because this information is not available for all individuals.

Concluding remarks

In conclusion, robin populations, which are only partially migratory and migrate short distances, only slightly shifted their wintering grounds, but seemed to respond to variation in winter temperature by year-to-year modification of migration distance. Conversely, individuals from the NE cluster, which almost entirely migrate long distances, have largely shifted their wintering grounds, and respond less tightly to variation in winter temperature than those of the NW cluster, although their response is opposite to what was expected. The novel analytic framework we developed allows extending previous findings by Visser *et al.* (2009) from a local to a continental scale and may prove suitable for retrospectively investigating the consequences of climate change across a broad range of migratory species, providing much needed information on the effects of climate change at the individual level.

4.6 Acknowledgments

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Data Accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.5fn37 (Ambrosini *et al.* 2016).

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PART II

Chapter 5

Effects of livestock farming on birds of rural areas in Europe

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5.1 Abstract

In the last decades, profound modifications of agricultural practices occurred in Europe, including the introduction of modern livestock farming. These modifications negatively affected the fauna of rural areas, as indicated by the large demographic declines suffered by several populations of birds typical of these habitats. The impact of agricultural practices on bird populations has been widely investigated, while the effect of livestock farming has seldom been assessed. To fill this gap, we carried out a quantitative meta-analysis of the existing scientific literature and evaluated the size of the effects of livestock farming on birds of rural areas in Europe. We only found 26 papers on this topic, from which 72 effect sizes could be estimated. The Barn swallow (*Hirundo rustica*) was the species on which most studies focused. Livestock farming positively influenced presence and distribution of Barn swallows in breeding habitats, while it did not significantly affect reproduction of this species. Effects on other bird species typical of rural habitats were non-significant. The positive effect on the insectivorous Barn swallow might be mediated by the enhanced insect abundance where livestock is reared. In addition, habitat features typical of rural settings where livestock is reared (e.g. cattle-sheds or large hayfields) positively affected Barn swallows independently of actual presence of livestock at a setting. Presence of livestock at rural setting therefore seems beneficial to Barn swallows, but not significantly to other bird species typical of rural habitats. The effect of livestock farming on birds of rural habitats has been under-investigated to date.

5.2 Introduction

In the last centuries, increasing human populations caused large-scale clearance of woodlands and the expansion of land used for agriculture worldwide (Meyer and Turner II 1992). In Europe, extended agricultural habitats favoured several bird species that use rural habitats as breeding, wintering or staging sites. Some species have become so typical of farmland that, irrespective of their phylogenetic relationships, they are now grouped under the term ‘‘farmland birds’’ (Harrison 1988; Lefranc 1997). Despite being an artificial habitat, European farmlands support nearly 120 bird species of conservation concern, most of which are exclusively dependent on this habitat for breeding or wintering (Tucker 1997).

The changes that occurred in farming practices since the middle of the last century probably negatively affected the fauna of rural habitats (Anderson et al. 2001; Donald et al. 2002; Fuller et al. 1995; Gregory et al. 2005). For example, European farmland birds showed stronger demographic declines than birds of other habitats during the last decades (Fuller et al. 1995; Donald et al. 2001, 2006), and overall their populations were reduced by 57 % between 1980 and 2013 (PECBMS 2015). Such changes include, among others, the mechanization of agriculture and the extension of arable fields at the expense of less productive areas, such as hedgerows, field boundaries and pastures, which, in contrast, are suitable habitats for foraging and nesting by birds (Lefranc 1997; Gillings and Fuller 1998; Evans et al. 2003; Henderson et al. 2007), the simplification of crop rotation, which caused a reduction of mixed farming systems and an increase in cereal and silage production (Vickery et al. 2001), an increase in the use of fertilizers and pesticides (Geiger et al. 2010) and changes in grassland management (Donald et al. 2002). The resulting simplification of farmland habitats, in turn, probably caused a reduction in food availability for birds, particularly invertebrates and seeds, and of nesting sites, with deleterious effects on their populations (Benton et al. 2003; Eggers et al. 2011).

Several studies have investigated the effects of changes in field management on birds (Evans and Robinson 2004; Evans et al. 2007; Henderson et al. 2007). However, these changes often occurred in parallel with changes in animal husbandry since farms often tended to specialize in either arable crops or livestock (Robinson and Sutherland 2002). In particular, rearing practices shifted from traditional ones, where a small number of heads is reared in a relatively large number of farms, to modern ones, where hundreds or even thousands of heads are reared in a relatively small number of large, industrialized and factory-like zootechnical plants. Such changes in zootechnical practices, in particular the abandonment of grazing, may also have contributed to a decrease in the suitability of farmland ecosystems for birds and, consequently, may have caused their decline (Evans et al. 2005).

In this study, we aimed at quantifying the magnitude of the effects of livestock farming practices on the distribution and reproduction of birds of rural habitats in Europe. To this end, we systematically reviewed the scientific literature on this topic and performed a quantitative meta-analysis. In particular, we considered studies investigating the effects of both indoor livestock husbandry, such as cow-rearing in cattle-sheds, and outdoor ones, such as extensive grazing on pastures.

While reviewing the literature, we observed that the majority of the studies on the effects of livestock farming on birds of rural habitats focused on a single species: the Barn swallow (*Hirundo rustica*). Indeed, several studies related the abundance, distribution and breeding success of Barn swallows to the presence of livestock farming, which is considered an important ecological factor that influences both food availability and quality of nest sites for this species (Møller 1983, 2001; Ambrosini et al. 2002a, b; Ambrosini and Saino 2010; Evans and Robinson 2004; Turner 2006; Grüebler et al. 2010; Orłowski and Karg 2011, 2013; Willi et al. 2011). Consequently, in this meta-analysis, besides investigating the effect of livestock farming on birds of rural habitats (see ‘‘Methods’’ section), we focused particularly on the Barn swallow, because the largest amount of information is available on this species.

Presence of livestock may have both direct and indirect effects on birds. We considered as direct effects the changes in the distribution or reproductive success of birds observed when livestock was reared or when there was grazing at a site when the study was conducted. These effects are thought to be mainly due to a larger abundance of the invertebrate fauna at, or very close to, the study site due to livestock presence. Indeed, the invertebrate fauna is important not only for strictly insectivorous species, but also for other species during chick rearing (Newton 2004; Henderson et al. 2007; McMahon et al. 2010; Orłowski and Karg 2011, 2013; Willi et al. 2011). In contrast, we considered as indirect effects the changes in the distribution or reproductive success of birds that were observed in a study, but linked to environmental or habitat features of settings where livestock was reared, but that do not imply presence of animals at the setting when the study was conducted. Such indirect effects include, for instance, larger availability of favourable nesting sites for Barn swallows and other species (e.g. European starling *Sturnus vulgaris*) in a farm due to the presence of rural buildings devoted to animal rearing, such as traditional cattle-sheds, piggeries and stables, which often remain accessible to birds even when livestock is no longer reared at that farm (Møller 2001; Turner 2006; Grüebler et al. 2010; Ambrosini and Saino 2010; van den Brink 2003; Willi et al. 2011; Von Post and Smith 2015). Similarly, hayfields and meadows, which are the preferred foraging habitats of Barn swallows and other birds, are usually larger arounorlod farms where livestock is reared compared to farms with no livestock (Møller 2001; Ambrosini et al. 2002a; Evans et al. 2007; Grüebler et al. 2010; Sicurella et al. 2014). Importantly, differently from pastures, hayfields and meadows are not regularly grazed, but are left growing for hay production. Since livestock is usually absent from these fields, we consider their effects on birds as indirect ones. Such indirect effects are expected to contribute to improving the overall habitat suitability for birds. In this study we therefore evaluated separately direct and indirect effects of livestock presence on birds. Further details on the distinction between direct and indirect effect is provided in the Methods section.

Effects of presence of livestock farming in agricultural settings may affect both distribution and reproduction of birds. In this meta-analysis, we therefore assessed and compared the size of the effects of livestock on bird distribution by cumulating studies considering presence and abundance of bird species ('distribution' studies hereafter, see also Methods). Similarly, we evaluated the effect size of livestock farming on different estimates of the reproductive success of birds (e.g. clutch or brood size, fledging success, 'reproduction' studies hereafter, see also ''Methods'' section).

Overall, we expect that the presence of livestock is beneficial for birds (i.e. determines larger abundance and larger breeding success). In addition, we expect that both direct and indirect effects of livestock farming are beneficial for birds, with direct effects having a larger effect size than indirect ones.

5.3 Methods

Literature search

On October 31st 2015, we searched for published scientific papers that investigated the effects of livestock farming and grazing on birds of rural habitats in Europe. Our analyses focused on the 39 bird species included in the list of European common farmland birds (PECBMS 2015) and on the house martin, *Delichon urbicum*, the only species not listed among farmland birds but that commonly occurs in European farmland landscapes and for which we found one study relating its distribution to livestock farming (Willi et al. 2011). Hereafter we will refer to the group of species included in this meta-analysis as ‘‘birds of rural habitats’’. We used the search platforms Web of Science and Google Scholar with the following keywords: ‘‘bird’’, ‘‘farmland bird’’, ‘‘farming’’, ‘‘rearing’’, ‘‘livestock’’, ‘‘agricultural intensification’’, ‘‘grazing’’ and ‘‘pasture’’. Moreover, we searched for papers that focused on farmland birds and on the house martin using their Latin scientific and common English name (e.g. ‘‘*Hirundo rustica*’’ and ‘‘Barn swallow’’). We also used a combination of keywords with ‘‘bird’’ or the scientific names of species plus another keyword among the previous ones (e.g. ‘‘*Delichon urbicum*’’ ? ‘‘livestock’’). Although ‘‘agricultural intensification’’ was among the keywords, we carefully evaluated the papers containing this keyword in order to select only those reporting information about the presence of livestock or about presence of environmental features typical of livestock farming settings (e.g. traditional cattle-sheds; see Introduction and below for details). Finally, we checked the reference list of the resulting articles and of all the papers citing each publication resulting from this search in order to identify other papers that could be relevant for our review.

We limited the review to papers from Europe. In detail, we considered studies from Denmark, Germany, Hungary, Norway and Russia (1 paper each), Switzerland and Poland (2 papers each), the Netherlands (3 papers), the United Kingdom (6 papers) and Italy (8 papers). Studies from outside Europe were excluded because we aimed at including only studies conducted on a homogeneous group of bird species (European farmland birds plus the house martin). In addition, only five papers from other continents addressed the topic of the effect of livestock farming on birds (details not shown). We also excluded papers for which we could not assess size of the effects on individual species of birds of rural habitats. For example, we excluded the paper by Pavel (2004) because it compared nest failure due to trampling by grazing animals by cumulating data on two farmland species (the meadow pipit *Anthus pratensis* and the skylark *Alauda arvensis*) and on a non-farmland bird (the water pipit *Anthus spinoletta*).

Livestock species considered in the studies included in the meta-analysis were mainly cows, horses, pigs and sheep. Only two studies (van den Brink 2003, Orłowski and Karg 2013, both included in the meta-analysis) investigated the effects of poultry farming on birds, while some studies did not report separate effects of each livestock species, but only that of presence of livestock (e.g. they reported the effect of presence of either cattle, pigs or horses on a farm, as in Sicurella et al. (2014)). For this reason, we did not attempt to investigate separately the effects of different species of livestock on birds.

Since 15 out of the 26 reviewed papers that reported the effects of livestock farming on bird species focused on the Barn swallow (see ‘‘Results’’ section and Table S1), we investigated separately the effects of livestock farming on this species and on the other birds of rural habitats.

Effect size estimates

We extracted statistics on effects of livestock farming from all the results reported in the selected publications and transformed them into Pearson's product-moment correlation coefficients (Pearson's r) that was used as a measure of effect size because it indicates the strength of the relationship between two variables. More precisely, its squared value r^2 provides information about the proportion of variance explained by the predictor variable in a sample (Rosenthal 1991, 1994). In accordance with Cohen's rule, a 'small' effect size corresponds to a Pearson's r of 0.10 (i.e. r^2 =1% of the variance), a 'medium' effect size corresponds to an r of 0.30 (i.e. r^2 =9 % of the variance) and a 'large' effect size corresponds to an r of 0.5 (i.e. r^2 =25% of the variance) (Cohen 1988). Since r-values do not follow a normal distribution, they were Z-transformed (Zr) before the analyses using Fisher's transformation (Fisher 1928).

Where available, we extracted from the paper the absolute values of all Zr or r coefficients and the relative sample sizes. Hence, a 'paper' may report one or more effect sizes. The direction (sign) of the effect was then assigned based on the congruence of the results with the hypothesis that birds of rural habitats benefit from the presence of livestock farming. If the result of an analysis supported the hypothesis (e.g. birds were more abundant or had larger reproductive success where livestock was reared), we assigned a positive sign to the effect size. Conversely, if the result did not support this hypothesis, we assigned a negative sign. When studies reported statistics different from Zr or r coefficients, they were included as follows. The test statistics *F*, $\chi 2$ and *t* were first transformed into *r*-values using the equations proposed by Rosenthal (1991) and then into *Zr* using Fisher's transformation (e.g. $F_{1,91}$ =5.9 from Ambrosini et al. 2002a was transformed into *r* =-0.2468 and *Zr* =-0.2520; this effect size was given a negative sign because reproductive success was lower in farms with than without livestock). When no statistics were available or non-parametric tests were reported, we computed effect sizes from P values (e.g. P =0.0457, sample size =24 from Evans et al. 2015 was first transformed into a one-tailed p value, i.e. P =0.023, and then into *r* =0.4031 and *Zr* =0.4273). Alternatively, we used information about means and standard deviations reported in the studies to estimate effect sizes (e.g. mean number of nests in organic farms 2.40 ±3.38 SD, sample size =20 and in conventional farms 2.55±4.50 SD, sample size =20 from Kragten et al. (2009) were transformed into *r* =-0.0263 and *Zr* =-0.0263, with a negative sign because of the larger number of nests in conventional than in organic farms). When only approximations of P-values were reported, we considered the extreme upper bound of the range (i.e. for $P < 0.001$, we used P =0.001 and transformed this value into *r* and *Zr* as above) to compute the effect size. Hence, we were conservative in the estimation of effect sizes in these cases.

When the sign of the effect was unclear, we first tried to extract this information from other parts of the paper (e.g. discussion or graphs). If this was not possible, we asked the authors for further information. Similarly, we asked the authors for information on the size of the effects, when a paper reported undefined values (e.g. ‘ns’ or ‘P>0.05’). If the authors did not reply to our first request of information, we sent them a second request. If we received no answer to this second request, we excluded the effect size from the meta-analysis. Overall, only two effect sizes from two different papers were excluded for this reason (details not shown).

All the effect sizes that we could extract from each paper were included in the meta-analysis because we used a statistical approach able to account for multiple effect sizes from the same paper (see below). The sample units of the results from which we calculated effect sizes included in the meta-analyses were alternatively breeding pairs, colonies, buildings, farms, fields or even years (see Table S1 for details on sample unit at each study). However, due to the small number of studies found in the literature on the effect of livestock farming on birds, we cumulated all effects sizes irrespective of the sample unit.

Direct and indirect effects and effects on bird distribution and reproduction

We aimed at evaluating separately direct and indirect effects of livestock farming on birds (see ‘‘Introduction’’ section). We therefore ran separate meta-analyses on the effect sizes from analyses investigating the influence of: (1) presence of reared animals per se (direct effects); and (2) presence of environmental features typical of livestock farming settings (indirect effects). In detail, effect sizes from analyses relating bird distribution or reproduction to presence or abundance of livestock, presence of livestock farming activities, grazing intensity and insect abundance were considered as deriving from direct effects of livestock on birds. In contrast, effect sizes from analyses reporting the effects on birds of presence of buildings devoted to animal rearing, presence of hayfields, meadows or other non-grazed grasslands used to produce hay, or other features of the farmland habitats linked to the presence of livestock (e.g. presence of manure heaps), including the presence of organic farming practices (Lubbe and de Snoo 2007; Kragten et al. 2009), were considered as deriving from indirect effects of livestock on birds. Indeed, organic farming usually implies the use of manure as the only fertilizer (Kragten et al. 2009).

When a paper included analyses about different livestock species (e.g. Orłowski and Karg 2011), we included one effect size for each livestock species, and accounted for pseudo-replication of effect sizes from the same paper in the analyses (see below). If a paper reported only one effect size about the overall effect of animal husbandry, without any distinction among livestock species (e.g. Hart et al. 2002), we reported that single value.

In this study, we were interested in investigating the influence of livestock farming on the presence and on the reproduction of birds in rural habitats. However, papers sometimes reported different analyses dealing with ‘distribution’ or ‘reproduction’ of a species. For example, Ambrosini et al. (2002a) reported effects of livestock farming on both presence and size of Barn swallow colonies. In these cases, we included in the meta-analysis only the effect sizes calculated from the analyses that provided more information on either distribution or reproductive success of birds.

For instance, when both analyses on presence and abundance of a species were reported in the same paper, we only included effect sizes from analyses on species abundance and discarded those from analyses on presence. Similarly, effect sizes from analyses investigating fledgling success were preferred over those from analyses investigating brood size, which in turn were preferred over those from analyses on clutch size.

After this selection, we obtained 72 effect sizes from 26 papers. Table S1 reports details on all effects sizes (variance, sample size and unit, direction of the effect) and additional information (bird species, country, latitude and habitat type of the study area) as well as full reference and year of publication of each paper reporting effect sizes.

Meta-analyses

Different effect sizes extracted from the same paper cannot be considered statistically independent. We therefore used hierarchical linear models (aka linear mixed effect models,LMMs) assuming a Gaussian error distribution. Indeed, LMMs are able to account for nonindependence in the data and their use for meta-analyses is advocated precisely for analyses including multiple effect sizes from the same paper (Van den Noortgate et al. 2013). In this case, each effect size (*Zr*) was weighted by its variance (equal to sample size -3), while the random part of the model included paper ID and effect size ID, nested within paper ID, as random grouping factors. In addition, residual variance was constrained to be equal to 1 in all models. With this parameterization, we obtained a mixed model able to fit a three-level meta-analysis (*sensu* Van den Noortgate et al. 2013). The reasons for such a parameterization are fully described in Van den Noortgate et al. (2013).

We first investigated whether mean size of the effects of livestock farming on the Barn swallow, and on the other bird species differed significantly from zero by running separate LMMs including only the intercept as fixed predictor (Van den Noortgate et al. 2013). In these models, sizes of direct and indirect effects, and of effects on distribution and reproduction were considered together. Species was entered as a further random grouping factor (crossed with effect size ID and paper ID) in all the models run on bird species others than the Barn swallow. Second, we investigated in separate meta-analyses whether mean size of direct and indirect effects differed from zero for both the Barn swallow and the other bird species. In these analyses, we considered both effects on distribution and reproduction together, hence we ran four additional LMMs with the same fixed and random structure. Third, we evaluated separately whether mean size of the effects of livestock on bird distribution and on Barn swallow reproduction differed from zero. We did not run any analysis on the effects of livestock farming on the reproduction of other bird species because we only found three effect sizes from studies investigating this topic. Therefore, we ran three additional models with the same fixed and random structure as above. Fourth, we compared the size of direct and indirect effects and of effects on Barn swallow distribution and reproduction by running LMMs while including as predictors dichotomous moderator variables indicating whether a study investigated direct or indirect effects, or effects on the Barn swallow or on other species.

Analyses were run with the SAS 9.4 proc MIXED (SAS Institute Inc, Cary, NC) by a restricted maximum likelihood (REML) procedure. Residual variance was set to 1 by the PARMS statement as is necessary in meta-analyses run on *Zr* values. Degrees of freedom were estimated with the Sattertwhaite approximation. Since the separate meta-analyses revealed heterogeneity of variances between levels of the moderator (e.g. between direct and indirect effects), we allowed for heterogeneity of variances at the study and at the paper levels by the GROUP option in the RANDOM statement. We note that with these settings, marginal means estimates from the LMMs with moderator variables obtained by the LSMEANS statement gave identical results as mean estimates from the separate meta-analyses. However, these allowed comparing mean size of the effects between levels of the moderator variable.

In the Supporting Information, we provide the code for all the analyses.

Influence of latitude and year of publication

We investigated whether bird populations living at different latitudes were differently affected by livestock practices, by relating effect sizes to the latitude of the study areas. When the exact coordinates were reported, we rounded them to the nearest degree. If no coordinates were reported in the paper, we searched for the study area on Google Earth™ (Google Inc., Mountain View, CA) and considered the latitude (rounded to the closest degree) of its approximate centre. If a study was conducted in several locations or across a wide area, we calculated the mean value between the northernmost and southernmost latitudes of the study area(s) and rounded this value to the closest degree.

Moreover, we evaluated whether the effect sizes changed in accordance with year of publication of the paper, as expected if studies with large effects were likely to be published first, while studies with small effects were likely to take longer time to be published (Møller and Jennions 2001). For these purposes we ran two distinct LMMs where latitude and year of publication were the predictor variables, and the effect size ID and paper ID (accounting for possible pseudo-replications) were entered as random factors. These analyses were also replicated by considering either the effect sizes on the Barn swallow or those on the other species. In these latter analyses, species was entered as a further random grouping factor.

Publication bias analyses

Studies reporting null or non-significant results may remain unpublished and therefore do not appear in the scientific literature. Such a deficit, if existing, may lead to an overestimation of significant studies and can bias the average effect size resulting from a meta-analysis (Song et al. 2000; Møller and Jennions 2001). To partly account for this potential issue, we examined indirect evidence for the presence of publication bias in our dataset using different statistical methods. First, we used the Kendall rank correlation test between effect sizes and sample sizes across all the 72 effect sizes (Begg and Mazumdar 1994). A high correlation value would indicate that the funnel plot of effect sizes on sample sizes is asymmetric, which may be due to publication bias. Second, we estimated the fail-safe number using Rosenthal's method with alpha equal to 0.5 (Rosenthal 1979). This test estimates the number of studies with an effect size equal to zero needed to make the mean effect size not significantly different from zero. Third, we estimated the number of possible 'missing' studies using the 'trim and fill' method with $L_0{}^+$ estimator (Duval and Tweedie 2000). In this analysis, we assumed that the funnel plot of effect sizes on sample sizes should be symmetric to the mean effect size in absence of publication bias. The model determines the number of 'missing' studies that would produce a symmetric funnel plot and finally estimates the new mean effect size that would be obtained by adding these studies to the original sample. These analyses were run by the *metafo*r library (Viechtbauer 2010) in R 3.0.3 (R Core Team 2014).

We ran the publication bias analyses only when the mean effect sizes resulting from the models differed significantly from zero (see Table 1) because we aimed at assessing whether publication bias may have introduced Type-I errors in our analyses. Our meta-analyses included different effect sizes obtained from the same paper, which are therefore non-independent. The LMMs we used for the analysis accounted for this non-independence of effect sizes (see 'Meta-analyses' above), but we are aware of no procedure for the test of publication bias that accounts for non-independence of effect sizes. We therefore repeated the publication bias analyses twice. First, we ran the analyses by considering all effect sizes as independent. Second, we calculated the mean *Zr* value within each paper by running random effect meta-analytic models on all the *Zr* values from the same paper with the *metafor* package in R, and then ran the publication bias analyses by including the mean *Zr* value for each paper reporting more than one effect size. This latter procedure corresponds to considering all effect sizes reported in the same paper as repeated measures of the same effect size. When both the analysis run considering multiple effects sizes from the same study as fully independent and that run considering them as fully dependent were non-significant, we concluded that publication biases did not affect our conclusions.

When running the publication bias analyses on mean within-paper *Zr* values, we were faced with the problem of assessing the correct sample sizes. For example, the same paper may report multiple effect sizes calculated on the same sample of farms or on different samples of farms. Even more complex situation may occur. For example, breeding success can only be estimated for the subsample of farms where Barn swallows were present among a random sample of farms in an area, which can both host breeding swallows or not (e.g. Ambrosini et al. 2002a). Importantly, sometimes papers were not crystal-clear in reporting which analysis was run on which subset of sample units. This sometimes determined the impossibility of properly assessing an overall sample size to be attributed to each paper (but note that we could always identify the sample size for all the analyses from which we calculated individual effect sizes). To solve this problem, we calculated the mean, minimum and maximum sample size among all the *Zr* values we mediated to run publication bias analyses on mean within-paper effect sizes. We then re-ran the Kendall's *tau* correlation test by using each of these within-paper estimates of effect sizes, and compared the results. We note that the analyses of the fail-safe number and the trim-and-fill number of missing studies can be run by including in the model the variance of the mean within-paper effect size obtained by the random effect model used to calculate it, so that these analyses do not require estimating within-paper sample sizes.

In the Supporting Information, we report the R code for running all the publication bias analyses.

5.4 Results

Reviewed studies

We identified 26 published papers reporting statistics suitable for a meta-analysis. Fifteen (58 %) papers focused on the Barn swallow only, ten (38 %) on other species but the Barn swallow, one paper (4 %) focused on both the Barn swallow and the house martin. From these papers, we extracted 72 effect sizes (reported in Table S1). Forty-seven of them (65 %) were about the Barn swallow, 33 (70 %) and 14 (30 %) of which reported respectively direct and indirect effects. In addition, 39 (83 %) and eight (17 %) of these effect sizes focused respectively on distribution and on reproduction of the Barn swallow. Twenty-five effect sizes (35 %) were about other birds of rural habitats, 17 (68 %) reported direct effects, eight

(32 %) indirect effects, 22 (88%) effects on distribution and three (12%) effects on reproduction. In detail, studies included in the present meta-analysis were on the house martin and on eight of the 39 species classified as European farmland birds (i.e. meadow pipit *Anthus pratensis*, northern lapwing *Vanellus vanellus*, red-backed shrike *Lanius collurio*, skylark *Alauda arvensis*, starling *Sturnus vulgaris*, whinchat *Saxicola rubetra*, white stork *Ciconia ciconia*, and yellow wagtail *Motacilla flava*; see Table S1).

Effect of livestock farming on farmland birds

Livestock farming had an overall medium effect (r = 0.265) on Barn swallows accounting on average for 7.0 % of the observed variance (Table 1 and Fig. 1). The mean size of direct effects was also medium (r = 0.325), explaining 10.5 % of the variance, while that of indirect effects was small (r = 0.147), explaining only 2.1 % of variance (Table 1). However, all these effects were significantly larger than zero (Table 1), and a meta-analysis with moderator variable revealed that mean size of direct effects of livestock farming on the Barn swallow did not differ significantly from that of indirect ones (difference: Zr = 0.190 ± 0.087 SE, $t_{11.2}$ = 2.19, P = 0.051), albeit the difference was only marginally non-significant. This suggests that presence of livestock (direct effects) tended to have, on average, slightly larger effects on Barn swallows than environmental or habitat features of settings linked to livestock presence, but that do not imply current presence of animals (indirect effects, see Introduction), and that both direct and indirect effects of livestock are beneficial to this species.

Livestock farming (both direct and indirect effects) had a positive and medium-sized effect on Barn swallow distribution ($r = 0.289$) explaining 8.3% of the variance, but did not significantly affect Barn swallow reproduction ($r = 0.156$ corresponding to 2.4% of variance explained; Table 1). The size of the effects on Barn swallow distribution did not differ significantly from that of effects on reproduction of this species (difference: $Zr = 0.140 \pm 0.184$ SE, $t_{3.49} = 0.76$, $P = 0.494$).

When other bird species of rural habitats than the Barn swallow were considered, no significant effect of lvestock farming was observed for any measure (Table 1 and Figure S1). The paucity of studies on reproduction of these species (equal to three) did not allow any analysis. Effect size did not change according to either latitude ($|t|_{8.02} \leq 0.96$, $P \geq 0.363$) or year of publication ($|t|7.89 \leq 1.20$, $P \geq 0.266$), and this held true when we considered either studies on the Barn swallows or on all the other species but the Barn swallow.

Tests of publication bias

The results from publication bias analyses were similar when run on all effect sizes separately (i.e. assuming independence of effect sizes reported in the same paper) and on mean effect sizes within a paper (i.e. assuming that effect sizes reported in the same paper are replicated measures of the same effect size) (Table S2).

In the analyses of the Barn swallow, the Kendall correlation test was significant and negative only when run on mean within-paper effect-sizes on reproduction with sample size set equal to the minimum sample size within papers (Kendall's τ = - 0.516, P =0.010). It was also marginally non-significant when this analysis was run on mean within-paper effect-size on all studies on the Barn swallow, but only when sample was estimated as the minimum sample size within papers (Kendall's τ =-0.367, P =0.052). In contrast, it was non-significant in all the other cases, including the analyses run on all effect sizes separately (Kendall's $|\tau| \leq 0.362$, $P \geq 0.079$). Rosenthal's fail-safe number was always larger than the threshold of 5n +10 (where n is the original number of effect sizes included in each analysis) suggested by Rosenthal (1979) as a conservative value for a fail-safe calculation (Table S2). In addition, Rosenthal's fail-safe number was also significantly larger than the 5n+10 threshold in all cases except for indirect effects calculated on mean within-paper effect sizes (see Fragkos et al. 2014 for confidence intervals for Rosenthal's fail-safe number). Finally, the number of 'missing' studies computed using the 'trim and fill' method was larger than zero (but always ≤4) only in three cases, when the analyses were run on mean within-paper effect sizes (Table S2). However, in all these cases, the mean effect sizes recomputed by accounting for this potential source of bias were still significantly larger than zero (Table S2), so confirming that possible asymmetries in the effect size distributions should not have affected our results. We thus concluded that, although there was some suggestion of possible bias in a small number of analyses, overall publication bias should not have affected our conclusions on the Barn swallow.

5.5 Discussion

This meta-analysis investigated the effects of livestock farming on the Barn swallow and on other bird species typical of rural habitats. We found evidence of a positive effect of livestock farming on Barn swallows, both when we considered the benefits provided by presence of livestock (direct effects) and those provided by features of the settings related to animal husbandry, like presence of rural buildings devoted to rearing animals or large extent of hayfields around farming sites (indirect effects). Direct benefits provided by livestock farming to Barn swallows are probably related to the larger abundance and availability of insects and other invertebrates around settings where animals are reared (Møller 2001). However, Barn swallow also seems to benefit from indirect effects of livestock presence, even if this is to a lesser extent. Indeed, use of natural fertilizers derived from manure is positively associated with richness and abundance of arthropod taxa (McMahon et al. 2010). Moreover, arthropod-rich environments, like hayfields, meadows and grasslands in general, are usually larger around farms with livestock, offering foraging habitat for insectivorous birds (Ambrosini et al. 2002a; Evans et al. 2007; Grüebler et al. 2010).

Livestock farming seems to affect Barn swallow distribution, while we found no conclusive evidence of any effect of livestock farming on the reproduction of this species. The Barn swallow is well-known to be strictly associated with livestock farming during the breeding period (Ambrosini et al. 2002a) and studies showed that colonies dramatically declined or went extinct after rearing or dairy activity ceased on a farm (Møller 2001; Ambrosini et al. 2002b, 2012; Willi et al. 2011). However, this meta-analysis highlighted that also presence of features related to animal husbandry at a farm may provide benefits to this species, even when livestock is not present (indirect effects). Interestingly, despite direct effects tended to have a larger influence on Barn swallows than indirect ones, the difference between them was marginally non-significant. For example, presence of traditional cattle-sheds at a farm was found to enhance colony size of Barn swallow, independently of the actual presence of livestock in them (Ambrosini et al. 2002a). In addition, a recent study observed that colony size increased with increasing extent of hayfields around the nesting site in farms without livestock, but not in those with livestock (Sicurella et al. 2014).

While it is known that disappearance of livestock farming may determine decline and even extinction of Barn swallow colonies (Møller 2001; Ambrosini et al. 2012), our results suggest that this species may benefit also by the presence of other features typical of animal farming settings. Some of these features may persist in agricultural settings for years after livestock farming ceased. For example, cattle-sheds and stables often remain accessible to birds for nesting even after animal husbandry activities stopped on a farm. These results therefore suggest that, despite maintenance of livestock should be prioritized as a strategy for contrasting the decline observed in some population of these species (Ambrosini et al.

2012; Sicurella et al. 2014), maintenance of large hayfields and of rural buildings used by swallows for nesting may represent alternative strategies whenever maintenance of livestock farming is impractical due e.g. to economic reasons (Sicurella et al. 2014). In addition, persistence of indirect benefits may explain, at least partly, the observed latency in the response of Barn swallow colonies to changes in the conditions of breeding habitats (Ambrosini et al. 2002b).

In contrast, our meta-analysis suggested that the effect of livestock on Barn swallow reproduction was almost null, accounting for less than 0.1% of the variance only. Such a small effect size on Barn swallow reproduction was unexpected, particularly because this species nests almost exclusively in buildings (Turner 2006), particularly in those devoted to rearing animals. In addition, several studies indicated that Barn swallows breed earlier, raise larger broods, produce nestlings of better quality, and have a higher probability of laying a second clutch in presence of livestock (Møller 2001; Ambrosini et al. 2002a). However, the size of the effects reported in these studies was small, probably because variation in clutch size and fledgling success, which are the components of breeding success recorded more often, is rather small in the Barn swallow (the coefficient of variation of clutch size is 0.20 as assessed on a sample of 16,135 first clutches observed in Northern Italy in 1999–2014, while that of fledging success is 0.55 as assessed on 18,941 first broods in the same area and period; R. Ambrosini unpublished data). Small variation in breeding success, in turn, may limit the options of observing large effects of livestock farming on them. In addition, breeding success of Barn swallows may be largely dependent on other effects unrelated to livestock farming, like presence of predators (e.g. mice *Mus musculus* and rats *Rattus ssp*., Eurasian hobby *Falco subbuteo*, sparrowhawk *Accipiter nisus*) (Møller and Nielsen 1997). For instance, in Denmark the breeding pairs of Barn swallows 'just' disappeared in the presence of barn owls *Tyto alba* that can lead to the extinction of large colonies in a year (A.P. Møller unpublished data).

We also investigated the hypothesis that the positive influence of livestock could be more evident at northern latitudes, where severe climatic conditions can determine larger variability in temperature and insect food availability during the breeding season (Møller 2001; Grüebler et al. 2010). However, we did not find any significant relationship between effect sizes and latitude of study areas, either on the Barn swallow or on the other species.

The size of the effects of livestock on other species of rural habitats was not significantly larger than zero in any case. This lack of effects of livestock on other bird species may be the result of the large heterogeneity of species considered. Indeed, the other bird species we considered included also not strictly insectivorous species, like the white stork, which were not expected to considerably benefit from the increasing insects abundance at setting with livestock. In addition, th Barn swallow probably depends more strictly on human activities and buildings than other birds. Indeed, European populations of this species breed almost exclusively in buildings (Turner 2006). Unfortunately, paucity of studies did not allow investigations within ecological groups of species like e.g. insectivores or granivores. Our meta-analysis therefore suggests that Barn swallow populations seem to benefit from the presence of livestock. Such benefits seem to arise both from presence of domestic animals per se, and from availability of nesting sites and foraging areas. Reduction of animal husbandry may therefore have concurred to the recent decrease of populations of Barn swallows (Møller 2001; Ambrosini et al. 2002a; Evans and Robinson 2004). In contrast, other bird species typical of rural habitats seem not to benefit from livestock farming. However, a positive effect of livestock on other species may have been masked by the large heterogeneity in the studies and in the species included in the analysis, as well as by the small number of papers that investigated this topic on other species.

During the last decades of the 20th centuries, the European Union introduced economic subsidies and agro-environmental schemes with the aim to improve agro-ecosystem biodiversity and to counteract the negative effect of agricultural intensification (Donald et al. 2001; Kleijn and Sutherland 2003). Farmland bird populations were expected to indirectly benefit from agro-environmental schemes, but precise evaluation of such effects has proven difficult, because effects seem to largely differ among species (Kleijn and Sutherland 2003; Hiron et al. 2013). Maintenance of livestock farming should therefore be included in conservation plans aiming at preserving Barn swallow populations in rural habitats (Ambrosini et al. 2012, Sicurella et al. 2014).

Importantly, in contrast to the large amount of information available on the effect of arable field management on birds (see Fuller et al. 1995; Anderson et al. 2001; Vickery et al. 2001; Donald et al. 2002; Gregory et al. 2005; and references therein), our extensive search of the literature revealed that only 26 published papers focused on the effect of livestock farming practices on single bird species of rural habitats. Future studies on this topic are therefore welcome, also because all the species we considered except for the house martin are farmland birds, which are declining at a continental scale.

5.6 Acknowlegments

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Chapter 6

Cloacal microbiota of Barn swallows from Northern Italy

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6.1 Abstract

Bird gut microbiota show large variation among geographical populations of the same species, probably because, differently from mammals, gut microbiota of birds is largely affected by extrinsic factors such as diet and environmental conditions. We analysed the cloacal microbiota (CM) of 12 Barn swallows (*Hirundo rustica*) from a colony in northern Italy by high-throughput DNA sequencing of the 16S rRNA gene. The CMs, dominated by bacteria of the phyla Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, did not significantly differ between adult females, adult males and fledglings. This first description of the Barn swallow CM in Italy, together with two previous studies from Europe, suggests that CMs of geographically different Barn swallow populations seem to be dominated by bacteria belonging to the same phyla but different genera. The intra-colony similarity of the CMs may be due to the exposure of individuals to the same local environmental conditions while on their breeding grounds.

6.2 Introduction

Recent studies have demonstrated that the gut microbiota can influence the physiology and behaviour of vertebrate species (Fraune & Bosch 2010) by affecting host physiology, improving nutrient assimilation, promoting detoxification and supporting immunological responses with consequences on host growth and survival (Mead 1997, Fraune & Bosch 2010). Moreover, the composition of the gut microbiota seems to influence host behaviour, particularly by influencing mate choice and reproduction (Kokko et al. 2002; Sharon et al. 2010). Alteration of gut microbiota may also exert negative effects, determining physiological disorders of the host, increasing its susceptibility to pathogens, and ultimately reducing its fitness (Muegge et al. 2011; Bik et al. 2016).

Little is currently known about the factors that shape the structure of intestinal bacterial communities. A recent review by Colston & Jackson (2016) highlighted that the host genetics and phylogeny influence the structure of bacterial communities in the gut of mammals, fishes and reptiles. In contrast, diet and geography seem the main factors affecting avian gut microbiome (Waite & Taylor 2014, see Colston & Jackson 2016 and references therein). Studies on birds also suggest that nestlings acquire their microbiota from the nest environment immediately after hatching or through the bolus regurgitated by parents, providing an example of vertical transmission of bacteria through generations (Lucas & Heeb 2005; van Dongen et al. 2013). Bacteria can be transferred also between adult birds during copulation (White et al. 2010; Kreisinger et al. 2015). This horizontal transfer of gut bacteria is favoured by the anatomy of the reproductive system of birds. Indeed, the avian cloaca serves both for defecation and for gamete transfer, creating the condition for sexual transmission of intestinal bacteria (Sheldon 1993; Lombardo 1998; White et al. 2010). These features suggest that birds could be a valuable model for investigating the processes that shape the bacterial communities of the intestinal trait (White et al. 2010).

We studied the cloacal microbiota (CM) of twelve Barn swallows (*Hirundo rustica*) from a colony in Northern Italy during the breeding season 2015. Only two published studies from Czech Republic have investigated Barn swallow CM so far (Kreisinger et al. 2015, 2017). In light of the large variability in the microbiota of bird populations from different geographical areas (Colston & Jackson 2016), our aim was to provide a description of the CM of a geographically different population. In addition, we investigated whether the structure of the bacterial communities of the CM differed among age classes (adults or fledglings) and sexes (adult males or adult females). CM is a subset of intestinal microbiota, and it is directly communicating with both the urogenital tract and the external environment. This makes it more susceptible to horizontal transfer of bacteria between individuals. Moreover, CM sampling is easily feasible by swabs (see Methods), while study of bacterial communities of more internal tracts of the gastrointestinal system would require the sacrifice of some individuals, which is ethically not acceptable for a protected species like the Barn swallow.

6.3 Methods

Study species and field procedures

The Barn swallow is a small, aerially insectivorous, long-distance migratory bird that breeds semi-colonially mostly in rural buildings (Turner 2006). Socially monogamous breeding pairs have 1-3 broods of 1-7 nestlings per breeding season. Offspring are altricial and are attended by both parents for approximately 20 days. Adult Barn swallows can be easily sexed during the breeding season from presence (females) or absence (males) of brood patch (Møller 1994; Turner 2006), while fledglings are monomorphic and cannot be sexed in the field until their first moult (Møller 1994; Turner 2006).

We sampled CM of four adult males, five adult females and three fledglings captured by mist nets in a breeding colony (Milan, Italy; 45° 28' 48.8" N, 9° 06' 05.3" E) on 24 July 2015. CM samples were collected using sterile DNA-free microbiological nylon swabs (minitip FLOQSwabs 516CS01, Copan, Italy) inserted ca. 10 mm inside the cloaca for approximately 20 s and gently twisted by approximately 360°. All samples were kept at +4 °C while in the field and brought to lab within 5 h from collection, where they were stored at -20 °C until processing. Six samples were stored in 2 mL aseptic microcentrifuge tubes (Eppendorf AG, Germany), while the others were stored in 1 mL of eSwab™ preservative (484CE eSwab™, Copan, Italy).

The study was carried out under permission of the local authority (Regione Lombardia, DGR 190 issued on 19/01/2015) responsible for authorizing animal studies in the wild. The owner of the riding school where we collected samples gave us the permission to conduce this work.

DNA extraction and sequencing

Total DNA was extracted from the swabs with FastDNA® Spin kit for Soil (MP Biomedicals, Solon, OH, USA). Dry swabs were directly inserted into the Lysing Matrix E tube. Microtubes containing the swabs stored in eSwab™ preservative were vortexed, and the whole content (approximately 1 mL) of each microtube was transferred to a Lysing Matrix E tube while adding 500 µL of Sodium Phosphate Buffer. Further steps of DNA extraction were performed according to manufacturer’s instructions. The V5-V6 hypervariable regions of the bacterial 16S rRNA gene were PCR-amplified by preparing 2 × 75 µL volume PCR reactions for each sample and including a 6-bp barcode at the 5’ end of each primer (Huber et al., 2007; Wang and Qian, 2009) to allow sample pooling and sequence sorting. Amplicons bearing different barcode pairs were pooled together to build libraries. Further library preparation with the addition of standard Nextera indexes (Illumina, Inc., San Diego, CA, USA; see also Gandolfi et al., 2017 for further details) and sequencing were carried out at Parco Tecnologico Padano (Lodi, Italy).

Reads from sequencing were demultiplexed according to the indices and the Uparse pipeline was used for the following elaborations (Edgar 2013). Forward and reverse reads were merged with perfect overlapping and quality filtered with default parameters. Chimera checking was run on pooled unique reads before the OTU clustering, and allowed removing 6764 (10.5%) suspected chimera sequences. Singleton sequences (i.e. sequences appearing only once in the whole data set) were also removed. Operational Taxonomic Units (OTUs) were defined on the whole data set clustering the sequences at 97% of similarity and defining a representative sequence for each cluster. Abundance of each OTU was estimated by mapping the sequences of each sample against the representative sequence of each OTU at 97% of similarity. Taxonomic classification of the OTU representative sequences was obtained by RDP classifier (Wang et al. 2007).

Sequences were deposited to EBI-ENA database with the accession numbers ERS1835461-72. Full datasets used in the present analyses are included in the Supporting Information available at the journal.

Statistical analyses

Alpha diversity

The number of sequences was used as an estimate of abundance of each OTU in a sample. We estimated the total OTU richness of each CM by the Chao1 index (Chao & Shen 2003) because this index estimates the total OTU richness after accounting for unobserved OTUs and its values can be compared among samples with different coverage. We then compared Chao1 indices between adult males, adult females and fledglings by ANOVA followed by planned comparisons of adult males and adult females (thus excluding fledglings), and adults (i.e. pooling adult males and females) and fledglings.

Beta diversity

We compared CM structures of adult males, adult females and fledglings by redundancy analysis (RDA). Abundance of each OTU was assessed on 12,000 randomly extracted sequences in order to give equal coverage to each CM. This number was slightly lower than the minimum coverage of our CMs. Also in this case, we performed planned comparisons by re-running RDAs on adult males and adult females only, and by comparing adults and fledglings and adjusting significance according to the false discovery rate (FDR) procedure (Benjamini & Yekutieli 2001). The Hellinger distance among CMs was used for all the analyses (De Cáceres et al. 2010; Legendre & Legendre 2012).

Rarefaction curves showed that bacteria diversity may be underestimated with 12,000 sequences (Fig. 1). We therefore re-ran these analyses on a random subsample of 50,000 sequences for the eight samples with a larger coverage and by normalizing the coverage to 50,000 sequences for the four samples with a lower coverage. The results never changed in that all significant tests remained significant, and all non-significant ones remained non-significant (details not shown), so we report only the results obtained with 12,000 sequences.

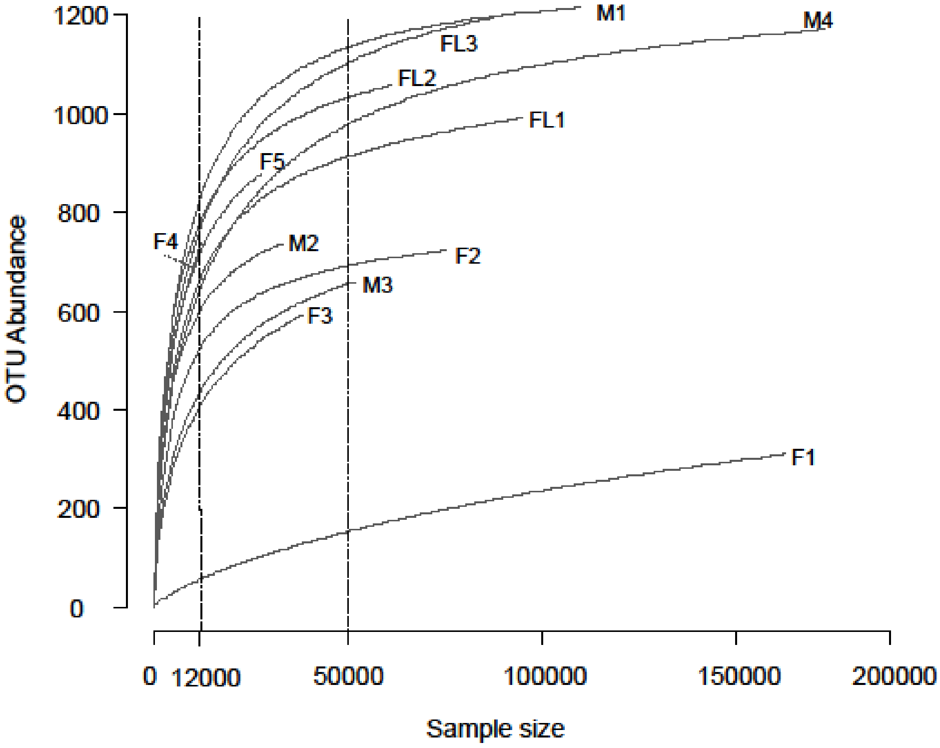

Figure 1. Rarefaction curves for the 12 CM samples. The vertical dotted lines represent 12,000 and 50,000 sequences. F: adult females, M: adult males, FL: fledglings.

6.4 Results

Composition of CM bacterial communities

We obtained 926,729 sequences clustered in 2482 OTUs, only 109 (4%) of which were present in all samples, 675 (27%) in more than 50% of the samples and 629 (25%) were exclusive of one sample. The number of OTUs in each sample ranged between 310 and 1214. CM of one adult female (#F1) was composed by a much smaller number of OTUs (n = 310) than that of the other individuals (n ≥ 592).

OTUs were classified in 32 phyla and 138 orders. Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes were the most abundant phyla, while Burkholderiales, Chlamydiales, Bacillales and Actinomycetales were the most abundant orders (Fig. 2). The CM of female #F1, which showed the lowest number of OTUs, was dominated by Chlamydiales (86% of sequences; Fig. 2).

Alpha and beta diversity of CM communities

Values of Chao1 indices (Table 1) did not differ significantly between adult males, adult females or fledglings ($F_{2,9} = 3.096$, $P = 0.095$). Planned comparisons also showed that they did not differ significantly between adult males and females ($t_9 = 1.419$, $P = 0.332$) and between adults (males and females pooled) and fledglings ($t_9 = 1.963$, $P = 0.149$).

RDA indicated that the CM structure did not differ significantly between adult females, adult males or fledglings ($F_{2,9} =1.265$, $P = 0.106$; Fig. 3) nor between adult males and adult females ($F_{1,7} = 0.938$, $P_{FDR} = 0.$

888) or between adults (males and females pooled) and fledglings ($F_{1,10}$ = 1.479, P_{FDR} = 0.306).

Table 1: Description of cloacal microbiome samples. For each sample, information about age (adult or fledgling) and sex of adults, Chao1 (±SE) index and the number of reads before and after quality filtering is reported.

ID Sample	Age/Sex	Chao1 index (± SE)	N.Reads	N.Reads after quality filtering
F1	Adult female	520.34 (± 44.85)	201604	170169
F2	Adult female	784.12 (± 18.80)	48413	42803
F3	Adult female	753.07 (± 33.98)	128489	108879
F4	Adult female	999.46 (± 43.82)	78069	55912
F5	Adult female	973.89 (± 20.22)	139840	102020
M1	Adult male	1246.58 (± 10.47)	147751	128722
M2	Adult male	747.50 (± 20.41)	60609	49615
M3	Adult male	1245.33 (± 18.57)	50157	44738
M4	Adult male	788.23 (± 13.94)	224544	188257
FL1	Fledgling	1121.49 (± 31.19)	111052	80295
FL2	Fledgling	1128.57 (± 17.58)	21906	17383
FL3	Fledgling	1298.53 (± 22.22)	90813	68463

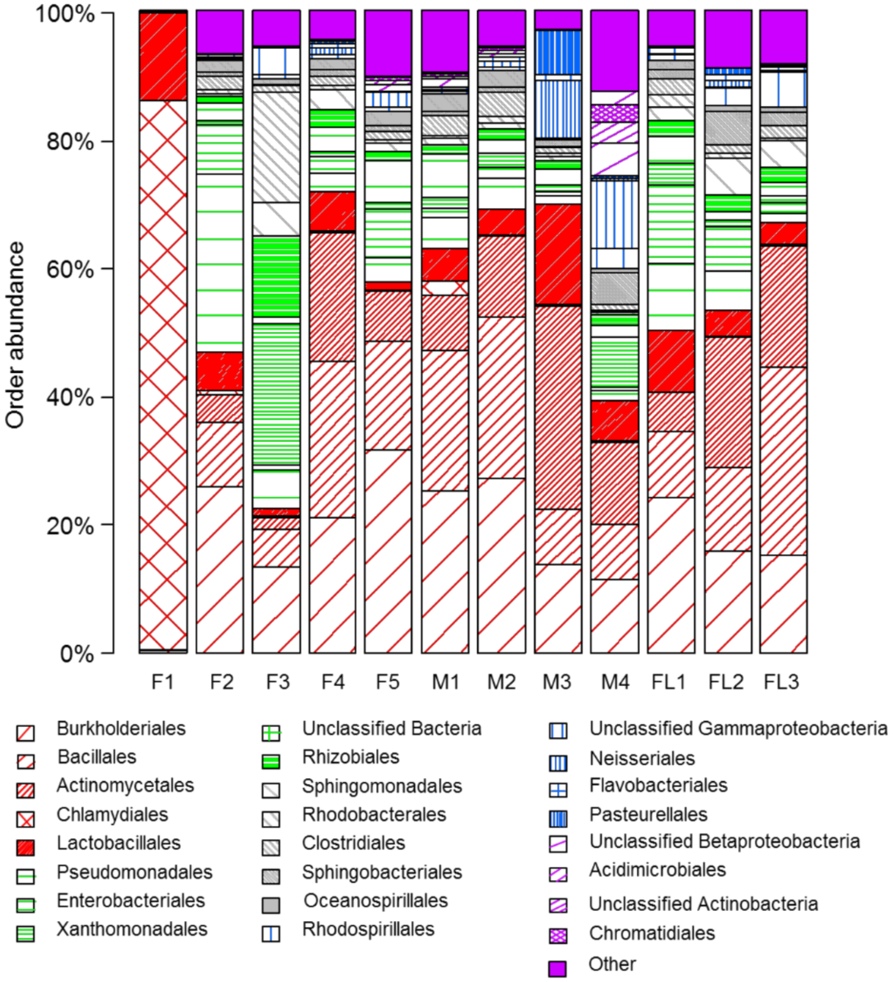

Figure 2. Relative OTU abundance classified at order level. F: adult females, M: adult males, FL: fledglings.

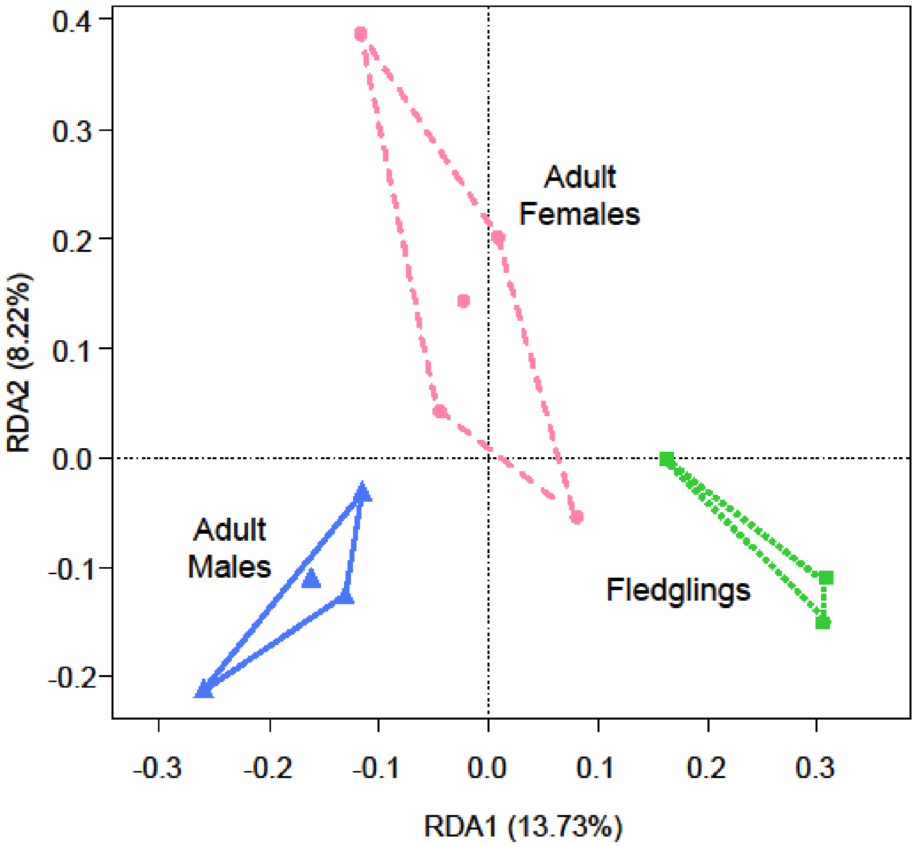

Figure 3. Biplot of first and second components from RDA of CM bacterial communities of 12 Barn swallows. Each symbol represents the CM of one individual. Different symbols and colours represent sex or age classes (pink dots = adult females, blue triangles = adult males, green squares = fledglings).

6.5 Discussion

The phyla Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes dominated Barn swallow CMs, consistently with the results on populations from the Czech Republic, which are ca. 580 km far from our study area, where a high abundance of the phylum Tenericutes was reported (Kreisinger et al. 2015, 2017). Proteobacteria and Firmicutes are common in the gastrointestinal trait of birds (Godoy-Vitorino et al. 2012; Waite et al. 2012; Waite & Taylor 2015). A more detailed description of the bacterial community at a lower taxonomic level showed that the most abundant genera differed from those observed in previous studies: the genera *Ralstonia*, *Pseudomonas*, *Aeribacillus*, and *Arenimonas* were abundant in our samples, while they were not found in the two previous studies in the Czech Republic (Kreisinger et al. 2015, 2017). *Ralstonia* spp. and *Pseudomonas* spp. are abundant microorganisms in many environments such as soil and water, and they have been found also in association with plants and animals (Brown 2014). *Aeribacillus* spp. are endospore-forming thermophilic bacteria closely related to *Geobacillus* genus, which has been described as ubiquitous in cold environments (Filippidou et al. 2015). *Arenimonas* spp. are aerobic non-spore-forming bacteria whose presence has been documented in soils and sediments. Interestingly, the two studies by Kreisinger and colleagues also found some differences in the CM compositions even of Barn swallow populations that bred at short distance from one another. Indeed, in one paper (Kreisinger et al. 2015), the most abundant genera were Candidatus *Hamiltonella*, *Corynebacterium*, *Acinetobacter*, and *Comamonas*, while in the other one (Kreisinger et al. 2017), the most abundant genera were *Mycoplasma*, *Catellicoccus*, *Serratia*, *Lactobacillus,* and *Dysgonomonas,* together with some members of the family Enterobacteriaceae that were not classified at genus level. Admittedly, these differences may be due, at least partly, to the different methods used in the two studies. Indeed, Kreisinger et al. (2015) collected CM by using swabs as in the present study, while Kreisinger et al. (2017) collected faeces to investigate faecal microbiota. However, faecal microbiota is generally considered as a proxy of bacterial communities of the lower intestine (Lewis et al. 2016). This difference in the methods therefore should not have largely affected the observed CM structure. Many genera that were abundant in the Czech Republic populations were among the least abundant or were even absent from our samples. Only the genera *Enterococcus*, *Staphylococcus*, and *Streptococcus* were consistently found among the ten most abundant genera in at least two out of the three studies on the species. These are all common and widespread bacteria. For instance *Enterococcus* spp. and related species are common, commensal members of gut communities in mammals and birds and also opportunistic pathogens (Byappanahalli et al. 2012).

The observed low overlapping of Barn swallow CMs is consistent with the hypothesis that environmental features of the areas where individuals live could be the main drivers of avian microbiota diversification and that CMs may therefore largely differ among geographical populations of the same species (Lucas & Heeb 2005; Waite & Taylor 2015). This confirms the importance of studies describing the microbiota of different and distant geographical populations of the same species. In addition, the ecological and evolutionary consequences of such inter-population differences will be a promising research avenue.

The CM of one adult female Barn swallow (#F1) was dominated by bacteria of the orders Chlamydiales (86%) and Lactobacillales (13%). Despite the large abundance of bacteria which are potential pathogens for vertebrates and a CM structure different from that of the other individuals, this female was recaptured in the colony during the year after the sampling (RA, personal communication) and thus survived to the following breeding season. We can hypothesize that the prevalence of Chlamydiales in the distal part of the gut may have a less detrimental effect on bird survival than in other parts of the digestive trait or that these bacteria are less detrimental to birds than to other vertebrates. This observation also confirms previous reports that gut microbiota of migratory passerines can include potentially pathogenic species without apparent effect on bird fitness (Lewis et al. 2016).

The majority of the 2482 OTUs found in the CM of Barn swallows had a low prevalence. Indeed, 25% of OTUs was found in only one individual while 27% in more than half of the individuals and only 4% in all the birds. The prevalence of rare OTUs and the large inter-individual variation in CM structure is consistent with the finding of previous investigations of the gut bacterial communities of birds (e.g. Lucas and

Heeb 2005, Hird et al. 2014, Lewis et al. 2016) and of other vertebrate taxa (Burr et al. 2005; Lucas & Heeb 2005; Ley et al. 2008; White et al. 2010). We did not observe significant differences between the sexes in the CM structure, consistently with a previous investigation on the Barn swallow in the Czech Republic. This may result from the horizontal transfer of bacteria among adults due to within pair and extra pair copulations (Kreisinger et al. 2015). We also did not observe a statistically significant difference in alpha or beta diversity between fledglings and adults. Our findings therefore contrast with the results of Kreisinger et al. (2017), which observed a higher alpha diversity of CM of adults than of nestlings and hypothesized that this was due to the ongoing formation of gut bacterial assemblage in nestlings.

Admittedly, the lack of significant differences in the structure of CMs among age and sex classes may be due to the rather small number of CMs considered in the present study. Indeed, our results show that males, females and fledglings form rather separate clusters, and future investigations on a larger sample may disclose differences in CM structures that were not revealed in the present study. Alternatively, it may be explained by the fact that, in birds, exogenous and environmental factors seem to influence the composition of the CM (Lucas & Heeb 2005; Waite & Taylor 2014) more than endogenous ones, and that Barn swallows breeding in the same colony are exposed to the same environmental conditions, including availability of insect prey. In addition, we sampled juveniles at a later age than Kreisinger et al. (2017), i.e. when they have already fledged and may have had time to acquire a CM more similar to that of adults. Finally, transfer of bacteria may occur not only among adults (see above), but also between adults and nestlings. Indeed, parents may transfer bacteria to nestling when feeding them, but they can also acquire bacteria from them e.g. when removing faecal sacs (Turner 2006).

This first description of the CM of Barn swallows in northern Italy and the third ever performed on this species (Kreisinger et al. 2015, 2017), although based on a rather small sample, thus suggests that CMs of geographically different Barn swallow populations seem dominated by bacteria belonging to the same phyla, while differences can be observed in the dominant genera.

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Chapter 7

Common swift feather microbiota described by high throughput DNA sequencing

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Chapter 7

7.1 Abstract

We provide the first-ever investigation of feather microbiota by high throughput DNA sequencing for any bird species by describing bacteria found on the innermost tertial feather of 22 adult Common swifts (*Apus apus*). We found feather microbiomes dominated by Bacillales, Actinomycetales, Burkholderiales, Sphingobacteriales, Sphingomonadales, Rhizobiales, Pseudomonadales, Clostridiales, Rubrobacterales and Lactobacillales. Bacterial communities changed with individual body condition with a significant increase of the ubiquitous genus *Ralstonia* (Burkholderiales). Three clusters of feather microbiomes could be identified, characterized by bacteria typical of seawater, plants and soil and unrelated to conditions at the breeding grounds. We hypothesized that feather microbiomes may reflect, at least partially, airborne bacterial communities of the environments where individuals spent non-breeding periods, or of those they crossed during migration, rather than breeding environment. If confirmed, this evidence may disclose the possibility to use feather bacteria as intrinsic marks for tracing non-breeding origin and routes of migratory birds.

7.2 Introduction

Besides being involved in several fundamental functions such as flight, thermoregulation and sexual displaying (Møller 1991; Leclaire et al. 2014), the plumage of birds hosts bacterial communities, like fur, skin or any other animal surface. The bird feather microbiota includes not only infective pathogens (see Hubálek 2004, Benskin et al. 2009) or feather-degrading bacteria (Justyn et al. 2017), but may also include beneficial ones (Ruiz-Rodríguez et al. 2009; Martínez-García et al. 2015). The increasing evidences of the central role of microorganisms in shaping the life histories of their hosts are stimulating the investigation of bird-bacteria interactions.

The feather microbiota is supposed to be under a strong selective pressure due to the intense solar radiation and the low nutrient concentration in the plumage, as well as the oil preening sanitization of feathers (Gunderson 2008). Moreover, feather microbiota changes significantly during the breeding season (Saag et al. 2011; Kilgas et al. 2012; Fülöp et al. 2017) because of the more intense intraspecific interactions that occur during this phase of the annual life-cycle, particularly during courtship, nest building, mating and parental care (Goodenough et al. 2017). In addition, in passerines, females usually have higher feather bacterial load than males and the microbiome of their feathers reflects more strictly that of the nest site (Czirják et al. 2010; Fülöp et al. 2017). Less is known, however, about the variation of the feather microbiota with individual conditions or with the load of other ectoparasites, particularly those that can contribute to feather degradation, like feather-eating chewing lice (Mallophaga) (González-Braojos et al. 2015).

Up to date, several studies on plumage bacteria relied on culture-based analyses that investigated the microbial load and composition by focusing on a few isolated and culturable bacteria (Møller et al. 2009; Schneegurt et al. 2016). They were particularly interest in feather-degrading bacteria (Lucas et al. 2005; Czirják et al. 2010; Saag et al. 2012), which include keratinolytic ones belonging to the genus *Bacillus spp.* (Burtt & Ichida 1999; Shawkey et al. 2003; Justyn et al. 2017). These bacteria cause a structural damage to the plumage that may reduce flight performances, thermal isolation or water-proof properties, as well as alter plumage coloration, thus affecting also mate choice and reproductive success of individuals (Grande et al. 2004; Shawkey et al. 2007). However, culture-based analyses are known to underestimate the total bacteria diversity and abundance (Rappé & Giovannoni 2003; Madsen 2015). To overcome this limitation, a few studies used culture independent methods to characterize feather microbiota (Shawkey et al. 2005; Bisson et al. 2007; Bisson et al. 2009). However, to the best of our knowledge, no investigations of the feather microbiota of a free-living species have been attempted so far by using high-throughput DNA sequencing, which is currently the technology that allows the deepest and most detailed characterization of bacterial communities (Brock et al. 2012; Caporaso et al. 2012).

In the present paper, we describe the feather microbiome of 22 adult Common swifts (*Apus apus*) by high-throughput DNA sequencing of the

16S rRNA gene. To the best of our knowledge, this work provides the first description of feather bacteria for this highly aerial migrator, which shows a peculiar behaviour of spending its life almost entirely on the wings and reaching the ground only for nesting (Hedenström et al. 2016), and the first-ever investigation of bird feather bacteria by high throughput DNA sequencing for any bird species.

Adult Common swifts may acquire feather bacteria from the atmosphere in the areas where they spent the different phases of their annual life-cycle (Bisson et al. 2009). In addition, they may acquire feather bacteria from the nest environment, as observed for other species (Kilgas et al. 2012). In this species, both parents share the parental duties including egg incubation (Lack & Lack 1951; Cramp 1998). However, males and females differ in the degree of nest attendance, with females generally feeding nestlings more and visiting the nest more frequently than males. In addition, feather microbiota may change during the different phases of reproduction (Gunderson et al. 2009) e.g. because Common swifts may gather and transfer bacteria from and to the nest environment, including eggs or chicks, while visiting the nest. We thus expected feather microbiota of this species to differ between the sexes and the breeding stage of each individual. In addition, we investigated whether it varied according to season, body size and condition, and arthropod ectoparasite load

7.3 Methods

Study species and data collection

The Common swift is a medium-size (16–17 cm; wing-span 42–48 cm) species and a long distance migrant (Cramp 1998). This species spends almost the entire life-cycle flying except for nesting, which occurs in cavities (Hedenström et al. 2016). Adults lay only one clutch of two-three eggs per year. The incubation lasts 20–22 days and both adults take care of the offspring until fledging that occurs within 37–56 days (Lack & Lack 1951; Cramp 1998).

We captured 22 adult Common swifts at their nests in a 'swift tower' in San Paolo (Brescia province) in the North of Italy (45°22′10.57″ N, 10°01′34.01″ E) in April-June 2013. The individuals were gently manipulated by an operator wearing latex gloves sterilized with alcohol immediately before the capture of each bird. The innermost tertial feather was picked and individually put in a sterile Petri dish. We characterized the bacterial community of one tertial feather because this feather is exposed to similar environmental conditions as remiges, but its removal would not impair the flight performance of the bird. We also recorded weight with a spring balance (accuracy 0.5 g), maximum flattened wing chord with a stop ruler (accuracy 0.5 mm), keel length (an index of body size) with a digital calliper (accuracy 0.01 mm), the presence of louse flies (*Crataerina pallida*), an obligate parasite of Common swifts that feeds on host blood, and the number of characteristic round holes left in tail and wing feathers by feather-eating chewing lice (Mallophaga: *Dennyus ssp*). We assume that hole number reliably reflects the intensity of chewing lice infestation as in the Barn swallow (*Hirundo rustica*) (Møller 1994). Breeding stage was calculated as the time-span between the hatching date and the date when the adult was captured in its nest (either positive if individuals were captured after hatching or negative if they were captured before). Finally, we recorded clutch size laid by each female or by the social mate of each male, and used the ratio between weight and keel length as an index of body condition. For accidental reason, some measure were not available for all individuals.

The study was carried out under permission of the local authority (Regione Lombardia Decreto 190 del 19/01/2015) responsible for authorizing animal studies in the wild and with the permission of the owner of the tower in San Paolo (BS) hosting the colony.

Molecular sexing

Individuals were sexed after PCR amplification of the sex-specific avian CHD1 gene. Total genomic DNA was extracted from feathers using the commercial 5 PRIME, ArchivePure DNA purification kit (5 PRIME, Hilden, Deutschland). The samples were initially rehydrated using a 0.9% NaCl solution for 10 min, with constant agitation. Only the vascolarized basal portion of the calamus was taken and chopped into small pieces. The calamus fragments were placed into 300 μL of the kit Cell Lysis Solution. Subsequently, the 'Protocol 14: DNA purification from 50 to 100 mg fresh or frozen solid tissue' of the ArchivePure DNA Purification Manual was followed except for adjusting quantities to 1/10 of the suggested volumes due to paucity of DNA present in the samples. Genomic DNA was finally resuspended in 30 μL of DNA hydration solution and stored at -20 °C.

A region of CHD1 gene was amplified using primers CHD1F 5’-TATCGTCAGTTTCCHTTTCAGGT-3’ and CHD1Rs 5’-CCTTTTATTGATCCATCAAGTC-3’, modified from Lee et al. (2010). The amplification was performed in a 15 µL reaction mixture, with 1–5 ng of genomic DNA, 1X PCR buffer (5 PRIME), 1.5 mM of Mg^{2+}, 0.3 µL of each primer (stock 10 mM), 1.5 µL of dNTPs (stock 2 mM) and 1 U Taq DNA polymerase (5 PRIME). Amplification was conducted under the following conditions: 94 °C for 5 min, 35 cycles at 94 °C for 45 sec, 55 °C for 45 sec and 72 °C for 1 min and further extension at 72 °C for 7 min. The amplification products were separated on 2.5% agarose gel and visualized after ethidium bromide staining. A single band identified a male and two different bands identified a female. Positive controls obtained from adults of known sex were included in the sexing protocol.

Bacteria DNA extraction and sequencing

Total DNA was extracted from half of the collected feather with FastDNA® Spin kit for Soil (MP Biomedicals, Solon, OH, USA). Half feather was directly inserted into the Lysing Matrix E tube. DNA extraction was performed according to manufacturer’s instructions. Since the amount of extracted DNA was very low, a nested PCR approach was used to prepare the samples for sequencing, which was performed by MiSeq Illumina (Illumina, Inc., San Diego, CA, USA) using a 250 bp × 2 paired-end protocol. Firstly, the nearly full length 16S rRNA gene was amplified from each sample with the universal primers 8F and 1495R (Tabacchioni et al. 1995; Frank et al. 2007) in 2 × 50 µL volume PCR reactions, performed with GoTaq® G2 Green Master Mix (Promega Corporation, Madison, WI, USA) and 1 µM of each primer. The cycling conditions were: initial denaturation at 95 °C for 4 min; 27 cycles at 95 °C for 30 sec, 55 °C for 45 sec, and 72 °C for 2 min and a final extension at 72 °C for 5 min. The amplicons were purified with the Wizard® SV Gel and PCR Clean-up System (Promega Corporation, Madison, WI, USA) and used as template for the subsequent amplification step. This second PCR was performed in 2 × 75 µL volume reactions with GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA) and 1 µM of each primer. We used primers 783F and 1046R (Huber et al. 2007; Wang & Qian 2009), which amplify a fragment containing the hypervariable regions V5-V6. Both primers were added with Illumina adapters at 5′ position. The cycling conditions were: initial denaturation at 98 °C for 30 sec, 20 cycles at 98 °C for 10 sec, 47 °C for 30 sec, and 72 °C for 5 sec and a final extension at 72 °C for 2 min. Finally, a third PCR was performed in 3 × 50 µL volume reactions by using 23 µL of the purified amplicons (Wizard® SV Gel and PCR Clean-up System, Promega Corporation, Madison, WI, USA) from the second step as template and 0.2 µM of each primer. Primers contained regions complementary to the Illumina adapters and standard Nextera indexes (Illumina, Inc., San Diego, CA, USA). The cycling conditions were: initial denaturation at 98 °C for 30 sec, 15 cycles at 98 °C for 10 sec, 62 °C for 30 sec, and 72 °C for 6 sec and a final extension at 72 °C for 2 min. After the amplification, DNA was quantified using Qubit® (Life

Technologies, Carlsbad, CA, USA). Sequencing was carried out at Parco Tecnologico Padano (Lodi, Italy).

Reads from sequencing were demultiplexed according to the indices and elaborated as reported in Musitelli et al. (2017). Briefly, forward and reverse reads were merged with perfect overlapping, suspected chimeras and singleton sequences (i.e. sequences appearing only once in the whole data set) were removed and Operational Taxonomic Units (OTUs) were defined at a 97%. Taxonomic classification of the OTU representative sequences was obtained by RDP classifier (Wang et al. 2007).

Sequences will be deposited to EBI-ENA database upon paper acceptance. Full datasets used in the present analyses are included in the Supporting Information 2 in electronic format and temporary available in google drive open-directory at:

https://drive.google.com/open?id=0B9Z15I6qtYQ3dGVEQ1FGYUhjaGc

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Statistical analyses

Alpha diversity. The number of sequences was used to estimate the abundance of each OTU in a sample. For each sample, we evaluated the total OTU richness by the Chao1 index that accounts for unobserved OTUs and its values can be compared among samples with different coverage (Chao & Shen 2003). In contrast, the number of OTUs observed in each sample was calculated on a random subsample of 2000 sequences, the sample size at which the majority of rarefaction curves reached a plateau (Figure S1). We analysed Chao1 index and the observed number of OTUs by Generalized Linear Models (GLM) assuming, respectively, a Gaussian or a Poisson distribution corrected for over-dispersion.

Beta diversity. The abundance of each OTU was assessed on the 2000 randomly extracted sequences per sample (see above) in order to give equal coverage to each bacterial community. The Hellinger distance among bacterial communities was used for the analyses, because of its desirable properties for multivariate statistical analyses: indeed, it is metric, decreases the importance of OTU abundance over occurrence and avoids the double-zero problem when comparing OTU composition between samples (De Cáceres et al. 2010; Legendre & Legendre 2012).

A preliminary investigation of bacteria community structure by principal component analysis (PCA) (Figure S2) suggested the presence of three groups of individuals with different bacterial communities. We thus investigated this pattern with hierarchical clustering using Hellinger distance among samples and a complete linkage method with the *hclust* function of R. This analysis confirmed the existence of three clearly distinct clusters (Figure 1a, see also the Results), which were therefore included in the analyses. Variation in bacterial communities was analysed by redundancy analysis (RDA), followed by post hoc pairwise comparison whose significance was adjusted by the False Discovery Rate (FDR) method (Benjamini & Yekutieli 2001). Variation in abundance of each bacterial genus according to the predictors that were significant in RDA was investigated by Poisson generalized linear models corrected for

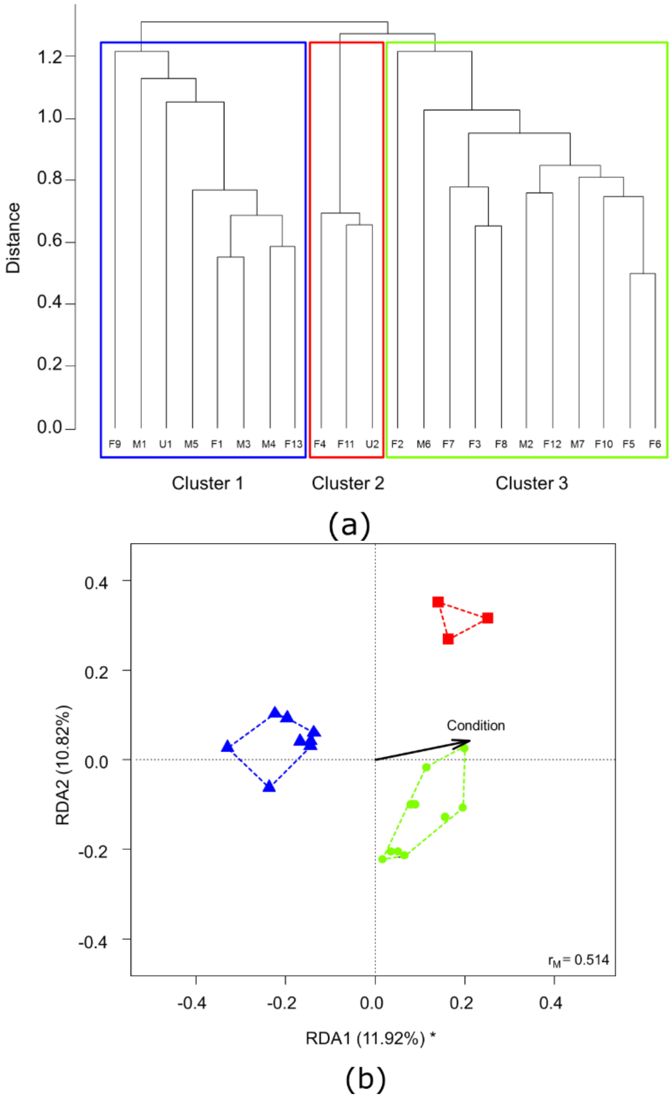

Figure 1 a) Hierarchical clustering of the bacterial communities from the feathers of 22 common swifts. b) Biplot of the principal components from a RDA of the bacterial community of 21 feather samples. Female #8 was excluded because keel length was not vailable. Each point represents the bacterial community of one bird. Blue triangles, red squares and green circles denote first, second and third cluster, respectively. The amount of variance explained by each axis is shown.

over-dispersion whose significance was adjusted by the FDR method. All the analyses were performed in R 3.2.2 (R Core Team 2016).

7.4 Results

We obtained 58194 sequences clustered in 598 OTUs, 7 (1%) of which were present in all samples, 165 (28%) in more than 50% of samples and 91 (15%) were exclusive of one sample. The number of OTUs in each sample ranged between 118 and 258.

The OTUs were classified in 44 orders, 10 (22%) of which occurred in all samples (Actinomycetales, Bacillales, Burkholderiales, Clostridiales, Lactobacillales, Pseudomonadales, Rhizobiales, Rubrobacterales, Sphingobacteriales and Sphingomonadales) (Figure 2) and 275 genera, 11 (4%) of which occurred in all samples, 75 (27%) in more than 50% of samples and 65 (23%) exclusive of one sample. Nine genera (*Hymenobacter*, *Lentibacillus*, *Methylobacterium*, *Nocardiopsis*, *Ralstonia*, *Rhodococcus*, *Sphingomonas*, *Staphylococcus* and *Virgibacillus*) had coverage > 1000 sequences and were considered abundant genera (Figure S3). As suggested by PCA analysis (Figure S2), the cluster analysis confirmed the presence of three distinct clusters that included respectively 8, 3 and 11 individuals (Figure 1a). The Bacillales and Actinomycetales were the two most abundant orders and dominated the bacterial communities of the first and second cluster respectively.

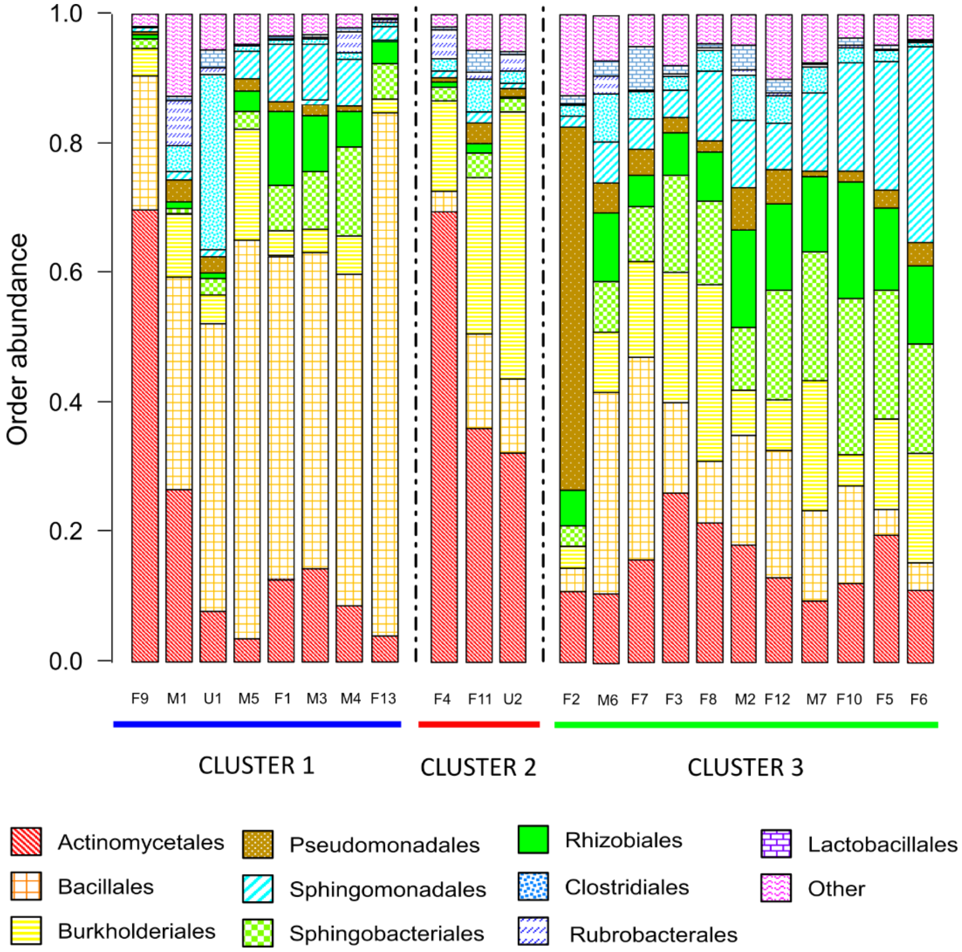

Figure 2. Relative OTU abundance classified at order level for the 22 common swift feathers. The three clusters are reported. Samples are reported in the same order as they appear in the dendrogram in Figure 1a.

Molecular sexing revealed that 13 individuals were females and 7 were males. Unfortunately, we failed in amplifying DNA from two individuals, which therefore could not be sexed and were removed from all analyses including sex among the predictors. Clutch size in the nest attended by male #4 was 5 eggs. Since Common swifts lay clutches of 2-3 eggs, rarely of 4 eggs (Cramp 1998; Sicurella et al. 2014), we hypothesized that this exceptionally large clutch was due to nest parasitism, and we excluded this value from the analysis.

Alpha diversity

Chao1 and number of OTUs did not change significantly according to sex or to any other variable ($F_{1,19} \leq 1.940$, $P \geq 0.180$). The number of OTUs differed significantly among the three clusters ($F_{2,19} = 6.901$, $P = 0.006$), but not Chao1 indices ($F_{2,19} = 3.215$, $P = 0.063$). In particular, cluster 2 had more OTUs than clusters 1 and 3 ($z \geq 2.479$, $P \leq 0.033$), which, in turn, did not differ to one another ($z = 1.523$, $P = 0.273$).

Beta diversity

RDA indicated that feather bacterial communities changed with individual condition ($F_{1,19} = 2.080$, $P = 0.008$). *Ralstonia* (Burkholderiales) was the only genus whose abundance increased significantly with individual condition (coef: 7.895 ± 1.629, $z = 4.846$, $P_{FDR} < 0.001$). No variation between sexes was found, nor with any morphological or phenological variables, or with clutch size ($F_{1,18} \leq 1.127$, $P \geq 0.287$).

The RDA confirmed that bacterial community structure significantly differed among clusters after controlling for condition ($F_{2,17} = 3.165$, $P = 0.001$), with significant difference between each pair of clusters (Pseudo-$F_{1,8} \geq 2.257$, $P_{FDR} \leq 0.014$). Abundance of six genera differed significantly among clusters ($F_{2,17} \geq 7.317$, $P_{FDR} \leq 0.035$). Post-hoc tests indicated that *Lentibacillus* and *Virgibacillus* (Bacillales) were more abundant in cluster 1 than in cluster 3, *Ralstonia* in cluster 2 than in cluster 3, *Hymenobacter* (Sphingobacteriales), and *Methylobacterium* (Rhizobiales) in cluster 3 than in cluster 1, and *Rhodococcus* (Actinomycetales) was more abundant in cluster 2 than in the other two clusters.

Group dispersion (multivariate variance) was homogeneous among clusters ($F_{2,18} = 2.121$, $P = 0.149$). Individuals belonging to different clusters also differed in condition ($F_{2,18} = 2.724$, $P = 0.044$) with a significant difference between clusters 1 and 2 ($z = 2.414$, $P = 0.041$; $|z| \leq 2.112$, $P \geq 0.085$ in the other cases).

Table 1. Description of feather bacterial communities from 22 Common swifts. For each sample, information about sex, Chao1 (±SE) index, the number of OTUs, the number of reads before and after quality filtering, and the total mapping reads are reported.

ID sample	Sex	Chao1 index (± SE)	No. OTUs	No. reads	No. reads after quality filtering	No. mapping reads
F1	Female	295.78 (± 18.82)	193	89084	56543	21508
F2	Female	235.55 (±21.32)	154	174448	108165	46895
F3	Female	313.48 (±17.59)	213	227959	109336	37021
F4	Female	221.62 (±17.34)	118	123984	57619	24670
F5	Female	263.14 (±11.02)	195	158644	77553	30764
F6	Female	307.45 (±26.03)	192	117228	65944	21549
F7	Female	312.62 (±25.14)	202	205929	116958	28522
F8	Female	279.43 (±19.45)	185	276440	136399	50989
F9	Female	230.09 (±20.53)	121	232982	116163	52191
F10	Female	265.85 (±14.66)	193	206714	132046	44944
F11	Female	216.00 (±12.93)	163	19801	7054	2050
F12	Female	266.16 (±9.79)	211	149780	86222	31138

F13	Female	256.20 (±22.35)	134	154969	105890	50343
M1	Male	218.37 (± 12.45)	160	104467	53444	29002
M2	Male	344.00 (±18.43)	258	228798	134904	44388
M3	Male	315.15 (±21.71)	190	42628	25191	10372
M4	Male	279.43 (±19.45)	179	19857	8085	2235
M5	Male	241.73 (±14.26)	159	180932	113521	36140
M6	Male	194.05 (±12.68)	148	111730	64928	24059
M7	Male	252.33 (±11.23)	199	154855	101047	47686
U1	Undetermined	270.80 (±22.61)	172	134920	41747	14505
U2	Undetermined	208.89 (±22.35)	130	126936	79033	27804

7.5 Discussion

We reported the first-ever description of the feather microbiota of a long distant migratory bird obtained by high-throughput DNA sequencing. The majority of the OTUs identified are rare and unique of different individuals. This high diversity of bacteria in Common swift feathers confirms the results by Bisson et al. (2007), who reported a high intra-specific variability in the composition of the plumage bacterial community, larger than inter-specific variability.

The structure of feather bacterial communities changed with individual condition, as assessed by the ratio between weight and keel length. In particular, abundance of *Ralstonia*, a ubiquitous genus belonging to Burkholderiales, increased in individuals in better conditions. This relationship was unexpected and, admittedly, we have no explanation for it. As opposed to previous studies on others birds (Saag et al. 2011; Kilgas et al. 2012; Goodenough et al. 2017), Common swift feather bacteria did not differ among sexes. This result can be because sexes are strictly monomorphic, share parental duties, and, therefore, are exposed to the same bacteria when entering nest holes. We also found no variation in bacteria communities with capture date. This suggest that Common swift feather microbiome does not strictly reflect air bacteria in breeding areas, as they show both seasonal variation (Bertolini et al. 2013) and variation at shorter time scale (Franzetti et al. 2011). We also found no variation with breeding stage, which suggests that exchange of bacteria with the nest environment does not contribute significantly to shape Common swift feather microbiome.

In contrast, we found that feather microbiomes could be divided into three clusters. Cluster 1 was dominated by OTUs belonging to the order Bacillales (with the exception of the male #9 dominated by Actinomycetales), cluster 2 by Actinomycetales and Burkholderiales, while no order was clearly prevalent in cluster 3 (except for male #2 dominated by Pseudomonadales). At genus taxonomical level, bacterial communities of cluster 1 showed large abundance of genera *Virgibacillus* and *Lentibacillus* (Bacillales) which are often described as moderately halophilic and are frequently retrieved in marine habitats, salt lakes, hypersaline soils or in salted seafood preparations (Zhang et al. 2016; Jung et al. 2017; Ok Jeon et al. 2017). Moreover, some *Virgibacillus* strains have been described as important cell factories for the production of extracellular proteinases (Montriwong et al. 2015; Sinsuwan et al. 2015). It is possible that these bacteria may have keratinolytic activity. Individuals in cluster 2 were dominated by the genera *Rhodococcus* (Actinomycetales) and *Ralstonia* (Burkholderiales). These bacteria are widespread in several environments, especially in soils and sediments. Burkholderiales are also commonly found in association with plant and animal surfaces (Brown 2014). Finally, bacterial communities of cluster 3 were characterized by large abundance of genera *Hymenobacter* (Sphingobacteriales) and *Methylobacterium* (Rhizobiales). These bacteria have been frequently indicated as main members of phyllospheric bacterial communities

(Vorholt 2012; Bulgarelli et al. 2013; Gandolfi et al. 2017). Individuals belonging to these groups also differed in their condition, which suggests that birds in poor condition may host different bacterial communities. Indeed, individuals in cluster 1 were in poorer conditions than those in cluster 2 and hosted larger amount of potentially keratinolytic bacteria. However, we stress that cluster 2 is composed of three individuals only, so all these speculations should be treated very cautiously. Importantly, the results showed that the three cluster structure does not depend on variation in individual conditions, as it appeared also after accounting for this latter variable in the analyses, and should therefore be related to other variable we did not account for.

The composition of plumage microbial communities is considered dynamic and open to the influence of local environments (Bisson et al. 2009). For instance, the microbial community in the plumage of American redstarts (*Setophaga ruticilla*) differed among habitats both in the breeding and in the wintering grounds (Bisson et al. 2007). In addition, tropical environments are thought to be more important sources of feather bacteria of migratory birds than temperate breeding sites (Bisson et al. 2009). These considerations led us to hypothesize that the three clusters we observed, which were unrelated to individual conditions and characterized by bacteria typical of different environments, may reflect different wintering habitats and/or migration routes of Common swifts. Each cluster was also characterized by bacteria that are generally predominant in a specific environment, i.e. seawater for cluster 1, soil for cluster 2 and vegetation for cluster 3. Intriguingly, Åkesson et al. (2016) identified three main routes followed by Common swifts for crossing the Sahara desert during spring migration: one crossing the western Sahara, one crossing central Sahara and one along the Arabian Peninsula. We therefore hypothesized that bacterial communities reflect, at least partially, different wintering or staging areas and/or migration routes visited by their host. Indeed, these migration routes imply, for instance, different crossing of the Mediterranean Sea, while the eastern route across the Arabian Peninsula and the Middle East is mainly on land. Unfortunately, we have no information on the non-breeding grounds and of the migration routes of the individuals we sampled. Different wintering or migration environments, in turn, may affect both condition of individuals during the following breeding season and feather bacteria. Thus, the association between feather bacteria and host condition does not necessarily contrast with the hypothesis that feather bacteria may maintain a signature of the non-breeding and migration environments, which, if confirmed by future studies, may disclose the possibility to use feather bacteria as an intrinsic mark for tracing origin and routes of migratory birds.

7.6 Aknowledgments

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Chapter 8

Parent-absent begging in Barn swallow broods: causes of individual variation and effects on sibling interactions and food allocation

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Chapter 8

8.1 Abstract

Altricial offspring solicit parental care using complex begging displays. Although such solicitations are mainly performed towards parents, nestlings of several birds often beg when parents are not at the nest. This parent-absent begging (PAB) has been interpreted as a mistake in correctly detecting parental arrival, but it may also reliably signal individual need to nest mates, potentially affecting intrabrood competition for food. Here, we focused on the proximate factors that may determine frequency and intensity of PAB, including brood size and sex ratio, as well as individual age, sex and body mass, in natural Barn swallow (*Hirundo rustica*) broods, under conditions of normal and experimentally reduced food intake. We also investigated the relationship between PAB and begging intensity at parental arrival and food intake in the two different experimental conditions. The frequency of PAB was larger after food deprivation than before, indicating that PAB reliably reflects hunger level. In addition, nestlings increased their own begging intensity upon parental arrival after performing PAB. Concomitantly, siblings decreased begging effort, irrespective of sex or body mass, but this occurred only when nestlings were normally fed, suggesting that PAB diminishes scrambling for food by nest mates. Finally, such a reciprocal begging modulation resulted in a larger chance of obtaining food at the subsequent feeding event for nestlings performing PAB under normal food provisioning. Within-brood signalling in the absence of parents can therefore play an important role in

determining competitive strategies among siblings and affecting parental decisions on food allocation in altricial broods.

8.2 Introduction

Offspring of altricial species are entirely dependent on the resources provided by parents during the first stages of their life (Trivers 1974; Clutton-Brock 1991). Since resources are limited, competition among siblings over parental care is the norm (Clutton-Brock 1991; Mock and Parker 1997). However, from the standpoint of any individual offspring siblings represent precious carriers of half of their own genes, thus contributing to increase inclusive fitness (Hamilton 1964). As the marginal benefit of acquiring additional units of parental investment increases with declining individual state (Godfray 1991, 1995), offspring have to balance the direct gain of receiving more care with the indirect cost of subtracting resources to siblings. Maximization of inclusive fitness will thus entail the adoption of condition-dependent behaviours by different offspring, which should shift from strong competition, potentially leading to the siblings' death, to moderation of selfishness, for instance by assuring a large share of resources to kin in poor condition (Elgar and Crespi 1992; Mock and Parker 1997; Bowers et al. 2013). The latter case is expected to occur more frequently whenever the individual fitness benefit of receiving additional parental resources is low (e.g. when the 'altruist' offspring is in good condition) and/or is not balanced by the cost sustained to obtain them (e.g.

when brood mates are more motivated to obtain it, thus leading to harsh competition among siblings).

In many taxa, sibling competition over parental care is mediated by begging behaviour, a combination of acoustic, visual and postural displays that offspring use to solicit food provisioning (Kilner and Johnstone 1997; Wright and Leonard 2002). The role of begging in mediating intrafamilial conflicts is still debated (reviewed in Kilner and Johnstone 1997; Wright and Leonard 2002; Mock et al. 2011), since begging has evolved either to manipulate parental feeding decisions to the offspring's advantage (Macnair and Parker 1979; Parker and Macnair 1979; Parker et al. 2002), or as a reliable signal of need used by parents to optimally invest care among the brood (Godfray 1991, 1995). In general, it is acknowledged that begging solicitations increase with offspring hunger and that parents vary food provisioning according to begging level (see Kilner and Johnstone 1997; Mock et al. 2011 for reviews). Finally, begging is costly in terms of energy expenditure (Kilner 2001; Boncoraglio et al. 2012) and increase of predation risk (Haskell 1994; McDonald et al. 2009).

Besides begging towards attending parents, offspring of several bird species often display begging also when parents are not at the nest (e.g. Budden and Wright 2001; Leonard and Horn 2001; Leonard et al. 2005; Bulmer et al. 2008; Roulin et al. 2000, 2009). Because this so-called parent absent begging (PAB) does not seem to provide any direct benefit in terms of food intake, it has been considered a misdirected behaviour, that in some cases might adaptively reduce the probability of missing parental feedings

(Budden and Wright 2001; Leonard and Horn 2001; Dor et al. 2007). This hypothesis has received some support especially in passerines, where PAB can be elicited by several external stimuli (e.g. movements by nest mates, moving shadows near the nest, incidental noises, adult birds other than parents flying near the nest; see Leonard and Horn 2001; Leonard et al. 2005; Dor et al. 2007). However, in other taxa (e.g. Apodiformes, Strigiformes) nestlings continuously perform PAB between parental feeding visits, suggesting that such a behaviour might serve to signal individual need and willingness to compete for the next feedings (Roulin et al. 2000, 2009; Dreiss et al. 2010). PAB might thus mediate negotiation among siblings over priority in access to food at subsequent feeding events, by allowing nestlings to adopt the optimal strategy between outcompete nest mates or refrain from competition against needier siblings, since their own chances of getting fed should be low (Roulin et al. 2000, 2009; Johnstone and Roulin 2003; Roulin 2004; Dreiss et al. 2010). To date, such a ‘sibling negotiation hypothesis’ has been demonstrated in the barn owl (*Tyto alba*)only (Roulin 2004; Roulin et al. 2000, 2009; Dreiss et al. 2010).

In a recent experiment involving dyads of size-matched Barn swallow (*Hirundo rustica*) nestlings, we have shown that PAB reliably reflected current hunger level and could be used by siblings as a reliable cue to assess how intense begging behaviour should be upon parental arrival (Romano et al. 2013). In this species however nestlings seem not to ‘negotiate’ by reciprocally exchanging information with siblings about their willingness to compete for parental food, as occurs in barn owls. Indeed, PAB does not occur continuously between all feeding visits, suggesting that it may actually be an error in detecting parent arrival to the nest. Nevertheless, it can be exploited by nestlings to adjust their ‘selfish’ vs. ‘altruistic’ behaviour (Romano et al. 2013). Here, we broadened the scope of our previous study by investigating the occurrence of PAB and its consequences for sibling interactions and food intake in entire Barn swallow broods in two contrasting conditions: under natural feeding regime and after a period of food deprivation. This approach allowed us to test whether PAB functions as a reliable signal, potentially affecting the begging behaviour of different nestlings, also in a natural context with multiple receivers of various competitive ability. In the present study we in fact investigated whether any response to PAB varied among nestlings differing in long-term condition, as gauged by the body mass relative to siblings which is a reliable proxy of hatching order, and sex, as previously shown for begging towards attending parents (Saino et al. 2001; Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011). In addition, differently from our previous study, where strong asymmetry in the hunger level among nestlings occurred, here all nest mates were always tested under the same level of satiation. This experimental design thus reflects a more realistic situation, simulating the variation in the ecological conditions to which the entire brood is exposed (e.g. sunny vs. rainy days). A further novel aspect of this study compared to the previous one is the examination of the nestlings’ behavioural responses to PAB events performed by at least two nest mates simultaneously (simultaneous PAB).

Moreover, we also investigated the role of several factors that can potentially affect the occurrence of PAB, including traits of individual offspring (age, sex, body mass) and of the brood environment (hunger level, brood size, brood sex ratio), which are known to affect begging behaviour toward attending parents in the Barn swallow (Saino et al. 1997, 2000, 2001, 2008a; Bonisoli-Alquati et al. 2011). Importantly, despite PAB has been examined in several bird species, no study has tested the possibility of a sex-specific variation in this peculiar behaviour.

We expected nestlings to increase PAB frequency and intensity after food deprivation as reported in the Barn swallow (Romano et al. 2013) and in other species (Leonard and Horn 2001; Roulin 2001; Dor et al. 2007 ; Bulmer et al. 2008). We then evaluated whether nestlings adjust begging strategies according to their own and/or siblings' PAB, possibly affecting food intake at the first subsequent parental feeding visit. According to our previous study (Romano et al. 2013), we expected nestlings to increase begging intensity after performing PAB (i.e. because they are particularly hungry) and to reduce begging intensity after a sibling had performed PAB compared with feeding events not preceded by any PAB. However, we had no a priori prediction about the behaviour of nestlings following simultaneous PAB. Furthermore, if a nestling performed PAB, its probability of obtaining food was expected to increase at the subsequent feeding visit. Since food deprived nestlings should be less prone to reduce begging after a sibling's PAB (Romano et al. 2013), we also expected that begging modulation after PAB should be less pronounced when nestlings were food deprived. Finally, as female and larger nestlings have been shown not to escalate begging when interacting with needy siblings (Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011), we predicted that female and larger nestlings reduced competitive effort after siblings' PAB signals more than their male or smaller nest mates.

8.3 Materials and Methods

Study species

The Barn swallow is a small, insectivorous migratory passerine that breeds semi-colonially inside rural buildings (Møller 1994). Females lay 1–3 clutches of 3–7 eggs per breeding season and incubate them for ca. 14 days (Møller 1994). Both parents provide food to nestlings, which fledge when 18–21 days old. At each food provisioning visit only one nestling is fed. Competition for food among siblings is mediated by calling, gaping and posturing upon feeding visits (Boncoraglio et al. 2008; Romano et al. 2012), but nestlings also perform PAB, mostly elicited by diverse external stimuli, such as vocal calls or flying of adults near the nest, and movements of nest mates (Romano et al. 2013). Begging intensity increases with hunger level, and parents preferentially feed those nestlings that beg more conspicuously (Boncoraglio et al. 2008; Romano et al. 2012).

Data collection

The study was performed in May–August 2012 in three Barn swallow colonies (= farms) near Milan, Northern Italy. Nests were inspected every second day to record breeding events. Only broods composed by at least three nestlings were included in the experiment: the brood size of nests used was 3 (N = 4 nests), 4 (N = 8), 5 (N = 7), or 6 (N = 3) nestlings. A few minutes before starting the first trial (see below), we marked each nestling with an individual aluminum ring and weighted it with an electronic scale (nearest 0.1g). We then computed relative body mass as the difference between individual body mass and mean body mass of all nestlings in a brood (mean coefficient of variation of body mass within broods: 5.9%; range: 2.25–9.33%). Taking the day of hatching of the first egg as day 0, trials were performed at the age of 8 (N = 2 nests), 9 (N = 8), 10 (N = 8), or 11 (N = 4) days. We recorded nestlings' traits and behaviour before day 12 after hatching, when peak body mass is attained. The relative body mass at this age reliably reflects the within-brood size/age hierarchy (Saino et al. 2001; Bonisoli-Alquati et al. 2011).

Behavioural data were collected from all broods (N = 22). Beginning between 07:00 and 08:00 h, broods were video recorded in their natural nests for 90 min (before food deprivation trial). All but one of the nestlings were removed from the nest for a 90-min period of food deprivation, and then returned to the nest for a second period of 90 min video-recording (after food deprivation trial; see Boncoraglio et al. 2008; Romano et al. 2011). Because the Barn swallow is an aerial forager feeding almost exclusively on flying insects, feeding trials were carried out only on sunny days, thus reducing variation in food availability due to weather conditions. Nestlings were temporarily marked with small white spots on their head to make them individually recognizable. The aim of the food deprivation phase was to experimentally increase hunger level, similarly to what may naturally occur in the case of heavy rain. We decided to leave one nestling, randomly chosen, in the nest during the food deprivation of its siblings to limit parental stress and reduce the risk of nest desertion. We left in the nest the normally fed nestling during the after food deprivation trial to avoid altering the size and the sex ratio of the brood between the two trials. However, because its satiation level was different from that of its food-deprived siblings, data for this nestling were excluded from the analyses concerning the after food deprivation trial.

A small drop of blood (ca. 20μl) was collected after the trials for molecular sexing (see Saino et al. 2008b), and the sibling sex ratio of each nestling calculated as the proportion of its nest mates that were male. On the whole we included in the study 97 nestlings (48 males and 49 females; normally fed nestlings: 9 males and 13 females).

Behavioural analyses

We recorded all PAB bouts, defined as any occurrence of postural begging in the absence of parents close to the nest (see Romano et al. 2013). Begging displays occurring after the departure of parents from the nest (see Leonard and Horn 2001) or within 5 s of parental arrival, and all cases of PAB performed when the parents were evidently flying around the nest were excluded from the analyses (see Leonard and Horn 2001). The maximum intensity of postural begging of all nestlings at each parental feeding visit and in the absence of parents was scored using a four-level scale (0 = no movements; 1 = gape open; 2 = gape open and neck stretched; 3 = intense begging with fully stretched neck and tarsi; see also Romano et al. 2012, 2013). We also recorded which nestling obtained the food during each feeding visit. Individual identification of nestlings was possible for the vast majority (ca. 90%) of cases.

When a nestling performed more than one PAB bout during the same inter-feeding interval, we considered only the last bout before parental arrival, because neither begging intensity of nestlings at subsequent parental arrival nor the probability of being fed was different after single or multiple PAB (see Romano et al. 2013). We adopted this procedure also because our aim was not to test the ‘sibling negotiation hypothesis’, which posits that nest mates are senders and receivers of sequential begging signals during periods of parental absence (e.g. Roulin et al. 2009; Dreiss et al. 2010, 2013), but only to investigate whether Barn swallow nestlings can use different kinds of signals, including those performed by siblings before parental arrival, to modulate their competitive behaviour when facing nest mates in different conditions. The analyses were also limited to the first feeding event following a PAB, because our previous findings showed that PAB does not affect both begging intensity and probability of obtaining food at the second (and subsequent) parental feeding visit (see Romano et al. 2013).

Importantly, we examined whether simultaneous PAB by two or more nestlings affected nestlings begging behaviour at parental arrival and food intake as compared to PAB bouts performed by single nestlings. Indeed, two or more nestlings were observed to simultaneously display PAB (28% of total PAB bouts), and this may affect subsequent behaviour of nest mates differently from the case when a single nestling performs PAB.

Because the effect of food deprivation on begging behaviour decreases as the nestlings are fed during the feeding trial (Romano et al. 2012), the analyses of PAB intensity, begging intensity at parental arrival and food intake were limited to the initial third of all feeding events of the after food deprivation trial (hereafter, we define each third of all feeding events as 'feeding period'; see Romano et al. 2012, 2013 for details), including on average 17.10 ± 6.16 SD (range 7–29) feedings. This decision is justified by the observation that PAB frequency considerably decreased from initial feeding period to last feeding period in the after food deprivation trial (Poisson mixed model including 'feeding period' as a three-level fixed factor, and nest and nestling identity as random effects: $F_{2,22} = 27.16$; $P \leq 0.0001$; mean PAB bouts per nestling (± ES) initial feeding period: 2.87 (0.50); middle feeding period: 2.25 (0.34); last feeding period: 1.11 (0.28); all pairwise comparisons were statistically significant: $t_{22} \geq 2.34$; $P \leq 0.029$). Conversely, we used all feeding events occurring in the before food deprivation trial (28.78 ± 8.49 SD feedings h^{-1}) because there was no reason to expect a variation in PAB frequency during this feeding trial. Finally, the analysis of variation in the number of PAB bouts (PAB frequency) by individual nestlings before and after food deprivation was limited to the initial feeding period also for the before food deprivation trial, because it was appropriate to use the same reference scale in both trials.

Statistical Analyses

We relied on repeated-measures mixed models using different distributional assumptions and appropriate link functions because of the different nature of the dependent variables (PAB intensity and begging intensity: ordinal variables; PAB frequency: count variable; probability of begging and nestling obtaining food: dichotomous variables). In all models we included nest and nestling identity as random grouping factors in the random part of all models, and we allowed random variation in the intercepts between each level of both factors. Our models were therefore ‘‘random intercept models’’ (sensu Schielzeth and Forstmeier 2009).

Variation in PAB frequency was analysed in Poisson mixed models, while variation in PAB intensity was analysed in multinomial mixed models for ordered outcomes (using the cumulative logit link function, which is suitable for cumulative probabilities; Schabenberger 2006). Models included feeding trial (before or after food deprivation) and sex as dichotomous fixed factors. Brood size, age, sibling sex ratio and relative body mass were included as covariates. Because including all two-way interactions between these factors would lead to model overfitting, given the relatively small sample of 22 independent broods, we decided to test only the interactions between feeding trial and all of the other factors.

Variation in begging intensity at parental feeding visits according to PAB was analysed in a multinomial mixed model for ordered outcomes (see above). The model included sex as fixed effect and a four-level factor (PAB category) indicating if the feeding event was, from the viewpoint of any focal nestling, 1) preceded by own PAB (individual PAB), 2) preceded by a simultaneous PAB by the focal nestling and at least one sibling (simultaneous PAB), 3) preceded by a PAB by one or more siblings (siblings' PAB), or 4) preceded by no PAB by any nestlings (no PAB). The latter category represents the average begging intensity when PAB did not occur (baseline begging intensity). Importantly, since nestlings which have just received a food item should reduce begging intensity because of temporary satiation, the latter two PAB categories did not include begging data of the nestling that was fed at the previous feeding event with respect to the feeding event under scrutiny. This procedure therefore allowed us to account only for variation in begging intensity not due to the contingent hunger level of nestlings (results were similar when all nestlings in all feeding events were included; details not shown for brevity). For the analysis of begging intensity, we ran separate analyses for each trial rather than a model including all data because: 1) before and after food deprivation trials included a different number of nestlings (i.e. data of normally fed nestlings were excluded from the analysis of the after food deprivation trial); 2) the sample size for each PAB category level was highly unbalanced between the trials so that any effect of e.g. begging intensity on the probability of receiving food would be masked by feeding trial and vice versa. However, for the sake of completeness we ran further models (excluding data for the normally fed nestling also from the before food deprivation trials) to investigate differences in the response to PAB between feeding trials, while testing the interaction term between feeding trial and the other factors.

Furthermore, the effect of PAB on the probability that a nestling begged at parental visit (probability of begging) was analysed in a binomial mixed model with begging occurrence (0 = no begging, 1 = begging) as a dichotomous dependent variable. Sex, PAB category and their interaction were included as fixed effects.

Finally, we tested whether the probability of obtaining food differed between feeding events preceded by own PAB or by no PAB in a binomial mixed model. This model included PAB category and sex as well as their interaction as fixed effects. Feeding events preceded by siblings' PAB were excluded from this analysis to avoid duplicating the data (see also Romano et al. 2013). In addition, since the category 'simultaneous PAB' included all the cases when at least two nestlings performed PAB, feedings after simultaneous PAB were omitted here because the probability of being fed by a nestling that had performed PAB should be closely correlated with the proportion of nestlings that did perform PAB, and not because of the PAB itself. As the probability of obtaining food strictly depends on the number of competitors, brood size was added as a covariate.

Feeding events preceded by a simultaneous PAB by all nestlings were excluded from the analyses of begging intensity, probability of begging and probability of obtaining food. In all models, degrees of freedom were conservatively set equal to the number of nests included in the analysis (DF = 22). Final models were obtained by removing in a single step all non-significant interactions, while retaining all main effects in the model. When multinomial models did not converge, we repeated analyses both without random effects and using linear mixed models including random effects. Qualitatively similar results were always obtained (details not shown for brevity).

All analyses were run with the SAS 9.1 GLIMMIX procedure (SAS Institute 2006). Differences between groups within each trial were explored by post hoc tests in binomial models (LSMEANS statement in SAS syntax). However, since the marginal means cannot be computed for multinomial models (Schabenberger 2006), we conducted specific planned comparisons in these cases (see also Romano et al. 2013). For ease of interpretation, in the 'Results' we show mean PAB score and mean begging score values. Sample sizes may differ slightly among analyses because of missing data for some individuals.

Ethical Note

When removed from nest, nestlings were kept in a cloth bag at ambient temperature. At each measurement session, nestlings were handled only for a few minutes. No obvious negative effects of handling nestlings or of blood collection were detected. The white spots on their head were applied by a non-toxic marker, and disappeared within a few days. During videotaping we could not detect any effect due to the presence of recording equipment and of the white marks on the nestlings' head on parental and offspring behaviour. The camera was installed near the nest the day before feeding trials to allow parents to habituate to it (the first feeding was brought to the nest within 10.45 min ± 7.75 SD since the beginning of the trial). The study was carried out under permission of the local authority (Regione Lombardia #M1.2011.0002141) responsible for authorizing animal studies in the wild.

8.4 Results

Individual variation of PAB before and after food deprivation

PAB displays accounted for approximately 16% of all begging bouts, with 23% of feeding events being preceded by one or more PAB by at least one nestling. Variation in PAB frequency and intensity was not related to brood size, sex, sibling sex ratio, age, or relative body mass (Table 1). As expected, nestlings performed more PAB when food deprived than when normally fed. However, PAB intensity did not vary between the before and the after food deprivation trial (Table 1). The effect of all the above mentioned variables did not differ between the before and the after food deprivation trial, as indicated by the absence of any statistically significant effect of their interaction with feeding trial.

Table 1. Final models showing the effects of feeding trials (before or after food deprivation), relative body mass, age, sex, sibling sex ratio and brood size on PAB frequency and intensity in 22 Barn swallow broods. Interaction terms dropped from final models (see Methods) are also shown.

	Coefficient (SE)	$F_{1,22}$	*P*
PAB frequency			
Final model			
Feeding trial	0.464 (0.165)[a]	7.92	0.01
Relative body mass	-0.046 (0.074)	0.38	0.54
Age	0.395 (0.274)	2.08	0.16
Sex	0.027 (0.263)	0.01	0.92
Siblings sex ratio	0.874 (0.665)	1.72	0.20
Brood size	-0.196 (0.251)	0.61	0.44
Excluded variables			
Relative body mass × Feeding trial	0.205 (0.151)	1.84	0.19
Age × Feeding trial	-0.025 (0.238)	0.01	0.92
Sex × Feeding trial	0.394 (0.346)	1.29	0.27
Siblings sex ratio × Feeding trial	0.614 (0.541)	1.29	0.27
Brood size × Feeding trial	-0.245 (0.190)	1.65	0.21
PAB intensity			
Final model			
Feeding trial	0.098 (0.187)[a]	0.28	0.60
Relative body mass	0.061 (0.109)	0.27	0.61

Age	-0.074 (0.163)	0.20	0.66
Sex	0.074 (0.256)	0.08	0.78
Sibling sex ratio	0.047 (0.429)	0.01	0.91
Brood size	0.136 (0.116)	0.96	0.34
Excluded variables			
Relative body mass × Feeding trial	-0.116 (0.170)	0.46	0.50
Age × Feeding trial	0.051 (0.268)	0.04	0.85
Sex × Feeding trial	0.097 (0.383)	0.06	0.80
Siblings sex ratio × Feeding trial	-0.649 (0.644)	1.01	0.32
Brood size × Feeding trial	0.209 (0.230)	0.82	0.37

a: positive coefficients indicate increasing PAB frequency in the after food-deprivation trial compared to the before food-deprivation trial

Table 2. Final models showing the effects of PAB category and sex on begging intensity and probability of begging during parental feeding visits, and on the probability of obtaining food in 22 Barn swallow broods, before and after food deprivation. In the analysis of the probability of obtaining food only feeding events preceded by individual PAB or by no PAB were included (see Materials and Methods). Two-way interaction terms were dropped because they never attained statistical significance (see Materials and Methods).

	Before food deprivation				After food deprivation			
	Coefficient (SE)	F	DF	P	Coefficient (SE)	F	DF	P
Begging Intensity								
PAB category		30.97	3, 22	<0.001		2.69	3, 22	0.07
Sex	0.021(0.063)	0.11	1, 22	0.74	-0.105(0.253)	0.17	1, 22	0.68
Probability of begging								
PAB category		25.43	3, 22	<0.001		3.74	3, 22	0.026
Sex	-0.009(0.083)	0.01	1, 22	0.92	-0.161(0.154)	1.10	1, 22	0.31
Proability of obtaining food								
PAB category		4.55	1, 22	0.044		0.32	1,22	0.58
Sex	-0.113(0.115)	0.97	1, 22	0.33	-0.318(0.220)	2.10	1, 22	0.16
Brood size	-0.527(0.067)	62.29	1, 22	<0.001	-0.547(0.116)	22.04	1, 22	<0.001

Effect of PAB on begging behaviour at parental visits

Begging intensity varied according to PAB category both before and after food deprivation (Table 2). However, variation in begging intensity in response to PAB was somewhat different between the two trials (Fig. 1a).

In the before food deprivation trial, nestlings begged more intensely during feeding visits following individual and simultaneous PAB than in those preceded by no PAB or by a siblings' PAB (planned comparisons, all $t_{22} \geq 6.23$, all $P \leq 0.001$; Fig. 1a, left panel). Importantly, nestlings reduced their begging intensity after a siblings' PAB compared to their baseline begging intensity ($t_{22} = 2.89$, $P = 0.008$; Fig. 1a, left panel). Finally, no difference was found in the begging intensity following individual versus simultaneous PAB ($t_{22} = 1.03$, $P = 0.31$; Fig. 1a, left panel).

After food deprivation, nestlings begged more intensely after individual PAB than at feeding events preceded by a siblings' PAB ($t_{22} = 3.47$, $P = 0.002$) or by no PAB ($t_{22} = 2.55$, $P = 0.018$; Fig. 1 a, right panel). Interestingly, the reduction of begging after siblings' PAB compared to baseline begging intensity, which emerged before food deprivation, was not observed when all nestlings were food deprived ($t_{22} = 0.95$, $P = 0.35$; Fig. 1 a, right panel). Finally, no differences between other PAB categories emerged (all $t_{22} \leq 1.71$, all $P \leq 0.10$; Fig. 1a, right panel).

The model including data from both trials showed that nestlings begged more intensely when food deprived than normally fed ($F_{1,22} = 11.31$, $P = 0.003$) and that they greatly varied their begging intensity according to PAB category ($F_{3,22} = 35.04$, $P \leq 0.001$; Fig. 1a). The relationship between begging intensity and PAB category did not significantly differ between the sexes (interaction effect: $F_{1,22} = 0.01$, $P = 0.92$). However, the PAB category by feeding trial interaction was non-significant ($F_{3,22} = 2.47$, $P = 0.09$), suggesting that observed differences between trials were weak.

Analyses of probability of begging provided almost identical results to those concerning begging intensity, confirming all the differences among groups described above in the analyses performed both separately for each trial and those including all data (see Table 2 ; Fig. 1 b; details not shown for brevity).

To account for the potential effect of individual size rank hierarchy within the brood (i.e. reflecting hatching order) on begging intensity according to PAB category we repeated all the above analyses including the relative body mass as a covariate and the interaction between relative body mass and PAB category as a fixed effect. Relative body mass and the interaction between relative body mass and PAB category never attained statistical significance (relative body mass × PAB category: all $F_{3,22} < 0.93$; all $P > 0.29$; relative body mass: $F_{3,22} < 1.18$; all $P > 0.28$). All the other results were qualitatively similar to those showed in Table 2 (details not shown for brevity).

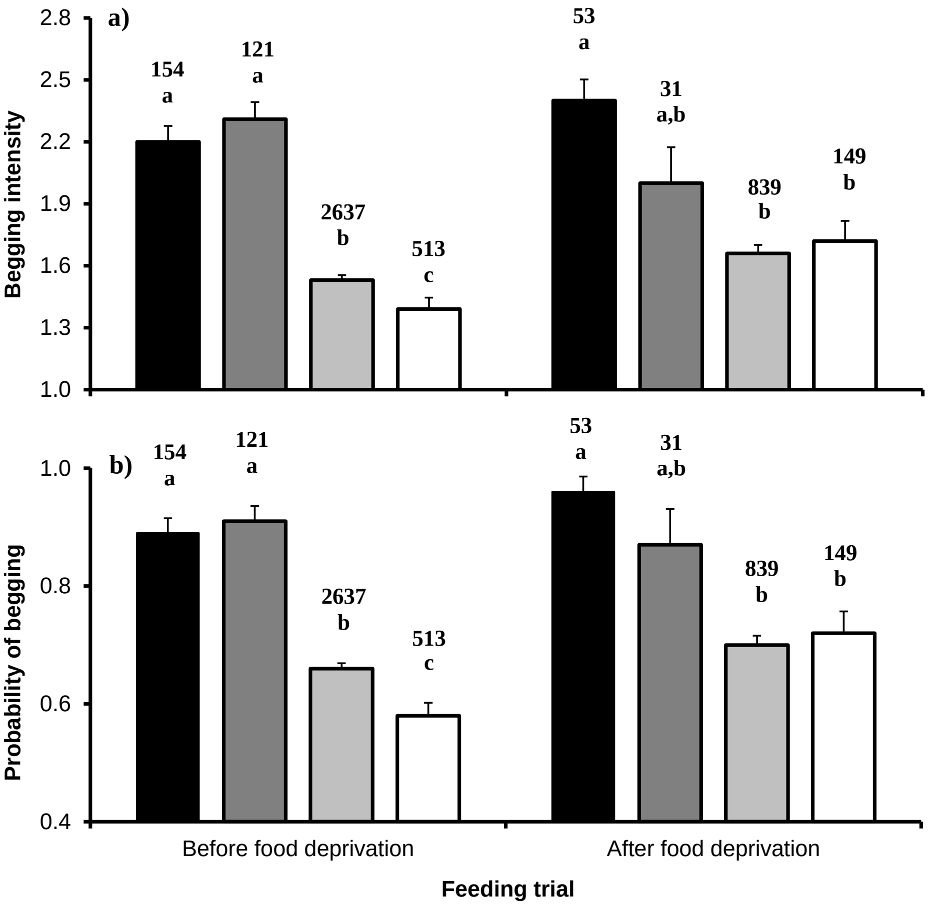

Figure 1. Mean (+ SE) (A) begging intensity and (B) probability of begging according to PAB category [feeding events occurring after individual PAB (black bars), simultaneous PAB (dark grey bars), no PAB (light grey bars) or sibling's PAB (white bars)]. Mean values are reported separately for the before (left panel) or the after food deprivation (right panel) trials. Numbers above bars indicate the number of observation for which we scored begging intensity and probability of begging, and SEs were calculated based on these data. PAB categories within each feeding trial that differed significantly [$P < 0.05$ according to planned comparisons in panel (A) or post-hoc tests in panel (B)] are denoted by different letters above bars.

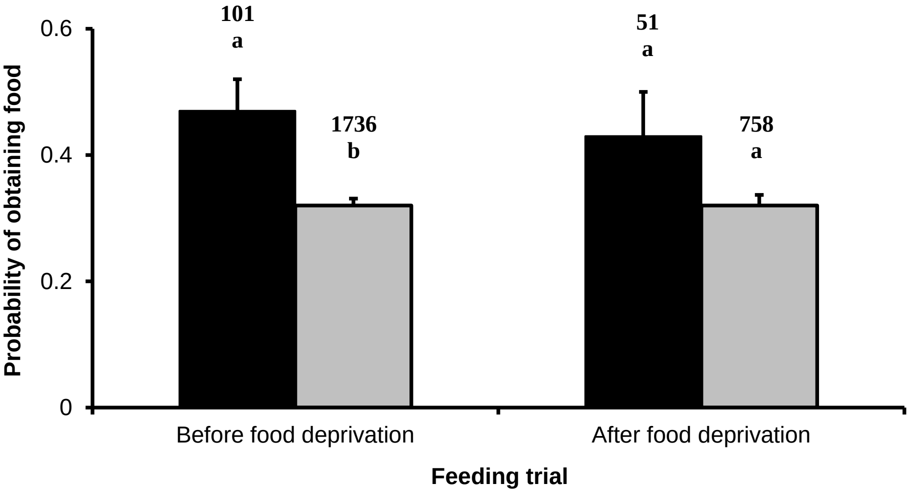

Figure 2. Probability (+ binomial SE) of obtaining food during the first parental feeding visit following individual PAB (black bars) or no PAB (light grey PAB) before and after a period of food deprivation. Numbers above bars indicate the number of feeding visits for which we were able to identify the nestling that had received food, and SEs were calculated based on these data. PAB categories within each feeding trial that differed significantly are denoted by different letters above bars.

Effect of PAB on food intake

In the before food deprivation trial nestlings obtained food more often after performing PAB than during feeding visits not preceded by PAB, while this was not the case after food deprivation (Fig. 2; Table 2).

Analyses ran on data from both trials showed that the probability of obtaining food was significantly affected by PAB category ($F_{1,22} = 4.69$, $P = 0.04$), and this effect of PAB category was similar in the before and the after food deprivation trial (PAB category × feeding trial: $F_{1,22} = 0.30$, $P = 0.59$; Fig. 2). Thus, also in this case the observed differences between trials should be taken with caution. Finally, the probability of obtaining food did not differ between trials ($F_{1,22} = 0.01$, $P = 0.95$) and sexes ($F_{1,22} = 2.54$, $P = 0.12$), while it was negatively affected by the number of competing nestlings ($F_{1,22} = 75.41$, $P < 0.001$).

8.5 Discussion

In this study, we have shown that PAB influences the begging behaviour of Barn swallow nestlings at the subsequent parental feeding visit. Nestlings, which had performed PAB increased the intensity and the probability of begging towards attending parents compared to their baseline begging levels. At the same time, siblings decreased their competitive efforts, but only when they had been normally fed rather than deprived of food. PAB can therefore influence competitive behaviour mediated by parent-present solicitations not only in dyads of similar size nestlings, as previously shown (Romano et al. 2013), but also in natural broods of 3–6 nest mates of different sex, size and competitive ability. This is also the case for the smaller ones, which have been previously observed to beg at higher intensity than their larger, senior siblings (Bonisoli-Alquati et al. 2011). The reciprocal fine-tuned modulation of begging behaviour according to PAB had consequences in terms of food intake in the before food deprivation trial, because the chances of being fed increased for nestling which had previously performed PAB. This was not the case after food deprivation, when nestlings did not reduce competition after sibling’s PAB.

We thus confirm that PAB reflects hunger state and may honestly signal willingness to compete for the next food item brought by parents (Romano et al. 2013). As a response, nest mates give up from competition, particularly when the indirect fitness gain of favouring food intake by a sibling in poor state is expected to exceed the cost of missing a parental feeding. This should be the case especially when harsh competition against hungry siblings, which will likely receive the next feeding, imposes a large direct cost of begging which may not be outweighed by the gain of obtaining food (Roulin et al. 2000; Johnstone and Roulin 2003). Conversely, when the marginal benefit of being fed is high, as is the case after food deprivation, nestlings appear to ignore PAB by siblings, likely because of selfish behaviour showing up at high levels of need (see also Romano et al. 2012, 2013). The observed behavioural modulation may thus be favoured by both natural selection and kin selection which have promoted the evolution of condition-dependent begging strategies minimizing the individual direct costs of harsh competition against hungry siblings, and limiting over-competition among relatives. PAB can thus function as a reliable cue used by offspring to identify the presence of very hungry siblings and modulate begging behaviour accordingly (see also Dreiss et al. 2012).

Admittedly, the present study is partly correlative and we cannot rule out the possibility that a reduction of begging following a sibling’s PAB compared to baseline begging intensity was caused by confounding variables not included in the analyses, or be the by-product of increased contingent satiation, with nestlings that were recently fed begging less than their siblings irrespective of PAB. However, because we have excluded from the analyses all feeding events in which a nestling had received food in the previous parental visit, the latter scenario is highly unlikely. Indeed, there is no reason to believe that nestlings hunger level during feeding visits not preceded by PAB should be systematically larger than those following siblings' PAB.

Another possible explanation for the reduction of competitive behaviour following siblings' PAB is that it was merely a response to the larger concomitant begging towards attending parents by the nestling that performed PAB. However, after siblings' PAB the probability of begging decreased markedly, implying that nestlings refrained from competition: we can thus safely rule out this alternative explanation and argue that the begging reduction mainly reflects a response to behavioural displays during the absence of parents.

No difference was found in begging intensity at parental arrival between feeding events preceded by individual or simultaneous PAB, indicating that nestling behaviour after individual PAB is mainly determined by individual contingent need, irrespective of the competitive environment. This interpretation is corroborated by the lack of any effects on PAB intensity and frequency of brood size, sibling sex ratio, relative body mass, age and sex, which are known to affect parent-present begging in the study species (Saino et al. 1997, 2000, 2001, 2008a ; Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011). The main driver of PAB occurrence is therefore contingent hunger level, as suggested by the increase in PAB frequency when nestlings were hungry (see also Romano et al. 2013; Budden and Wright 2001; Roulin 2001; Bulmer et al. 2008).

The lack of variation in PAB frequency and intensity according to brood size is not surprising, because Barn swallows adjust food provisioning rate according to the number of offspring (Saino et al. 1997, 2000). This was the case in the present study, as the total food items delivered to the nest strongly increased with increasing brood size, resulting in no variation in the per capita number of feedings received according to brood size (details not shown), and thus possibly in no difference in hunger level between nestlings reared in small or large broods. A similar explanation could be used to interpret the lack of effect of relative body mass on PAB frequency and intensity. Latehatched, smaller nestlings usually beg more intensely than larger nest mates, and the same should thus be expected for PAB. However, since they usually receive more food (Bonisoli-Alquati et al. 2011), they are expected to be on average more satiated than larger siblings. Hence, PAB cannot be considered as a reliable indicator of the individual long-term condition relative to nest mates, as conversely begging towards attending parents does (Saino et al. 2001; Bonisoli-Alquati et al. 2011), but it rather only mirrors the satiation level.

Male and female Barn swallow nestlings have been shown to beg at different intensity according to rearing conditions (Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011; Romano et al. 2011). However, such a difference was not evident in the present dataset (see Table 2). Hence, a lack of sex differences in the begging response to PAB is not surprising. Since a sex-related variation in begging intensity has been demonstrated to occur according to experimental manipulation of nestlings' long-term conditions (e.g. hatching order or immune-stimulation during the first days of life; see Bonisoli-Alquati et al. 2011; Romano et al. 2011), it can be speculated that the very short-term nature of PAB in displaying need may not represent a signal strong enough to differently affect behaviour of either sex.

Although in other species the occurrence of PAB was shown to vary according to nestling age (Budden and Wright 2001; Leonard and Horn 2001; Dor et al. 2007 Bulmer et al. 2008), this was not the case in the Barn swallow (see Romano et al. 2013). PAB seems therefore not linked to the degree of sensory and cognitive development of the offspring and thus to their increasing ability in discriminating parental arrival from other 'wrong' stimuli (Budden and Wright 2001; Dor et al. 2007; Bulmer et al. 2008 ; but see Leonard and Horn 2001). While the small range of ages at which the nestlings were tested (8-11 days after hatching) could be the cause of no variation in the frequency of PAB, this finding implies that the present results are not confounded by any age effects. Yet, additional studies are needed to investigate the role of PAB at different developmental stages (i.e. close to hatching or fledging).

In the Barn swallow, PAB appears mostly elicited by diverse external stimuli, such as movements by nest mates, flying adults or noise around the nest, and may not be directed towards siblings (Romano et al. 2013), similarly to other passerines (Budden and Wright 2001; Leonard and Horn 2001; Leonard et al. 2005; but see Bulmer et al. 2008). PAB displays could thus be considered as ‘false alarms’, resulting from selection for very fast begging responses to limit the chances of missing delivered food (Leonard and Horn 2001; Leonard et al. 2005, Dor et al. 2007). Hence, when asymmetries in the satiation level among nest mates occur (Romano et al. 2013) or when nestlings are hungry (present study), PAB does not confer any advantage in terms of food intake, while the cost of performing it is maintained. However, the concomitant variation of begging effort by interacting siblings in response to PAB suggests that it can also serve as an adaptive tool to reduce unnecessary scramble competition. We emphasize that the outcome of sibling competition in response to PAB is in line with the ‘sibling negotiation hypothesis’ (Roulin et al. 2000; Roulin 2001; Johnstone and Roulin 2003), but that the mechanism may not. Indeed, sibling negotiation has been proposed based on observations of nestling behaviour in the barn owl, a species where continuous and reciprocal communication among siblings negotiating priority in access to food occurs before every feeding visit (Roulin et al. 2009; Dreiss et al. 2010, 2013). Different interpretations of PAB as a misdirected behaviour or as a reliable signal of need may therefore not be mutually exclusive.

Together with previous studies of the Barn swallows (Boncoraglio et al. 2008; Bonisoli-Alquati et al. 2011; Romano et al. 2012, 2013), our findings indicate that nestlings modify their begging when nest mates are more hungry depending on their own current level of satiation. Considering the relatively high frequency of PAB bouts, we believe that such a begging modulation can have a profound influence on food share among offspring, implying that parents do not have the total control over final allocation of resources (see Godfray 1991, 1995). Nestlings may thus impact on parental food allocation not only by outcompeting smaller, weaker nest mates and monopolizing resources (Kacelnik et al. 1995; Kilner 1995; Cotton et al. 1999), but also by moderating their selfishness (Stamps et al. 1985; Romano et al. 2012 ; sensu Marques et al. 2011; Martìn-Galvez et al. 2011).

Parent-offspring communication usually involves both postural and vocal begging displays (Wright and Leonard 2002). It is thus possible that these different components of begging may also play a distinct role in the communication among siblings in passerine broods, as already suggested in the barn owl (Roulin et al. 2000, 2009 ; Dreiss et al. 2010, 2012, 2013). Here we examined the consequences of postural PAB in affecting the intensity of competition among interacting siblings. Despite in the Barn swallow the intensity of postural begging is strictly linked to vocal displays (our unpublished data), an open question therefore remains about the possibility that vocal PAB may act in a different, more subtle way to communicate hunger state to siblings in the nest. Future studies are therefore needed to address this issue.

In conclusion, we have shown that PAB plays an important role in determining competitive behaviour among siblings and their food intake in natural Barn swallow broods, and that such behaviour does not vary according to sex and relative nestling size. We encourage further studies on other bird species to investigate the generality of the role of intra brood signalling during interfeeding visits on the outcome of sib–sib competition and on parental food allocation decisions.

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General discussion

This thesis aims at providing evidence about the effects of climate changes and habitat alterations on migratory behaviour of birds. A necessary preliminary step to the analysis of this set of relationships is a thorough understanding of bird movements and migration patterns. In this thesis, I contributed to these studies by developing a novel method for representing migration routes from the analysis of ring recoveries or from the analysis any dataset that includes information on the positions where individuals were observed, provided that at least two observations per individual are available, for instance sight-resight data of whales or other marine mammals (chapter 2). Thanks to its generality, this method can therefore complement previous investigation on the ecology and behaviour of several migratory species. Chapter 2 presents the results of the application of this new method to the Barn swallow, which was chosen as a model species because of the large datasets of ring recoveries hosted at the EURING and SAFRING databanks. The general pattern of migration disclosed by this novel and automated method for representing migration routes is consistent with previous descriptions of the migration of the species throughout Europe (Ormerod 1991, Turner 2006). In particular, results highlighted that Barn swallows seem to avoid direct crossing of the Mediterranean Sea by moving between North Africa and western and central Europe at Gibraltar, the Balearic Islands, Sicily and Sardinia. Populations breeding in Eastern Europe probably move through the Middle

East, Turkey, and the Balkan Peninsula. Paucity of data from Africa prevented depicting the migration routes of this species to the south of the Sahara desert. This is particularly unfortunate because this information would have contributed to shed light on the routes used for crossing the Sahara Desert, which is the main geographical barrier the Barn swallows cross during their migration, and to the distribution of individuals in the wintering grounds (i.e. on migratory connectivity; Webster and Marra 2005). Despite these limits, this method for representing migratory routes can integrate previous analyses of the progression of the migration (Ambrosini et al. 2014), in order to offer a more complete description of the migration behaviour of birds over wide geographical areas.

New technologies are revolutionizing the study of bird migration (Bridge et al. 2011). Beside the traditional analyses of ringing data, new tracking devices are available, which provide new and detailed information on migration movements of several species (see e.g. Finch et al. 2017). Thanks to these new technologies, the non-breeding grounds of the geographical populations of Barn swallows studied during by PhD were known with reasonable precision. Indeed, a few years ago, a number of individuals were tagged with miniaturized light-level geolocators (Liechti et al. 2015), and I could use this information for investigating the influence of the ecological conditions at the non-breeding staging (‘wintering’) grounds on the dynamic of my study populations (chapter 3), which has suffered a sharp decline in recent years (Ambrosini et al. 2012, Sicurella et al. 2014). In addition, previous studies provided detailed information on the timing of Barn swallow migration and on the routes these birds follow (Ambrosini et al. 2014, Liechti et al. 2015). These pieces of information were used for analysing the relationships between environmental features in non-breeding grounds, particularly primary productivity, which is considered a general index of conditions faced by birds during wintering (Szép and Møller 2005, Szép et al. 2006, Gordo and Sanz 2008, Grande et al. 2009, Ockendon et al. 2014), and population dynamic of the Barn swallow in the breeding grounds (chapter 3). This study indicated that the primary productivity in the non-breeding ranges in central-Africa at the time when Barn swallows arrive from autumn migration and at stopover areas in North Africa during spring migration affect the year-to-year variation of population size (i.e. number of breeding pairs) in a breeding area of northern Italy. This analysis thus integrated information from different phases of the annual life-cycle of a migratory species, and demonstrated, for the first time, that the arrival at the wintering ranges and the possibility to refuel before crossing of the Mediterranean Sea during spring migration are the two most critical phases for Barn swallows breeding in Italy. This may occur because the long migration journeys across two main geographical barriers seriously challenges the physiological condition of individuals, threatening their survival and deceasing their reproductive performances (Møller 1994, Balbontín et al. 2009). Species migrating long distances probably experience divergent patterns of climate and environmental variability, which may reduce their ability to cope with climate trends (Saino et al. 2011). Importantly, the geographical variation in the rate of climate change implies that such divergence increases with the distance between the geographical areas where migrants spend different phases of their annual life-cycle. Identifying areas and periods that are critical for determining population dynamics, may shed light on the processes driving the decline of several populations of migrators and help planning more effective conservation actions (Ambrosini et al. 2011).

This work also revealed that the variability of ecological conditions in the breeding quarters seems to affect the population dynamics of Barn swallows in northern Italy only to a small extent, consistently with the results of previous investigations conducted on the same species in the same geographical area (Sicurella et al. 2014). However, ecological conditions at the breeding sites strongly influence the distribution of individuals. In fact, previous investigation on the breeding area (Ambrosini et al. 2002a, Sicurella et al. 2014) indicated that this species benefits from the presence of hayfields and livestock activities, and generally from the preservation of traditional management and extensive agricultural systems. The same seems to occur also in other areas of the European breeding range of this species (Møller 2001, Evans et al. 2003b, Donald et al. 2006). Importantly, the Barn swallow distribution is strictly related to the distribution of livestock farming activities (Møller 2001, Ambrosini et al. 2002b, 2012). In contrast, the distribution, abundance, and demographic trends of several other species typical of agricultural habitats (‘farmland birds’) are usually related to the extent of different crop types, including pastures and hayfields, or, generally, to indices of the intensity of agricultural practices (Evans et al. 2003a, 2007, Faria et al. 2012). Large extent of pastures and hayfields, in particular, is generally considered a feature of agricultural landscape that is beneficial for several farmland birds (Evans et al. 2003a, Faria et al. 2012, Sicurella et al. 2014), but its distribution, in turn, is linked to farming practices. It is therefore possible to hypothesize that livestock may be beneficial for birds both because pasture and hayfield extent is linked to the presence and the abundance of livestock, and because presence of livestock per se may favour bird populations e.g. because manure favours proliferation of insects. In chapter 5, I propose a review of the scientific literature aiming at clarifying the effects of the presence of livestock farming on farmland birds. In particular, I investigated this topic through a quantitative meta-analysis, quantifying the magnitude of the effects of livestock farming practices on the distribution and reproduction of birds of rural habitats in Europe. This review showed that livestock activity favours Barn swallows, probably because it enhances the abundance of invertebrate prey and the availability of nesting sites and foraging areas. In contrast, we could not find any significant effect of livestock farming on other species, probably because of the few studies available in the literature.

Variation in the ecological conditions at arrival from autumn migration and along spring migration routes can explain the year-to-year fluctuations in the population consistency at the breeding quarters, but not the negative trend per se suffered from this species (chapter 3) which is often attributed to uneven rates of climate change in the breeding and wintering areas, and the consequent ecological mismatch suffered by populations (Saino et al. 2011). Changes in agricultural practices at the breeding areas probably act additively to these ecological processes, generally explaining only a small fraction of the negative trends of this species in northern Italy (Ambrosini et al. 2012, Sicurella et al. 2014). In this areas, the breeding populations declined by 53% between 2001 and 2014 (Ambrosini et al. 2012), a trend much more negative than that observed at continental scale, which is estimated at -16% between 2005 and 2014 (BirdLife International 2017), with large variations among geographical regions of Europe (Siriwardena et al. 1998, Robinson et al. 2003, Evans et al. 2003b). The links between ecological conditions experienced by Barn swallows during winter and migration and the demographic trends of different populations of this species thus need further investigation. Nevertheless, the results reported in this thesis can provide a guidance for future studies on both the Barn swallow and other migratory birds. In particular, researches should focus on the whole annual life cycle (thus including migration journeys, stopover sites, wintering, moult and breeding) and identify critical periods of the life cycle as well as the geographical areas where they occur.

Long-distance migrators are considered more sensitive to global climate change than short distance migrators (Lehikoinen et al. 2004, Rubolini et al. 2007, Both et al. 2009). With the aim of investigating the effect of climate change also on short distance migrants, chapter 4 focuses on the migratory behaviour of the European robin, a model species for studies on partial migration (Adriaensen and Dhondt 1990). Differently from the Barn swallow and other long distance migrants, short-distance and partial migratory species are expected to respond more quickly to changes in environmental conditions during winter (Berthold 1991). In particular, the study in chapter 3 aimed at quantifying the effect of climate change on the proportion of individuals that migrate each year, and the distance migrated, through the analysis of the large ring dataset available for this species across Europe. A high degree of migratory connectivity, and great differences in migration strategies among geographical populations of this species were found. In addition, the variability of migratory behaviour among individuals seems associated to fluctuations of winter temperatures at the breeding grounds. This suggests that individuals may be able to adjust their migration behaviour according to contingent climatic conditions, consistently with the hypothesis of plasticity in migration behaviour of short-distance migrators(Lehikoinen and Sparks 2010). Interestingly, the responses differed among distinct geographical populations of European robins that differ in their migration ecology (i.e. propensity to migrate or be resident and migration distance). Indeed, individuals of an entirely migratory population shifted their wintering grounds and, consequently, reduced the length of the migration journey, more than those of a partial migrant population. In contrast, individuals from the partial migrator population showed greater plasticity in their response to year-to-year variation in winter temperatures by remaining more in the breeding grounds or migrating shorter distance migrated during mild winters (chapter 3). These difference can be because European robin population migrating longer distances may be more limited in their phenological responses to year-to-year variation in ecological condition than those migrating shorter distances (Remisiewicz 2002). Populations of this species that differ in their migration strategies may thus respond differently to changes in climate conditions, and in ways similar to those observed between species that migrate long- and short-distances. However, the distinction between long- and short-migrants, and even between residents and migratory bird, is difficult, and arbitrary (Pulido and Widmer 2005). In the light of the large variability in migration propensity, distance and timing of migration observed among species and populations, the different migration strategies should be interpreted as different degrees of expressions the migration activity, where the resident and long-distance migrators are at the two extremes (Pulido et al. 1996).

The second part of my thesis deals with other aspects of the biology in two migratory birds. First, I described the structure of bacterial communities associated to two trans-Saharan migrants, the Barn swallow and the Common swift. The microbiota (i.e. the community of bacteria and other unicellular organisms associated to a metazoan), performs fundamental functions for the host, for instance it contributes to modulate the immune system function (Macpherson and Harris 2004), produces key amino-acids (Nalepa 1994, Ley et al. 2008a, b), and contributes to the defence against pathogens (Ruiz-Rodríguez et al. 2009, Martínez-García et al. 2015). Evidences also suggest that the microbiota can modulate individual behaviour (Kokko et al. 2002, Sharon et al. 2010, Archie and Theis 2011). The investigation of the microbiota is now possible at an unprecedented level of detail thanks to new technologies, such as the high-throughput DNA sequencing, that allow characterizing the microbial communities by studying the DNA extracted from a sample (i.e. the microbiome) (Brock et al. 2012, Caporaso et al. 2012). Chapter 6 reports the first description of the cloacal microbiome of a Barn swallow population breeding in Italy. This is the third Barn swallow microbiome described in Europe (two previous studies were from the Czech Republic; Kreisinger et al. 2015, 2017). The results suggest that, in general, the microbiota of geographically different Barn swallow populations are dominated by bacteria of the same phyla, but by different orders. Interestingly, comparison of the results from these three studies suggests that individuals from the same colony seem to have similar gut microbiomes, probably because they are exposed to the same local environmental conditions on their breeding grounds (chapter 6)..

The study in chapter 7 focused on the feather microbiota of adult Common swifts. This study showed that feather microbiomes could be divided into three distinct clusters, each one characterized by bacteria generally predominant in a specific environment (i.e. saltwater, soil and vegetation), and, apparently, not related to any feature of individuals or to conditions in the breeding grounds, as individuals were captured at the same colony. We thus hypothesized that feather microbiomes may reflect, at least partly, air bacteria of the environments where individuals spent the non-breeding periods, or of the areas they crossed during migration (Common swift spend the non-breeding period entirely airborne; Hedenström et al. 2016). Clearly, this conclusion is highly speculative, and needs further validation. However, if microbiomes, and feather bacteria in particular, may be related to migration behaviour, these studies may be a first step toward linking microbiology to migration ecology.

The last study of this Ph.D. thesis investigates the role of begging displayed in absence of parents (parent-absent begging or PAB) in driving the intra-brood interactions among Barn swallow nestlings. In this study, some nestling were prevented from being fed by their parents and this experiment was able to demonstrate that nestlings use information from the PAB displayed by their siblings to modulate the intensity of their begging at the next visit of the parents. This behaviour thus leads to an overall reduction of sibling competition. The results of this study also suggest that resources supplies at a nesting site affect food allocation from parents, and, most importantly, indirectly alter social intra-brood interactions that have potential consequences on nestling quality. In the case of the Barn swallow, which forage in a short range around the farms where breeding colonies occur (Ambrosini et al. 2002a) the ecological conditions that determine food availability are the presence of livestock farming and the extent of hayfields in the neighbourhood of a farm (Ambrosini et al. 2012, Sicurella et al. 2014). Thus, despite this last study investigated a process more related to the behavioural ecology of this species, the results provide a further, indirect, confirm of the importance of suitable habitat conditions at nesting colonies for the breeding biology of the Barn swallow.

Conclusions

The results of the studies included in the present Ph.D. thesis confirm that climate change and habitat modification are having significant impacts on European migratory birds (chapter 3, 4 and 5, see also Donald et al. 2001, Møller et al. 2010), which, in turn, are already responding to global changes by altering their migratory behaviour and phenology (chapter 4, see also Rubolini et al. 2007, Charmantier et al. 2008, Lehikoinen et al. 2013). Environmental changes occurring in the non-reproductive ranges exert important carry-over effects on the dynamics of breeding populations of long-distance migrants, including the Barn swallow (chapter 3). In particular, the ecological conditions during some specific critical phases of the annual life-cycle affect year-to-year variation in the population consistency (chapter 3, see also Szép 1995). These critical periods seem to be the arrival to the wintering ground after post-nuptial migration and possible stopover sites during spring migrations. So far, few studies attempted to identify of critical phases in the life-cycle of other migrants, probably because migration routes and wintering grounds are poorly known for most species, particularly small passerines. Populations of partial migrants also appear to be able to adjust their migration strategy to changes in ecological conditions more quickly than long-distance migrants. In this thesis, this has been demonstrated on the European robin with a study at continental scale which also demonstrated that different geographical populations of the same species that differ in their degree of migratoriness can show different patterns of response, similar to those observed, respectively in different short and long-distance migratory species (chapter 4).

Besides climate changes, the alteration of the breeding habitats is the other main anthropogenic factor responsible for variation in the distribution and the abundance of several bird species (Donald et al. 2001, 2006). In particular, chapter 5 indicates that livestock activities are elements of the rural habitats whose preservation may exert positive effect on Barn swallow populations, and potentially on other farmland birds.

Some of the studies reported in this dissertation (chapter 2, 3 and 4) could be conducted only thanks to the availability of long time series of data, such as those already available in the computerized databanks of organizations, like the EURING; that store information on ring-recoveries collected over long times. Despite the analysis of these data is hampered by several problems, the most relevant of which is the large spatial and temporal heterogeneity in sampling effort, these datasets, together with museum specimen collections, are the only ones allowing retrospective analyses of migratory behaviour. In this thesis, I present novel frameworks of analysis of ringing data (chapters 2 and 4) that can be applied to a broad range of migratory species, in particular for representing migratory routes and for investigating the effects of environmental and climate changes on bird populations. In addition, the application of microbiological techniques to the study of the bird ecology and behaviour can improve our understanding of bird migrations, connecting microbiology and migration ecology (Archie and Theis 2011).

Conclusions

In conclusion, all the studies collected in this thesis consistently demonstrate that bird are already showing adaptive responses to global changes. In particular, the sensitivity of migratory species to changes in ecological conditions experienced during all their life-cycles confirms their role of “winged sentinels” of global environmental changes (Wormworth and Şekercioğlu 2011).

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I dedicate this thesis to my son Giona with the wish that he will pursue his interests and passions (the music) with enthusiasm and resolution, and realize his dreams.

Supplementary materials

Supplementary materials to chapter 2:

Representing migration routes from re-encounter data: a new method applied to ring recoveries of Barn swallows (*Hirundo rustica*) in Europe

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Legend to supplementary material.

Appendix 1. Full details on analytical procedure, computation times with cells of different size (Table A1), maps of movements originating from re-encounters within the same migration event (i.e. in the same year), value of all parameters used for the analysis of Barn swallow recaptures (Table A2)

Supplementary material Appensix 2-5 are in electronic format and temporary available in google drive open-directory at: https://drive.google.com/open?id=0B9Z15I6qtYQ3dGVEQ1FGYUhjaGc

Note for kml files

The kml files in Appendices 4-5 can be opened in Google Earth™.

Appendix 2: Commented R 3.2.1 script for all the analyses.

Appendix 3: Table with Barn swallow population size at each European country (from BirdLife International 2017, http//wwwbirdlife.org).

Appendix 4: kml file with spring and autumn movement, direction and flight route maps form analyses with cells of 0.25 × 0.25 ° latitude × longitude. It includes layers showing movement maps, direction maps and flyway maps for both spring and autumn migration for all birds with cells of size 0.25° × 0.25°.

The movement layers also provide information on coordinates and dates of recovery of the bird, which can be displayed by selecting a movement on the map. In all files, spring migration is in yellow and autumn migration is in red.

Appendix 5: kml file with results of analyses i) with cells of different size; ii) on live re-recaptures or dead re-encounters. It includes layers showing movement and direction maps for spring and autumn migrations from analyses run with cells of size 0.5° × 0.5° and 1° × 1°, for analyses run separately on movements of live recaptures or dead recoveries of birds and flyway simulations with number of simulations from each European country proportional to Barn swallow population size in that country.

The movement layers also provide information on coordinates and dates of recovery of the bird, which can be displayed by selecting a movement on the map. In all files, spring migration is in yellow and autumn migration is in red.

SUPPLEMENTARY MATERIAL APPENDIX 1

Full details on each step of the procedure

The following part explains in details each step of the procedure for identifying migration routes from ring recoveries or sight-resight data. Each of the parameters to be set during the procedure is defined, as well as the values assigned to it in the analyses run on the Barn swallow dataset (see Table A1). We will focus on autumn migration, while briefly discussing at the end of each part the changes in the procedure necessary for analysing spring migration.

Identification of individual movements

STEP 1. In the first step, the range of years under analysis is defined by the parameters firstYear and lastYear. For the Barn swallow dataset, all the ring recoveries were considered by setting firstYear = 1908 and lastYear = 2011. The migration period (autumn or spring) to be analysed is defined by parameter aut. In our example, aut = TRUE identifies autumn migration (aut = FALSE identifies spring migration). In this step, the temporal limits of the migration periods are also set by parameters T0 and T1 (1 corresponds to January). In our example, T0 = 8 and T1 = 12, corresponding to August-December. Similarly, the limits of the study area are defined (i.e.: areaLonMin = -19, areaLonMax = 44, areaLatMin = 20, areaLatMax = 70 corresponding to 19° W − 18° E longitude, 35° N − 48° N latitude). Recoveries outside this period, records of birds recorded only once during autumn migration, or records outside the study area are discarded. This step assures that only records of birds observed at least twice during the selected migration period are retained.

STEP 2. The algorithm tries to identify the most probable migration track followed by each individual based on the information provided by ring recoveries under the assumption that individuals follow (approximately) the same migration routes every year (at least at the rather coarse spatial scale used for this analysis; see below). The criteria used for this task are:

- First, records of the same individual in the same year are ordered chronologically and connected to one another in this order because these records provide information on the actual movement of an individual during one migration event. Each pair of connected records forms the first parts of a track.
- Second, records of the same individual in different years are connected to one another and to the track parts created in the point above by a clustering procedure that connects each record to the (spatially) closest record or track irrespective of year of recovery. The cluster analysis is run with the *hclust* procedure in R. Figure 1 shows some examples of

how positions are connected depending on whether they are recorded in the same year or not. The logic of this clustering procedure is to both maintain the information on actual movements of individuals during the same migration period and to connect one position of the same individual to the closest one. We emphasise that the connection between records of the same individual in the same year is maintained during this step of the procedure. A pair of connected records forms what we will term '*movement*' hereafter. We note that it is possible to discard movements generated by connecting records of the same individual in different years and to retain only those connecting recoveries in the same year (see STEP 2a) by the parameter sameYear (= FALSE in our example; see also STEP 6 for how this selection actually occurs in the procedure).

STEP 3. Length of each movement is calculated (in km). We emphasise that, having no other information, we are assuming that individuals move straight between consecutive positions. Parameter ortho allows choosing between orthodromic (ortho = TRUE, as in our example) or loxodromic (ortho = FALSE) distance. Only movements in a given range of distances are retained (parameters rayMin = 100 and rayMax = 800 corresponding to 100-800 km in this example). These thresholds serve to discard both movements that are too long to reasonably represent straight migration movements between locations and movements that are too short to reasonably represent real migration movements done by the animal, i.e. movements due to dispersal, vagrancy or other pre-migration movements (see Ambrosini et al. 2014 for a similar approach). We note that excluding non-migratory movements by setting a lower distance threshold allows inclusion in the dataset of recoveries during months when individuals are clearly stationary. Indeed, although these birds were recovered outside the migratory phase, their positions can be considered representing the initial or the final destination of that individual.

Two weights used to model uncertainty in the movement direction are also calculated in this step. The first weight aims at accounting for ringing and recovery effort at the cells where each movement starts and ends. In details, this ‘effort’ weight is equal to the sum of the number of ringing data at the starting and ending cells of each movement. We emphasize that a more direct measure of ringing/recovery effort at each cell would be highly preferable. However, this information is unavailable for the vast majority of species and countries.

The second weight accounts for the fact that uncertainty in the actual direction followed by a bird during a movement should increase with movement length. Indeed, the shortest the movement the most probably it could be approximated by a straight line connecting starting and ending locations. This “length” weight is therefore set equal to the inverse of the length of each movement.

The third weight will be introduced and a proper combination of all these weights calculated in STEP 7. Weights will be used to calculate the weighted mean migration direction at each cell.

STEP 4. The direction of each movement is calculated as the clockwise angle between the North and the direction of the movement in degrees (azimuth). We assume that all individuals move southwards during autumn migration (i.e. movement directions should be between 90° and 270°) except for movements connecting consecutive records of the same individuals during the same year (STEP 2a). In such cases, movement direction is the direction from the earliest to the latest record. To ease the following steps of the analyses, directions between 180° and 360° are expressed as negative angles (e.g. 270° equalled -90°). It is possible to choose whether to include or exclude from the analysis movements with a direction opposite to that expected for the migration period under analysis (i.e. northwards movements during autumn migration and southwards movements during spring migration) by setting the no reversal parameter (= TRUE in the analyses of Barn swallows i.e. discarding reverse movements). Direction of movements connecting positions of the same individual in different years at approximately the same latitude are somewhat undefined. The operator can choose whether to retain or discard these movements by the no east west parameter (= TRUE in our example, i.e. we discarded approximately east-west movements) and the approxlat parameter, which sets the minimum difference in latitude for retaining a movement (= 0.2° in our example).

Geographical grid and assignment of movements to each cell

STEP 5. The size of the cell in which the study area is divided is set by the dimensionCell parameter (= 0.25 in our example, implying 2704 cells of 0.25° × 0.25° latitude longitude). Results obtained by using cells of different size are reported in Supplementary material Appendix 3. Cell size should be chosen based on data distribution, by balancing the benefits of small cells, which allow a detailed description of migration patterns, with those of large cells, which allow maximizing the extent of the geographical area covered by the analysis. Computation time, which increases non-linearly with cell number, should also be considered (see Table A2).

STEP 6. All movements intersecting any particular cell are identified and associated to the cell. Thus, each movement is usually associated with more than one cell, and each cell can have a number of associated movements. The parameter sameYear acts at this step of the procedure because it compares dates of recoveries connected by a movement created in the previous steps of the procedure, and, when it is set to TRUE, allows selecting only the movements of birds recaptured within the same year. The parameter sameYear was set to FALSE in the Barn swallow analyses for including all movements and to TRUE in the analyses restricted to movements within the same migration event.

Migration direction at each cell

STEP 7. A third weight to model uncertainty in movement direction is calculated and all weights to be included in the analyses are combined. This third weight rests on the reasoning that representing a movement by a straight line connecting starting and ending position is clearly a simplification of the real trajectory followed by an individual bird. Indeed, a bird may make detours that pass unnoticed by simply analysing ring recoveries. This implies that an individual may even not have actually crossed some cells that are associated with a movement in our procedure, particularly during long movements crossing several cells. We reasoned that this may occur more probably for cells in the middle of a long movement, while cells close to the starting or the ending points of the movement should have been crossed more likely. To account for such uncertainty, we introduced a “distance” weight whose value is calculated by first assessing the distances between the centre of a cell and the start or end of the movement, and then calculating the inverse of the smallest among these distances. The distance weight thus has large values for cells close to the start or the end of a movement, and small values for cells in the middle of long movements. Thus, differently from the effort and length weight whose values were associated to each movement, values of the distance weight change at each cell crossed by a single movement.

By setting the parameter weight.use = TRUE, it is possible to include weights in the assessment of migration direction at each cell (STEPs 8-11). Setting this parameter to FALSE implies that steps where weights are calculated are skipped by the procedure so to save time in the elaboration. Indeed, the weight calculation is computationally demanding (see Table A2). The operator can also select whether to include each of the length, effort or distance weights in the analyses by setting W.eff, W.len or W.dist to TRUE or FALSE. Weights are first rescaled individually so that the sum of the values of each weight over all the movements crossing the cell equals one. This step assures that all weights are given equal importance in the following calculations. In the analyses of the Barn swallows, we included all three weights.

If more than one weight is selected, weights should be combined to obtain an overall weight for each movement at each cell to include in the analyses. The operator can choose between summing (summa = TRUE) or multiplying weights (summa = FALSE). Summing values implies that weights "compensate" each other, while multiplying emphasizes the effect of small values. The resulting overall weight is then further rescaled so that values for all movements associated to a cell sum to one.

STEP 8. Migration directions are calculated based on the direction of the movements associated at each cell. Parameter minClust sets the minimum number of movements associated to a cell for running the cluster analysis (= 5 in our example). If few (< 5) movements are associated with a cell, migration direction at that cell is calculated as the circular mean direction or the weighted circular mean direction if weights are considered. The (weighted) circular variance of the (weighted) mean direction (i.e. the squared value of the standard error) is also calculated.

STEP 9. In contrast, if several (≥ 5) movements are associated with a cell, a cluster analysis is run for assessing whether all movements are in the same direction or not. Cluster analysis is based on a distance matrix between all movements intersecting a cell, where distance among movements is equal to the absolute value of the difference between movement directions (in degrees). The *pam* procedure from the CLUSTER library in

R is used to identify the occurrence of two potential clusters based on this distance matrix. The *pam* procedure is a clustering algorithm partitioning observations in a number of clusters defined *a priori* and returning a measure of the goodness of the classification of the data into a given number of clusters. This measure is called the ‘overall average silhouette width’ (*oasw*) and is a dimensionless coefficient ranging from -1 to +1. Increasing *oasw* values indicate better classification of the data (Rousseeuw 1987). Currently, it is not possible to modify the number of clusters at a cell by simply setting a parameter (but it is clearly possible to do it by modifying the R code). This is because we recommend to use cells as small as possible (see above), and thus to reduce cell size rather than increase the number of clusters at each cell. If the operator aims at using weights, as we did in the analyses of the Barn swallows, they are considered also in this clustering procedure.

STEP 10. If oasw value is lower than a value set by the oaswLimit parameter (= 0.5 in this example, oasw < 0.5 indicates ‘weak’ or no clustering structure in the data), the cluster analysis is discarded, and the (weighted) circular mean direction is calculated on all the movements associated to a cell.

STEP 11. In contrast, if oasw value is larger than the threshold (oasw ≥ 0.5 indicates reasonable or strong cluster structure) movements are divided into two clusters and the (weighted) circular mean direction is calculated separately for the two clusters. If the two mean directions so obtained diverge more than a threshold set by the angleDiv parameter (45° in the example), both mean directions are associated with the cell, otherwise the cluster analysis is discarded and the (weighted) circular mean direction is calculated on all movements. Hence, one or two migration directions can be obtained for each cell. Cells with double directions may represent areas of flyway divergence (i.e. migratory divides), areas where flyways cross, or areas of flyway convergence.

STEP 12. The directions at each cell obtained from the step above are represented by arrows drawn on a map ('direction map').

Spring migration can be analysed by simply setting parameter aut = FALSE in STEP 1. All other steps are similar to those of the procedure used for autumn migration, except that direction of movements connecting the positions where an individual was recorded *in different years* is assumed to be northwards (STEP 4) because, during spring migration, birds should move mainly northwards.

The procedure also produces a table reporting, for each cell with at least one direction 1) the cell ID; 2) the coordinates of its centre (longitude and latitude); 3) the number of movements crossing the cell; 4) the (weighted) mean and variance of all the directions associated with a cell; and 5) the (weighted) mean and the variance of the directions of movements in both clusters if the cell is a double direction cell. The table is returned in csv format and a full path to a directory for saving the file is required.

Flight route simulation

Simulations are run by randomly choosing the easternmost or westernmost direction at each cell with double directions if the rndDir parameter is set to TRUE. Otherwise, simulations are run by first considering only the easternmost direction at each cell with double directions (‘eastern simulations’), and then only the westernmost one (‘western simulations’) (rndDir = FALSE as in our example). Selecting either the easternmost or the westernmost direction at each cell with double directions during each simulation, avoids simulated flight routes to change direction repeatedly (i.e. it avoids producing ‘zigzag’ flyways). Indeed, zigzag flight routes may occur when, for instance, opposite directions are chosen in adjacent cells with double direction. Simulations are generated through the following steps.

STEP 13. The starting position of the simulated bird is chosen according to the startPosition parameter. When startPosition = 1, the starting position is chosen randomly within the study area. In detail, both longitude and latitude are selected as random numbers in the range of longitudes and latitudes of the study area using the runif command of R. When startPosition = 2, the starting position is selected randomly among the positions where a bird was observed during the migration period (i.e. the position of any recovery selected after STEP 1). When startPosition = 3, the starting position is selected randomly among the positions defining a movement (either the start or the end of a movement, i.e. all positions retained after STEP 6). When startPosition = 4 the starting positions are chosen randomly within a desired list of countries in a relative number initially proportional to the density of the species population per country. We set this option in the analyses of the Barn swallow and used population size obtained from BirdLife International (2017) (Supplementary material Appendix 5). The overall number of positions in all the countries is equal to twice numSimulation. When startPosition = 5, the longitude and latitude of starting positions are passed as a 2-columns table (*.csv) by the user where each row is a position and the two columns the longitude (“Lon”) and latitude (“Lat”) coordinates.

StartPosition = 1 therefore determines a spatially uniform distribution of starting locations. StartPosition = 2 allows starting simulations proportionally ringing and recovery effort while startPosition = 3 allows starting simulations proportionally to spatial distribution of movements on which all the analyses are based. StartPosition = 4 allows starting simulations proportionally to the spatial distribution of the study species, while startPosition = 5 allows fully custom choice of starting positions.

In all cases, the number of simulations is defined by the numSimulations parameter, which actually defines the number of eastern and the number of western simulations, so that the total number of simulations is twice numSimulations (but see STEP 14). However, when starting positions are chosen proportional to population size (startPosition = 4) or custom (startPosition = 5), numSimulations only sets the number of starting positions initially chosen by the procedure, while the number of simulations actually run is then reduced proportionally to data availability in direction map in STEP 14 (see below for details).

STEP 14. If startPosition = 4 or 5, only starting positions falling within cells with a migration direction (i.e. with one or two arrows in the direction maps; ‘full cells’ hereafter) are considered. This selection guarantees that starting positions are spatially distributed consistently with both the spatial distribution of a species and the spatial distribution of full cell. Imagine, for instance, a country hosting a large population of the species under study, but where ringing effort was spatially heterogeneous, so that full cells are concentrated only in a small part of the country. Setting the number of simulations from that country proportional to population size only would determine starting a disproportionately large number of simulations from the relatively small area where full cells concentrate. We decided to apply this selection criterion also to starting positions chosen by the operator to prevent simulations starting from empty cells, i.e. cells with undefined migration direction. We thus strongly advice that custom starting positions are carefully chosen not only within the study area, but also within full cells. Finally, we stress that when startPosition = 4 or 5, final number of simulations can be lower than the number of simulations set by the numSimulation parameter. The selection of starting positions is run for the eastern and the western simulation separately.

STEP 15. From the starting position, each simulated bird moves a constant length equal to 1/4 of cell size in the migration direction indicated by the arrow of that cell in the direction map. If the initial position is in a cell with double directions and parameter rndDir = FALSE (see above), the bird moves a constant length in the direction of the easternmost arrow during the eastern simulations, or in that of the westernmost arrow during the western simulation. In contrast, if rndDir = TRUE the easternmost or the westernmost direction is chosen randomly.

STEP 16. When an individual enters a different cell and that cell is full, it starts moving according to the migration direction of the new cell. Hence, at each step, the simulated bird moves a constant length equal to 1/4 of cell size according to the direction associated with the cell in which it is. If it enters a cell with double direction and rndDir = FALSE, the bird moves in the direction of the easternmost arrow during the eastern simulations, or in that of the westernmost arrow during the western simulation. In contrast, if rndDir = TRUE the easternmost or the westernmost direction is chosen randomly.

STEP 17. If a bird enters an empty cell (i.e. a cell with no assigned direction), it continues moving according to the direction it was following in the previous cell.

STEP 18. The simulation stops when either the bird exits the study area or it moves across a number of empty cells defined by the maxEmptyCells parameter. In our example, maxEmptyCells = 10, which means that the simulation stops when a simulated bird moves through more than 10 empty cells. This criterion speeds up the simulation procedure because it avoids the procedure to produce long straight tracks in areas with no data and that would be discarded in the following step of the procedure (see below). To avoid simulations to run indefinitely if directions in different cells determines the occurrence of loops in simulated flyways, a simulation stops after a the maximum number of iterations equal to four times the sum of the lengths of the two sides of the study area.

STEP 19. All positions after the last position that occurred in a full cell are discarded. This means that the first and the last position of a simulated flyway are always in full cells, but a simulated flight route can include traits through empty cells, provided these are shorter than the distance set by maxEmptyCells. A flight route is finally defined as all the positions of a simulated bird.

Flight routes can be smoothed in order to improve their graphical representation by setting the smoothing parameter (= TRUE in the example). The smoothing procedure we implemented works according to the following steps.

STEP 20. The starting point of a flight route and the two immediately following positions are selected. We will call these positions A, B and C, for simplicity. The procedure then calculates the orthodromic (great circle) or loxodormic (rhumb) distances (according to parameter ortho = TRUE or ortho = FALSE) between A and B, B and C and A and C. If the sum of the distances between A and B and between B and C is larger than twice the distance between A and C (i.e. AB + BC > 2AC), B is discarded, and the flight route re-drawn by connecting A and C directly.

STEP 21. The procedure is repeated by starting from the second position in the flight route and so on until the end of the route. In details, the procedure considers positions B, C and D if B was not discarded in STEP 19 or C, D and E if B was discarded, and so on.

Simulations can be generated also by starting from the final destination reached by the individuals and making each bird moving to a direction *opposite* to that indicated by direction maps. These “reverse” simulations are useful whenever there is large uncertainty in e.g. the position of individuals at the start of migration, the flight routes followed by birds when entering the study area, or the sizes of population in the areas migration starts from. For example, the reverse simulation is useful while considering the spring migration of species for which few data are available in the wintering grounds and the only information available is about their distribution in the breeding grounds. Reverse simulations can be generated by the revSim parameter (= FALSE in the simulations of autumn flyways of the Barn swallow, = TRUE in spring ones), which acts on STEPs 15-17 of the simulation procedure and makes bird moving through each cell toward a direction opposite to the mean direction(s) at that cell. This parameter does not affect the behaviour of the rndDir parameter i.e. it is possible to run reversed simulations by choosing always either the easternmost or the westernmost direction at each cell with double direction or to randomly choose the direction at each time a bird enters a cell with double direction. Once a direction is chosen, during reversed simulations the bird simply moves opposite to that direction.

Table A1. Values of the parameters entered in the procedure for reconstructing Barn swallow migration directions and flyways during spring and autumn migration.

Parameter	**Spring**	**Autumn**	**Descriptor**
aut	FALSE	TRUE	Analysis on autumn or spring. migration
T0	1	8	First month of migration
T1	7	12	Last month of migration
yearFirst	1908	2011	First year of migration
yearLast	1908	2011	Last year of migration
areaLonMin	-19	-19	Minimum longitude of study area
areaLonMax	44	44	Maximum longitude of study area
areaLatMin	20	20	Minimum latitude of study area
areaLatMax	70	70	Maximum latitude of study area
ortho	FALSE	FALSE	Whether the procedure calculates orthodromic (TRUE) or loxodromic distances (FALSE). The latter requires the *birdring* package (Korner-Nievergelt and Robinson 2014).
rayMin	100	100	Minimum length of a movement (in km)
rayMax	800	800	Minimum length of a

			movement (in km)
noreversal	TRUE	TRUE	Remove (TRUE) or include (FALSE) northward movements during autumn migration and southward moves during spring migration
noeastwest	TRUE	TRUE	Remove (TRUE) or maintain (FALSE) movements between points at the same latitude
approxlat	0.2	0.2	Minimum difference in latitudes for considering points at the same latitude
cellDimension	0.25	0.25	Dimension of a cell
minClust	5	5	Minimum number of movements for cluster analysis
oaswLimit	0.5	0.5	Minimum value of the overall average silhouette width for calculating double directions at a cell.
angleDiv	45	45	Minimum divergence (in degree) to separate double directions at a cell
numSimulations	500	500	Number of simulated flight routes
maxEmptyCells	64	64	Maximum length of simulated flight routes in empty cells during simulations

smoothing	TRUE	TRUE	Whether to smooth simulated flight routes or not
revSim	TRUE	FALSE	Whether to run simulations backwards (e.g. from the breeding grounds)
rndDir	FALSE	FALSE	Whether to choose randomly western or eastern direction in double cell during simulation
sameYear	FALSE	FALSE	Whether to select only movements of birds recaptured within the same year
startPosition	4	4	Whether to start simulation from 1) random points; 2) positions where a bird was observed; 3) recoveries defining moves; 4) points from input; and 5) points proportional to population density per country
countryList	(App. A5)	(App. A5)	List of countries from which to start flight routesimulation
W.length	TRUE	TRUE	Whether to use weight related to movement length
W.dist	TRUE	TRUE	Whether to use weight related to the distance of the cell from nearest movement vertex

W.eff	TRUE	TRUE	Whether to use weight related to number of records in the cells where movement vertexes are
summa	TRUE	TRUE	Whether to sum (TRUE) or multiply weights (FALSE)
weight.use	TRUE	TRUE	Whether to calculate weights for movements

Table A2. Run-times for processing the analyses of spring and autumn migration at different cell size (0.25° × 0.25°, 0.5° × 0.5°, 1° × 1° Lon × Lat). We also report run-times for processing spring and autumn migration at different cell sizes while considering weights. Analyses are run with an Intel® Core™ i3-3110 M CPU at 2.40 GHz and 4 GB RAM.

Cell size (Lon × Lat)	**Weights excluded**		**Weights considered**	
	Spring	**Autumn**	**Spring**	**Autumn**
0.25°×0.25°	01:25:08	1:32:41	3:16:30	6:30:10
0.5°×0.5°	01:11:13	1:04:01	1:01:40	1:57:17
1°×1°	00:49:25	0:42:25	0:43:58	0:50:09

Figure A1. Spring movement map based on recoveries of Barn swallows within the same migration event (i.e. birds that were recovered twice during the same year). Red lines represent movements connecting recovery positions of the same individual. Grey points represent ring recoveries during January-July while blue points indicate ring recoveries connected by movements.

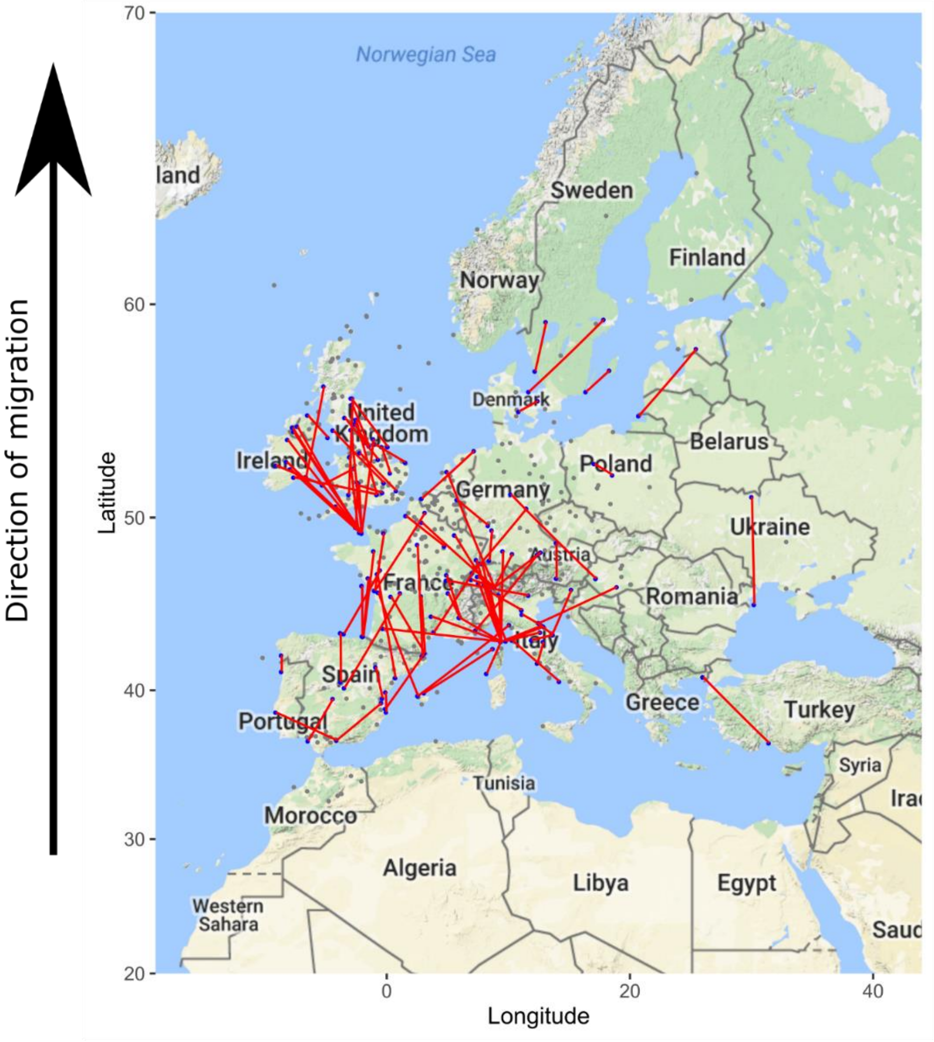
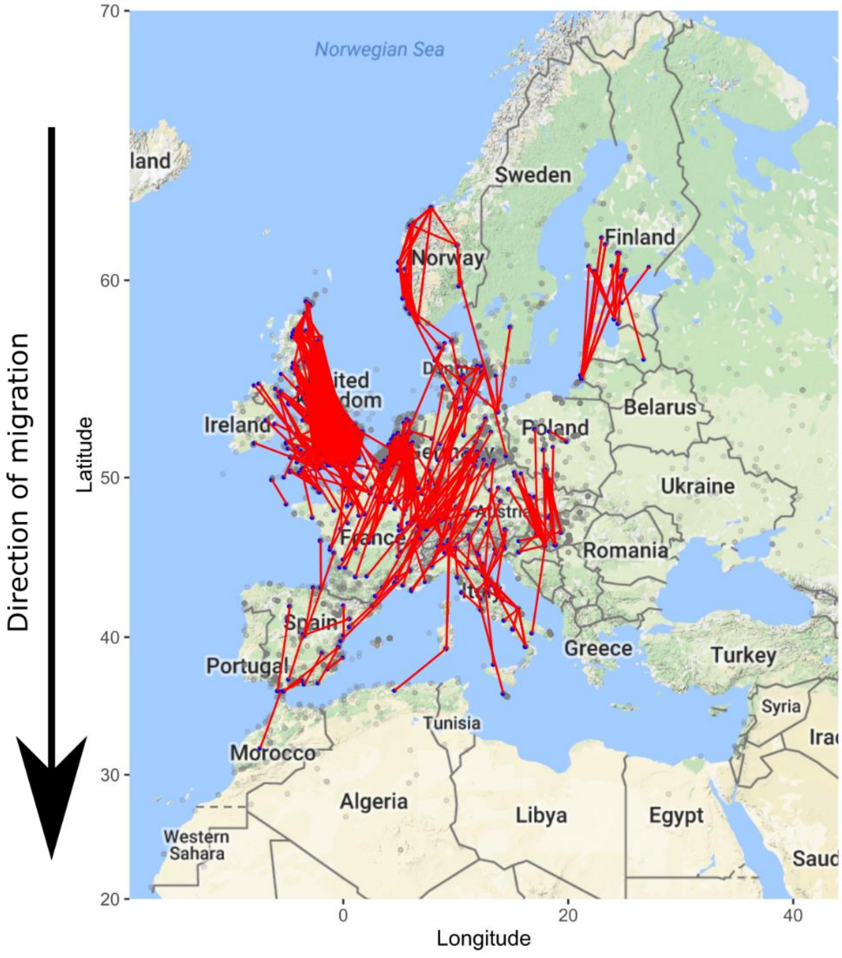


Figure A2. Autumn movement map based on recoveries of Barn swallows within the same migration event (i.e. birds that were recovered twice during the same year). Red lines represent movements connecting recovery positions of the same individual. Grey points represent all ring recoveries during August-December while blue points indicate ring recoveries connected by movements



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Korner-Nievergelt, F. and Robinson, R. A. 2014. Introducing the R-package “birdring.” - Ringing Migr. 29: 51–61.

Supplementary materials to chapter 3:

Environmental conditions at arrival to the wintering grounds and during spring migration affect population dynamics of Barn swallows *Hirundo rustica* breeding in Northern Italy

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Roberto Ambrosini

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Preliminary on total reproductive output

The main aim of this paper was to investigate the year-to-year variation in the mean number of breeding pairs of Barn swallow (*Hirundo rustica*) recorded in a sample of farms in the Parco Regionale Adda Sud (Northern Italy) from 1999 to 2014. In our main analysis we modelled variation in the mean number of breeding pairs according to variation in ecological conditions experienced by individuals at different stages of their life-cycle. In this analysis we also included among the predictors the reproductive success of first broods in the preceding year as a proxy of the total reproductive output of the population because reproductive success of first broods was available for all years, while that of second broods was available for a few years only (see below). Population size may be affected by the total reproductive output in the preceding year, as Barn swallows at their first breeding attempt mostly disperse only a few kilometres from their natal site (Scandolara et al 2014).

During the preliminary analyses showed in these Supplementary Materials we aimed at investigating whether the reproductive success of first broods may be used a proxy of the total reproductive success of both first and second broods. Reproductive output is determined by the breeding success of both first and second broods and by the proportion of pairs that produced a second brood, which, in turn, may be influenced by ecological conditions experienced by individuals during breeding. We hypothesized that these effects may act through three different mechanisms. First, at temperate latitudes, temperature and rainfall have been demonstrated to influence the reproductive output of bird populations (Brown & Brown 1999; Dunn & Winkler 1999), likely through an indirect effect of food availability on the body condition of parents. Second, previous studies have shown that Barn swallows breeding in farms where livestock is reared have larger reproductive success than those breeding in farms with no animal farming, due to both an overall larger offspring survival and a larger probability of laying a second clutch (Grüebler et al. 2010). Third, the extent of hayfields close to breeding sites has been identified as an important factor affecting nestling quality (Sicurella et al 2014) and, ultimately, their survival (Evans et al. 2007). We therefore accounted for temperature and rainfall, presence of livestock farming and hayfield extent at the breeding sites in our analyses of both the proportion of pairs raising a second brood and of the reproductive output of second broods.

Methods

Data on second broods were available to us only for a limited number of years. Indeed in 2008 and 2010-2014 a subsample of 37-48 farms selected among those censused during the period of first clutches (April- June) was censused also in July-August to assess number of breeding pairs and breeding success during the period of second clutches following the same protocol.

We could not capture and individually mark all Barn swallows at the farms we censused, owing to the large number of pairs and farms we censused at each year, so we could not precisely assess the proportion of pairs laying one, two or three clutches. However, we could assess the number of breeding pairs that, at each year, bred during the period of first clutches (April – June) and during the period of second clutches (July – August) based on the maximum number of nest simultaneously active during each period. Third clutches were not considered because of the few pairs laying a third clutch in each year (Turner 2006).

In all years we also recorded habitat conditions at breeding sites, particularly presence of livestock farming and extent of hayfields within 200 m from each farm, because these conditions are known to influence number of breeding pairs and breeding success (Møller 2001; Turner 2006; Grüebler et al 2010; Ambrosini et al 2012; Sicurella et al 2014).

Meteorological data

Data on air temperature and rainfall at breeding sites were obtained from the Agenzia Regionale per la Protezione dell'Ambiente (ARPA, www.arpalombardia.it). These data were collected at 1-day intervals at two meteorological stations located in the close proximity of the borders of the Parco Regionale Adda Sud (distance < 12 km; coordinates of the two stations are 45.44° N 9.50° E and 45.26° N 9.38° E). Mean temperature and total rainfall were calculated for each month from May to August in all years from mean daily temperature and rainfall data collected at these two meteorological stations.

Statistical analyses

In these preliminary analyses we tried to identify ecological variables that may account for the ratio between the mean number of breeding pairs that bred during the period of second broods and the mean number of breeding pairs that bred during the period of first broods and for the ratio between the mean breeding success of second and first broods. Both these analyses were based on the sample of farms where both first and second broods were censused at each year. Since in several years first broods only were censused, this analysis therefore aims at assessing whether any environmental variable may account for variation among years in the relative importance of broods produced during July – August (period of second broods) and of those produced during April – June (period of first broods).

The ratio between the mean number of breeding pairs that bred during the period of second broods and the mean number of breeding pairs that bred during the period of first broods and that between the mean breeding success of second and first broods are not limited between zero and one, because we cannot exclude that the mean number of pairs breeding during the period of second broods and their breeding success was larger than that of pairs breeding during the period of first broods. We therefore assumed these variables to be normally distributed, and we assumed a Gaussian error distribution in the analyses (Kolmogorov-Smirnov test for the ratio between breeding pairs of second and first broods: $D = 0.3935$, p-value = 0.24; Kolmogorov-Smirnov test for the ratio between breeding success of second and first broods: $D = 0.3333$, p-value = 0.4234).

We focused on meteorological and environmental conditions at breeding sites, which are known to influence breeding success of Hirundinidae. Any meteorological or environmental variable that affected either the ratio between the number of breeding pairs that bred during the period of second and first broods, or the ratio between breeding success of second and first broods may significantly influence year to year variation in the mean number of breeding pairs because it influenced the relative importance of the reproductive output of second broods. Specifically, we aimed at assessing whether the ratio between the number of breeding pairs that bred during the period of second and first broods varied according to i) the monthly total precipitation and ii) the monthly average temperatures from May to July in the Parco Regionale Adda Sud, iii) by the proportion of farms with livestock farming and iv) the mean hayfield extent within 200 m from each farm. Similarly, we modelled the ratio between breeding success of second and first broods according to i) the monthly total precipitation and ii) the monthly average temperatures from June to August in the Parco Regionale Adda Sud, iii) by the proportion of farms with livestock farming and iv) the mean hayfield extent within 200 m from each farm. In both cases, given the few data available (only 5 years) and the large number of predictors (8 variables) whose effect we aimed at testing, the analyses were performed following an Information Theory-based approach.

We produced all the Linear Models (LMs) assuming a Gaussian error distribution, including the all the possible combinations of the above mentioned predictors, by limiting to two the maximum number of predictors simultaneously included in each model (for a total of 37 models for each dependent variable, see table S2 and S3). In this way we aimed at avoiding model over-fitting and at testing the potential effects of all predictors. In both cases we then selected all the models with a ΔAICc < 2 (Burnham and Anderson 2002) with respect to the model with the lowest AICc, and then averaged model coefficients (Johnson and Omland 2004). These analyses were performed with the lm procedure in the stats package (R Core Team 2014) and the dredge and model.*avg* procedures in the MuMIn package (Barton 2014) in R 3.0.3 (R Core Team 2014).

Results and discussion

The model selection process indicated that the best model of the ratio between the number of breeding pairs that bred during the period of second broods and the number of breeding pairs that bred during that of first broods included only the intercept (AICc = 13.3), and any other model including meteorological and/or environmental variables as predictors had poorer fit (ΔAICc > 7.1 in all cases, see Table S2). Similarly, an intercept-only model (AICc = -5,1) fitted the ratio between mean breeding success of second and first broods better than any other model including meteorological and/or environmental variables as predictors (ΔAICc > 5.2 in all cases, see Table S3).

Hence, none of the variables we included seemed to influence the relative reproductive output of second and first broods. We therefore assumed that the number of chicks produced during first broods is a good proxy of the total annual reproductive output of the population and therefore we included it as predictor in the main analysis of variation in population size.

Table S2 and S3 see the electronic supplementary materials on the online version of the manuscript (doi:10.1007/s10144-015-0529-7).

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Supplementary materials to chapter 4:

Migratory connectivity and effects of winter temperatures on migratory behaviour of the European robin *Erithacus rubecula*: a continent-wide analysis

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Details on the migratory connectivity analysis and on cluster identification

The analysis of migratory connectivity and the following subdivision of European robins *Erithacus rubecula* into clusters of individuals showing similar migration behaviour was based on the framework of analysis proposed by Ambrosini, Møller & Saino (2009). We will not discuss the technical details of the analyses here, since we refer to the above mentioned paper, but will only highlight some aspects of this method that are relevant for the analyses presented in this paper.

Basically,, the analyses can be divided in two steps. The first aims at assessing the intensity of migratory connectivity, which can be defined as a measure of "how closely individuals of migratory species spatially cluster throughout the annual cycle" (Veen 2013). According to this definition, strong connectivity implies that individuals share similar breeding and wintering locations, while weak connectivity implies that individuals from different breeding populations mix at their wintering grounds and vice versa. The degree of migratory connectivity can be evaluated by observing the reciprocal position of individuals at the breeding and at the wintering grounds. Ambrosini, Møller & Saino (2009) proposed a quantitative measure of migratory connectivity based on the correlation between the matrix describing the distance between all pairs of individuals when they are at their breeding grounds and that describing the distance between the same individuals when they are at their wintering grounds. In particular, the degree of migratory connectivity can be assessed by a Mantel test on great circle (orthodromic) distance matrices between individuals calculated separately for the breeding and the wintering grounds (Ambrosini, Møller & Saino 2009). A strong correlation indicates that individuals maintain the same reciprocal positions, and therefore there is strong migratory connectivity. Conversely, if individuals mix, correlation between distance matrices will be low. It is important to notice that the only information included in this analysis is the distance between individuals at the breeding and the wintering grounds (i.e. information represented by the red arrows in Figure S1).

The second step of the analysis aims at identifying the clusters of individuals sharing breeding and wintering grounds, and it is based on a cluster analysis run on a combination of the matrices indicating the distance between individuals at the breeding and the wintering grounds. This analysis can be performed with the *pam* procedure in the *cluster* library in R 3.0.2 (R Core Team 2013). The *pam* procedure is a clustering algorithm that partitions observations in a number of clusters identified a priori. As a measure of the goodness of the classification of data into a given number of clusters, the procedure returns the overall average silhouette width (*oasw*), a dimensionless coefficient ranging from -1 to 1. Increasing *oasw* values indicate better classification of data (Rousseeuw 1987), and the best number of clusters in which data can be partitioned can be chosen as the number that maximizes the *oasw*. We stress that the only information included in the analysis also in this case is the one represented by the red arrows in Figure S1.

In summary, the only information included in the analysis of the intensity of migratory connectivity and in that run to divide robins into clusters is the reciprocal position of individuals at the breeding and the wintering grounds (red arrows in Figure S1), *not* the distance travelled by individuals during migration (black arrows in Figure S1). Indeed, these analyses will give the same results when applied to hypothetical individuals that migrated different distances (black arrows), but are in the same reciprocal positions (red arrows) as depicted in parts A and B of Figure S1. Hence, migration distance is not taken into account during cluster identification.

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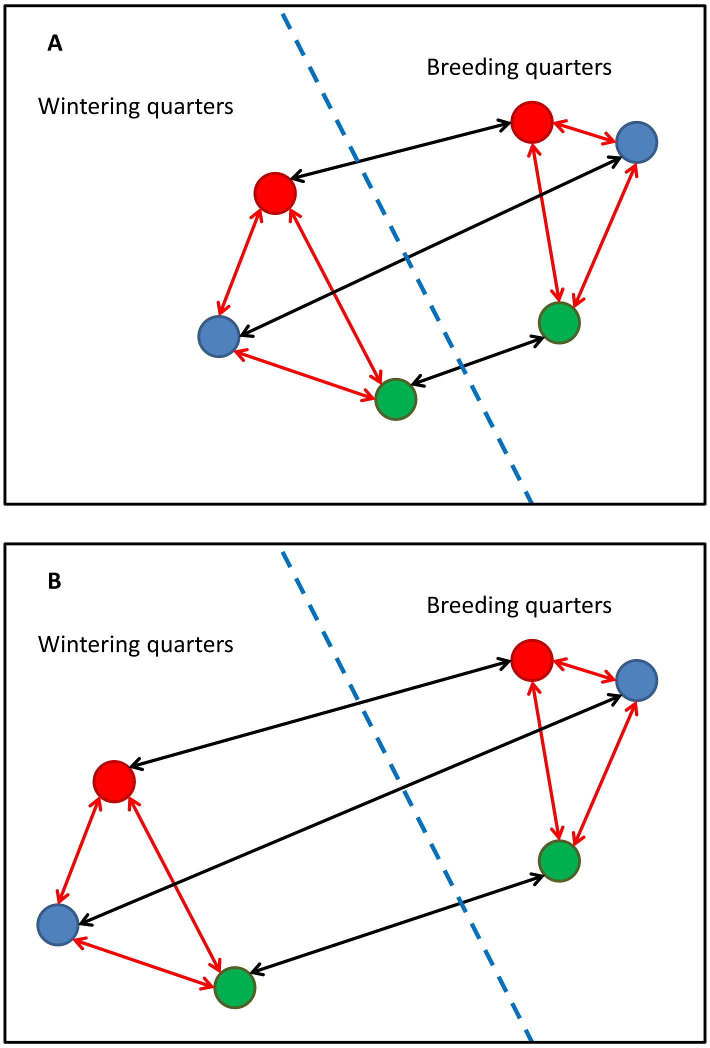
Figure S1. Schematic representation of positions of hypothetical individuals (dots of different colours) in the breeding and in the wintering quarters. Red arrows represent distances between individuals, black arrows distances travelled by individuals during migration. Migratory connectivity analyses are independent form migration distance (black arrows) and will return the same result when applied to individuals that migrate different distance (part a and b of the figure), but maintain the same reciprocal positions.



Additional details on methods

RATIONALE FOR DATA SELECTION CRITERIA

In Europe, ringing schemes provide data on birds ringed and recovered to the EURING Data Bank (EDB; see http://www.euring.com/). However, ringing schemes have historically adopted different procedures for processing and storing reports of birds that have been ringed and subsequently re-encountered (see http://www.euring.org/data_and_codes/obtaining_data/recovery_definitions.htm for full details on these different procedures). Differences in reporting procedures may largely affect the proportion of migrant birds if, for example, birds re-encountered close to the ringing site are not reported. However, all ringing schemes have always reported birds found dead. For this reason, we selected all records from individuals found dead in winter (EURING code 'condition' in 1-2; see du Feu *et al.* 2010 for details on EURING code). In addition, we discarded the following data from the analyses in order to reduce heterogeneity as far as possible (Paradis *et al.* 1998): birds that were in poor condition or had an accident when ringed (EURING code 'condition' in 4-5); birds that were kept for more than 13 h during ringing or birds that have been moved or held extensively during ringing (EURING code 'manipulated' equal to C, F, T, M); birds that were intentionally killed by man other than shot (EURING code 'circumstances' equal to 0 or 2); birds that were not found freshly dead (EURING code 'condition' equal to 3); birds for which the dates and places of ringing and/or recovery were not recorded accurately to the nearest 1 week for both the ringing and the finding date (EURING code ‘date accuracy’ in 4-8) or to the nearest 100 km for the ringing or finding places (EURING code ‘coordinates accuracy’ equal to 6 or 9).

After this selection, only four individuals had more than one record in either the breeding or the wintering period. In these cases, we selected respectively the northernmost and the southernmost one because these positions should reflect final destination of birds (see Ambrosini, Møller & Saino 2009 and Ambrosini *et al.* 2011 for a similar approach). The final database thus consisted of two geographical locations (one for breeding and one for wintering) for 1111 robins in the period 1947-2011.

CRITERIA USED FOR ASSESSING AGE TO ROBINS

For assigning age, we used the reported age code (EURING code ‘Age scheme’, du Feu et al. 2010) at ringing. Specifically, birds that were ringed in spring with age code ≥ 4 were classified as adults when found dead in winter, whereas birds that were ringed in spring with age code 1 or 3 and found dead in or before February of the subsequent year were classified as juveniles (214 individuals = 19.3%). In addition, all birds that were found dead in winter at least 12 months after ringing were classified as adults, irrespective of reported age at ringing (including birds whose age at ringing was not known, i.e. age code 0 or 2; overall 391 individuals = 35.2% were considered adults).

References

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Ambrosini, R., Rubolini, D., Møller, A. P., Bani, L., Clark, J., Karcza, Z., Vangeluwe, D., du Feu, C., Spina, F. & Saino, N. (2011) Climate change and the long-term northward shift in the African wintering range of Barn swallows *Hirundo rustica*. *Climate Research*, 49, 131-141.

du Feu, C.R., Clark, J.A., Fiedler, W. & Baillie, S.R. (2010) *The EURING Exchange Code 2000 Plus*. EURING, Thetford, UK.

Paradis, E., Baillie, S.R., Sutherland, W.J. & Gregory, R.D. (1998) Patterns of natal and breeding dispersal in birds. *Journal of Animal Ecology*, 67, 518-536.

Figure S2. Demographic indices in each country included in the study.

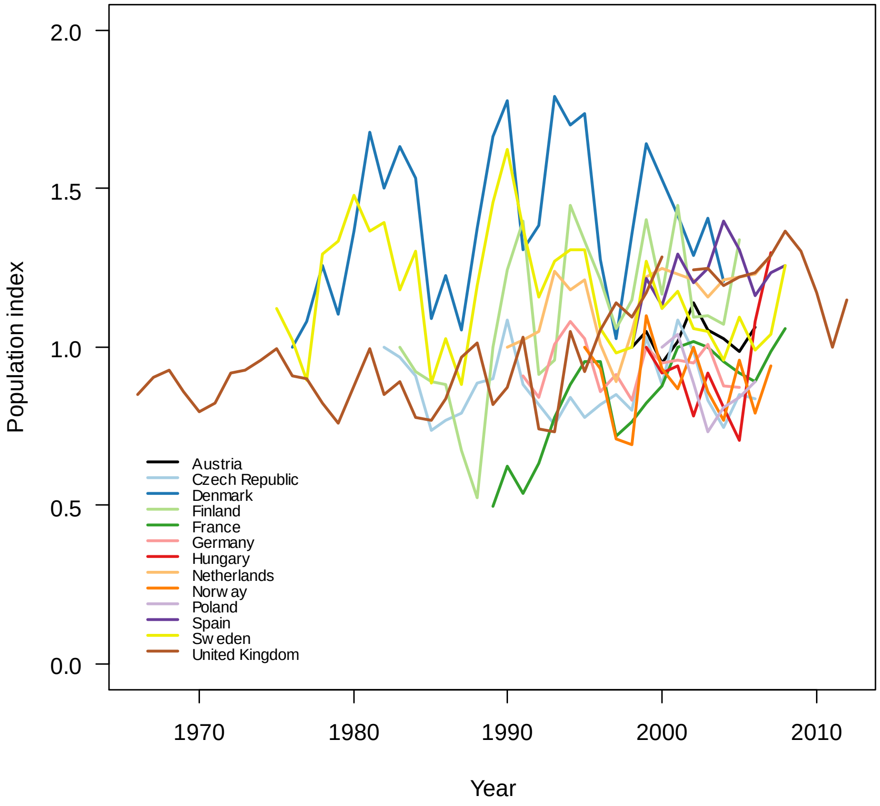


Figure S3. Frequency distribution of migration distance, defined as the great-circle distance between the ringing and the recovery sites. Only the 882 individuals that moved more than 0.571 km (see Methods) are shown.

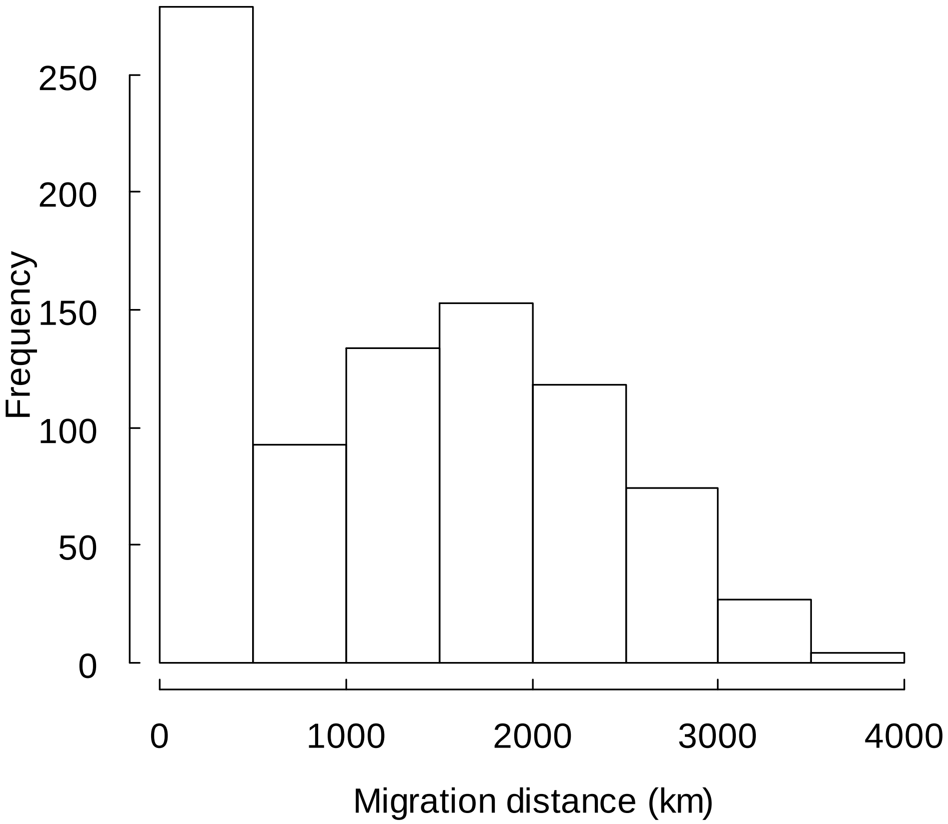
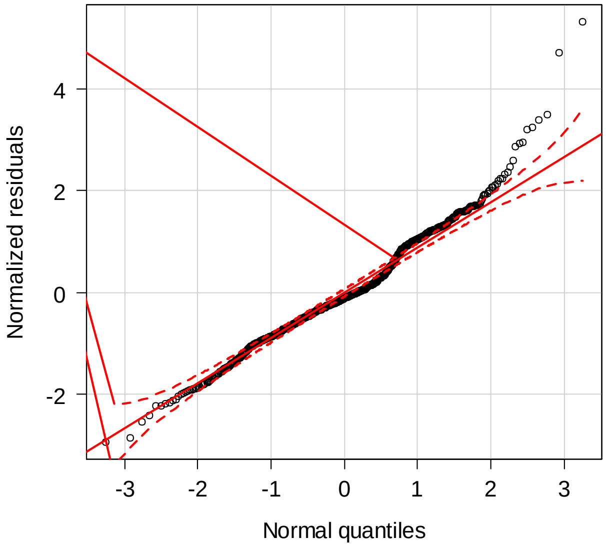
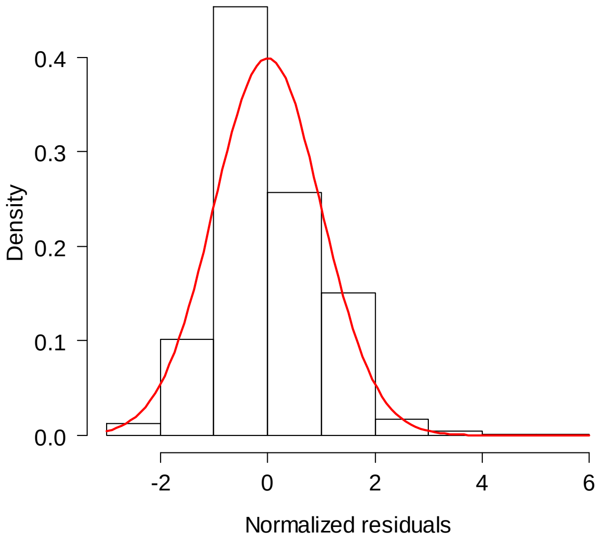


Figure S4. a) Qq-plot and b) histogram of normalized residuals from the LMM model of migration distance shown in Table 3. In b) a Gaussian curve has been superimposed on the histogram.

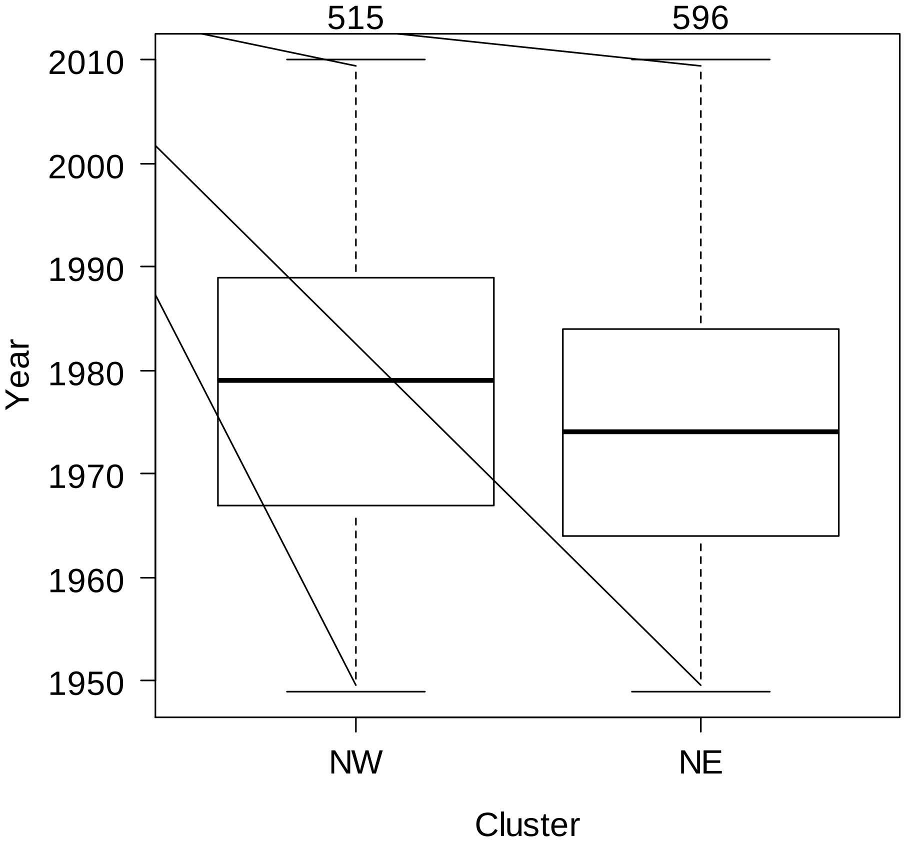


a



b

Figure S5. Boxplot of the years in which robins were recovered at their wintering grounds per cluster. The solid lines represent the median values, the top and the bottom of the boxes represent the first and the third quartiles, whiskers include 95% of data. Numbers above the graph denote sample sizes.



Analyses of migration propensity run on different datasets and including potentially confounding effects

Analysis of migration propensity including population indices indicated a significant decrease of migration propensity at increasing values of population indices (Table S1). This analysis also confirmed the increase in migration propensity with year, but not the variation in migration propensity with latitude and longitude (Table S1). However, size of the latitude effect was similar to that reported on the whole model (see Tables 2 and S1). Conversely, longitude seemed not to explain variation in migration propensity once that population index was accounted for. Non-significance of these effects may be due to the reduction in sample size, since both latitude and longitude were not significant ($\chi^2_1 \leq 3.19$, $P \geq 0.074$) in a model not including population indices, but restricted to the same dataset used for the analysis including population indices. In addition, power analysis indicated that power of the test to detect a significant latitude or longitude effect decreased to 0.434 and 0.595, respectively, when sample size was equal to that of the analysis including population index.

When we re-ran the analysis of migration propensity using different distance thresholds to differentiate between sedentary and migrant robins, we found that the effect of year was no longer significant when the threshold value was set to ≥ 3 km. In contrast, significance of both the latitude and longitude effects was confirmed when this threshold was set up to 100 km (other details not shown).

Finally, when we analysed migration propensity in a model including age and its interactions with winter temperature (sample size for the NW cluster was only 171 juveniles and 211 adults), beside all the predictors listed in Table 2, we found no significant effect of age or its interaction ($\chi^2_1 \leq 0.43$, $P \geq 0.512$, $|\text{effect size}| \leq 0.034$ in all cases). Removal of the non-significant interaction from the model did not result in a significant effect of age (details not shown). Significance of all the other effects listed in Table 2 did not change qualitatively with the exception of latitude that turned not significant ($\chi^2_1 = 0.90$, $P = 0.343$, effect size = 0.049; other details not shown).

By comparing the results of these additional analyses with those run on all individuals, it appeared that the increase in migration propensity of robins of the NW cluster through time was confirmed in all the analyses, but disappeared when a slightly larger distance threshold was used to differentiate sedentary from migratory birds. Geographical variation in migration propensity was not confirmed in the analysis including population indices, though latitude still had an effect of similar size than that observed on the whole sample, and only a longitudinal variation appeared in the analysis including bird age.

The fact that the proportion of migratory robins decreased in years when summer population index was larger contrasts with the hypothesis that individuals that could not find a suitable territory for wintering should be more prone to migrate, because this would determine an increase, rather than a decrease, in migration propensity in years with increased population. We can speculate that larger population indices are achieved in years with better general conditions, which in turn may prompt more birds to winter in their breeding grounds.

Table S1. Binomial GLMM of migration propensity (proportion of individuals that moved more than 0.571 km) including population index as a covariate and cell identity as a random grouping factor. The NE cluster was excluded from the analysis because almost all individuals of this cluster migrated. Sample size is 327 robins. Covariates (year, winter temperature, latitude and longitude) were centred to their mean values before the analysis. Significance of each term was assessed by likelihood ratio tests (χ^2 values and associated *df* and *P*). Coefficients are reported with the relative SE. Asterisks denote coefficients that differ significantly (** $P < 0.01$, *** $P < 0.001$) from zero. Pseudo-R^2 values were calculated according to Negelkerke (1991) while effect sizes according to Nakagawa and Cuthill (2007). The random effect (cell identity) variance is 0.204. Effects whose significance differs from that in the model in Table 2 are bolded.

Effect	**χ^2**	***df***	***P***	**Coef.**	**SE**		**Effect size**
Intercept	**0.01**	**1**	**0.930**	**-0.020**	**0.230**		
Year	24.94	1	< 0.001	0.077	0.015	***	0.262
Winter Temperature	3.10	1	0.078	0.163	0.092		0.095
Latitude	**3.19**	**1**	**0.074**	**0.157**	**0.087**		**0.097**
Longitude	**0.24**	**1**	**0.632**	**-0.047**	**0.096**		**-0.026**
Population Index	7.17	1	0.007	-3.418	1.271	**	-0.144

AIC = 422.1, Marginal Pseudo-R^2 = 0.135, Conditional Pseudo R^2 = 0.151

Analyses of migration distance run on different datasets and including potentially confounding effects

The analysis of migration distance including population indices did not confirm the significance of the interactions between cluster and year, winter temperature, latitude and longitude (Table S3). A model excluding these non-significant interactions disclosed a significant effect of year (coef. = -1.711 ± 0.760 SE km year^{-1}, $\chi^2_1 = 5.06$, $P = 0.024$, effect size = -0.099) and latitude *per se* (coef. =: 25.813 ± 4.280 SE km degree^{-1}, $\chi^2_1 = 36.38$, $P < 0.001$, effect size = -0.257), but indicated no variation in migration distance with winter temperature or longitude ($\chi^2_1 \leq 0.45$, $P \geq 0.503$, |effect size| ≤ 0.098; other details not shown).

Also in this case, the lack of any winter temperature effect may be due to the fact that analyses including population indices restricted the dataset, particularly for the NE Cluster (from 592 to 29 individuals, see Tables 3 and S2). Indeed, winter temperature, *per se* or in interaction with cluster, was not significant either in a model without population index among its predictors but restricted to the same dataset used for the analysis including it ($\chi^2_1 \leq 0.32$, $P \geq 0.568$, effect size ≤ 0.032, other details not shown). In addition, power analysis indicated that power of the test to detect any significant effects of cluster by year or cluster by temperature interactions decreased to 0.419 and 0.289, respectively, when overall sample size was equal to that of the analysis including population index, and to, respectively, 0.140 and 0.120 when sample size *at each cluster* was set equal to that of the analysis including population index. Conversely, the power of detecting a significant cluster by latitude interaction was still 0.807 when sample size was reduced to 215 individuals randomly chosen from the whole sample, but was only 0.383 when sample size was 186 individuals from the NW and 29 from the NE cluster.

When we re-ran the analysis using different values of the migration distance threshold, we observed that the cluster by winter temperature interaction turned non-significant when the threshold was set to ≥ 30 km, while the significance of all the other effects was unchanged (details not shown). However, when the distance threshold was set to ≥ 30 km sample size in the NW cluster was reduced from 290 to 87 individuals or less, and the power of the test to detect a significant cluster by year or cluster by winter temperature interaction decreased to 0.579 or less. Winter temperature *per se* was also non-significant when we re-ran the model excluding the cluster by winter temperature interaction while changing the threshold value (details not shown).

The cluster by year interaction also turned not significant when the threshold was set to ≥ 70 km, when the power of the test to detect a significant interaction was still 0.908 (sample size was 68 individuals in the NW cluster and 591 in the NE one). However, when we removed the non-significant interaction, year effect was significant for any distance threshold (details not shown). Finally, the cluster by latitude interaction was always significant for all threshold values (details not shown).

The model including age and its interactions with cluster and winter temperature, beside all the other predictors, indicated no significant effect of the age by cluster and of the age by winter temperature interactions ($\chi^2_1 \leq 2.37$, $P \geq 0.12$, |effect size| ≤ 0.059 in all cases). In contrast, after removal of the non-significant interactions, age was highly significant, and indicated that adult robins migrated significantly longer distances than juveniles (coef. = 55.269 ± 18.973 SE km, $\chi^2_1 = 9.76$, $P = 0.002$, effect size = 0.116). Significance of all the other predictors listed in Table 3 did not vary (details not shown).

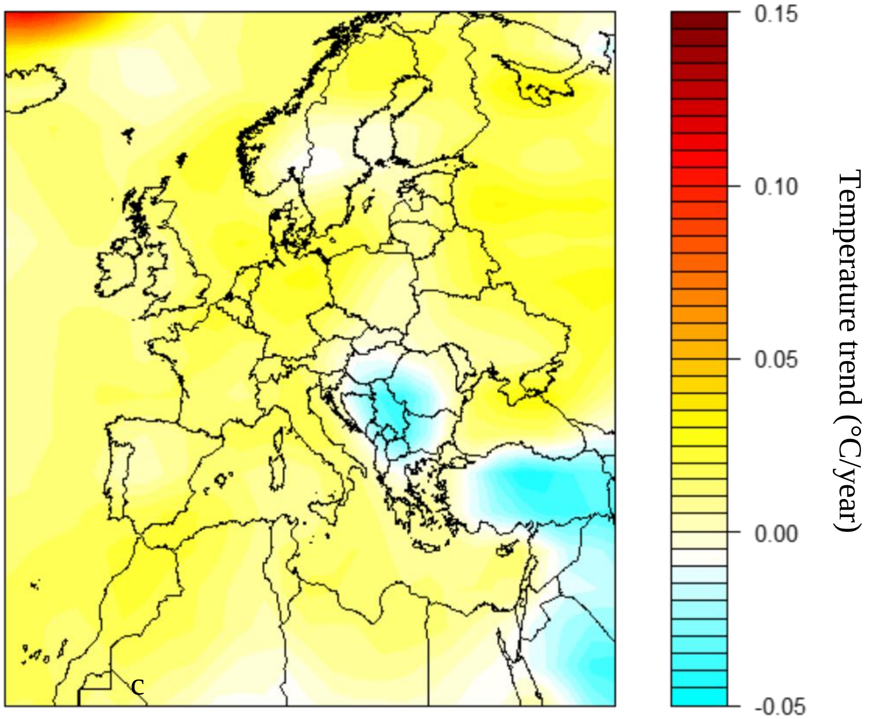
Hence, the significance of the cluster by winter temperature interaction was not confirmed in the analysis including population indices. However, the lack of significance in the analysis including population indices could be due to restriction of the dataset to recent (mainly post-eighties) years for most countries, as suggested by the fact that winter temperature was not significant in an analysis not including population index, but restricted to the same dataset, and to a lower power of the statistical test due to reduced sample size. Conversely, significance of the cluster by winter temperature interaction was confirmed by the analysis including bird age and by those run by setting migration distance to up to 30 km.

Table S2. Mixed model of migration distance (distance travelled by robins that moved more than 0.571 km, see Methods) including population index as a covariate and country as a random grouping factor. Sample size is 215 robins (NW: n = 186, NE: n = 29). Covariates (year, winter temperature, latitude and longitude) were centred to their mean values before the analyses. Significance of each term was assessed by likelihood ratio tests (χ^2 values and associated *df* and *P*). Coefficients (marginal means) are reported for each cluster with the relative SE. Asterisks denote coefficients that differ significantly (* $P < 0.05$, *** $P < 0.001$) from zero, and different letters denote effects that differ significantly ($P < 0.05$) in post-hoc tests. Pseudo-R^2 values were calculated according to Negelkerke (1991) while effect sizes according to Nakagawa and Cuthill (2007). The random effect (country) variance is 301044.8 and residual variance is 193675.4. Effects whose significance differs from that in the model in Table 3 are bolded.

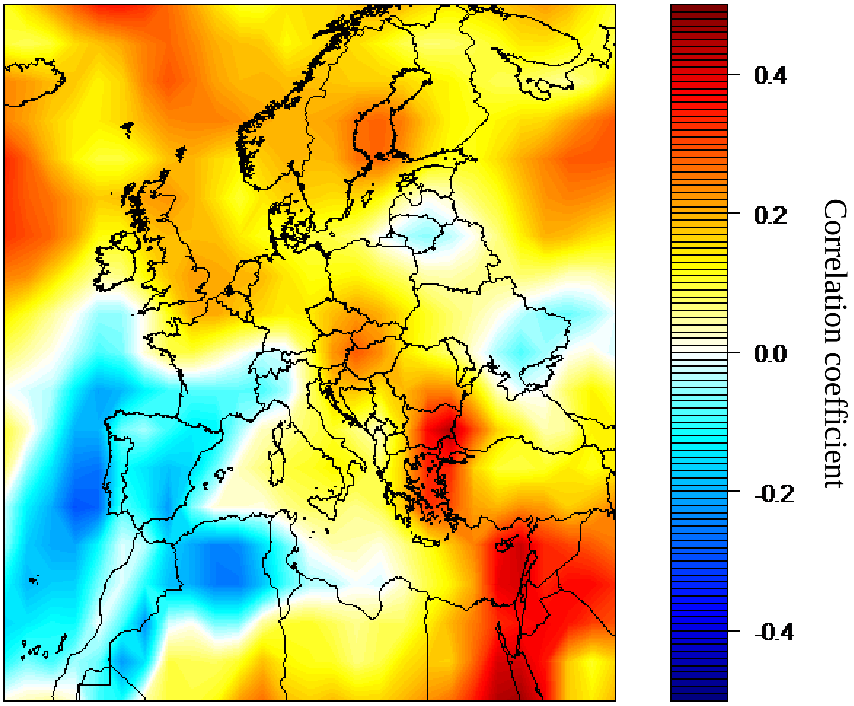
Effect	χ^2	***df***	***P***	**Cluster**	**Coef.**	**SE**			**Effect size**
Cluster	19.85	1	< 0.001	NW	606.530	197.253	***	a	
				NE	1530.407	163.707	***	b	
Year	4.75	1	0.129						
Winter temperature	0.06	1	0.808						
Latitude	34.53	1	< 0.001						
Longitude	0.21	1	0.647						
Cluster x Year	**1.89**	**1**	**0.169**	NW	-1.661	0.762	*		-0.122
				NE	-21.226	14.197			-0.084
Cluster x Winter temp.	**0.25**	**1**	**0.619**	NW	1.078	4.432			0.014
				NE	24.980	47.972			0.029
Cluster x Latitude	**1.39**	**1**	**0.238**	NW	25.135	4.278	***		0.315
				NE	69.376	37.400			0.104
Cluster x Longitude	**0.86**	**1**	**0.353**	NW	-2.362	5.155			-0.026
				NE	24.313	28.219			0.049
Population Index	0.46	1	0.496		37.880	55.703			0.038

AIC = 2560.2, Marginal Pseudo-R^2 = 0.308, Conditional Pseudo-R^2 = 0.9

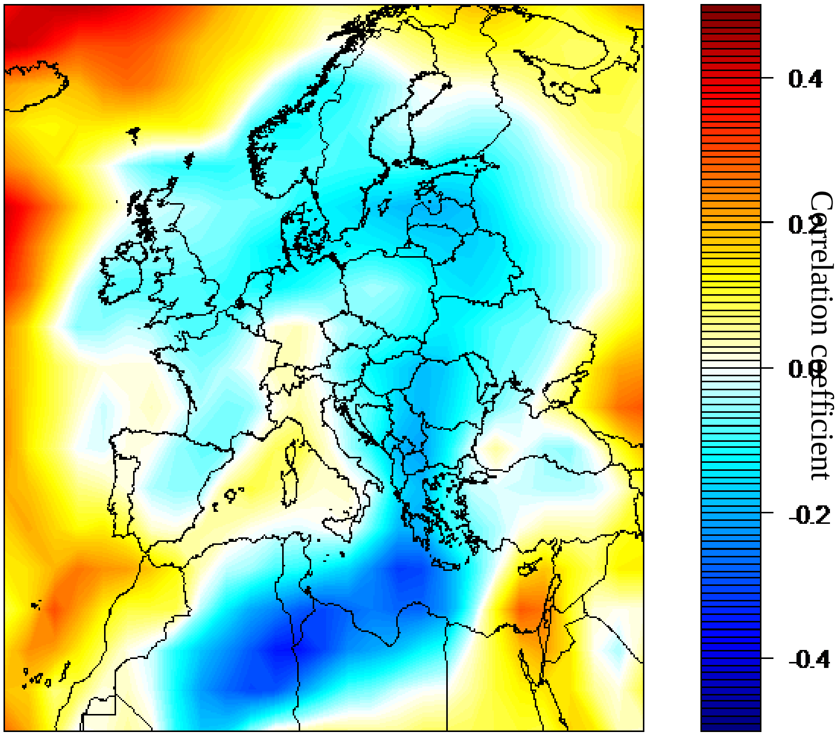
Figure S6. Maps of a) trends in winter temperatures in Europe, calculated as the slope of the minimum monthly mean temperature in each winter for the period 1948-2011; b) partial correlation coefficients between mean temperature in the warmest month in June-August (summer temperature) and mean temperature of the coldest month in December-February (winter temperature), after removing the effect of year (detrended correlations); c) detrendend correlations between mean temperature in the coldest month in September-October (autumn temperature) and winter temperature; d) detrended correlations between winter temperature in year *i* and in year *i*-1.



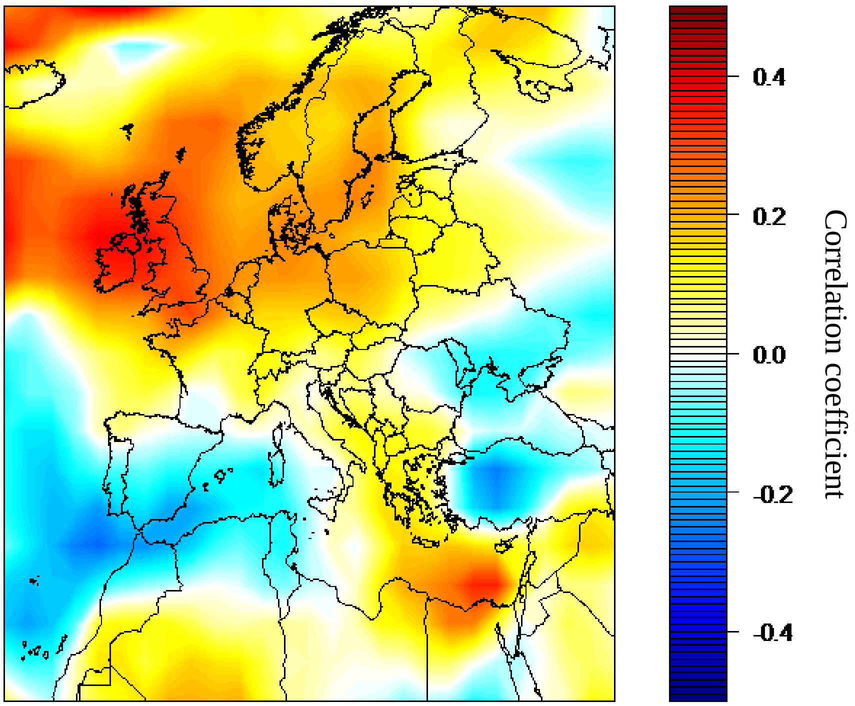
a



b



c



d

Supplementary materials to chapter 5:

Effects of livestock farming on birds of rural areas in Europe

Federica Musitelli • Andrea Romano • Anders Pape Møller
Roberto Ambrosini

Table S1 see the electronic supplementary materials on the online version of the paper (doi:10.1007/s10531-016-1087-9).

Table S2. Indirect publication bias analyses. Results of three indirect publication bias analyses are reported, for each subset of studies for which a mean effect size significantly larger than zero was observed, i.e. all tests on the Barn swallow except for that on reproduction (Table 1 in the main text). The table reports the number of effect sizes in each analysis, the Kendal τ correlation between effect sizes and sample sizes and the relative *P*-value, the fail safe number and three outcomes of the trim-and-fill test, specifically the estimated number of missing studies, the estimate of the mean effect size obtained after augmenting the observed data with the (estimated) missing studies, and its 95% confidence interval. Each test was run first by including all effect sizes, thus assuming complete independence of analyses reported into the same paper and, second, by including only the mean effect size within paper, thus assuming full dependence of effect sizes reported in each paper (see Methods for details). In the case of Kendall's τ, we reported the results of analyses run by correlating the within-study effect size with the mean within-study sample size.

Subset	Number of effect sizes	Kendall's τ	p	Fail safe number	Trim and fill		
					Missing	Estimate	95% CI
All effect sizes							
All	47	-0.116	0.262	6622*$^{+}$	0	0.272	(0.173 – 0.371)
Direct effects	33	-0.155	0.218	4456*$^{+}$	0	0.317	(0.183 – 0.452)
Indirect effects	14	-0.022	0.912	201*$^{+}$	0	0.162	(0.098 – 0.226)
Distribution	39	-0.098	0.394	6264*$^{+}$	0	0.296	(0.189 – 0.404)
Mean within-paper effect size							
All	16	-0.267	0.165	663*$^{+}$	4	0.213	(0.065 – 0.362)
Direct effects	14	-0.231	0.279	494*$^{+}$	3	0.262	(0.082 – 0.442)
Indirect effects	9	0.222	0.477	61*	0	0.124	(0.078 – 0.170)
Distribution	14	-0.341$^{\#}$	0.101	644*$^{+}$	4	0.235	(0.087 – 0.382)

*: values above the threshold $5n + 10$ (where n is the original number of studies) proposed by Rosenthal (1979) as a reasonable, conservative critical value against which to test a fail-safe calculation.

$^{+}$: values that differ significantly from the $5n + 10$ threshold according to Fragkos et al. (2014).

[#]: when sample size was estimated as the minimum sample size within papers, Kendall's τ was equal to -0.516, *P* = 0.010.

References

Fragkos KC, Tsagris M, Frangos C (2014) Publication bias in meta-analysis: confidence intervals for Rosenthal's fail-safe number. Int Sch Res Notices 2014:825383. doi: 10.1155/2014/825383

Rosenthal R (1979) The "file drawer problem" and tolerance for null results. Psychol Bull 86:638–641

Code for all the analyses run with SAS 9.3

We here provide commented code for all the analyses run with SAS 9.3 including steps for proper data selection from Table S1.

```
/* SAS code for analyses reported in Musitelli et al.
Effects of livestock farming on birds of rural areas in
Europe*/

/* data import
put your path to file Table S1.xlsx provided as Supplementary
Information*/

proc import datafile  = "C:\...your path to file ...\Table
S1.xlsx"
out = meta replace dbms = xlsx;
run;

*analyses on Barn swallow;

data meta_swallow;
set meta;
if barn_swallow EQ 1;
run;
```

```
proc mixed data = meta_swallow cl covtest method=REML;
title 'Barn swallow all';
class ID ID_paper;
model zr = / solution ddfm=satterthwaite cl;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
weight N3;
parms 1 1 1 / hold=3;
run;

data meta_swallow_dir;
set meta;
if barn_swallow EQ 1 AND type_effect EQ 1;
run;

proc mixed data = meta_swallow_dir cl covtest method=REML;
title 'Barn swallow direct effects';
class ID ID_paper;
model zr = / solution ddfm=satterthwaite cl;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
weight N3;
parms 1 1 1 / hold=3;
run;

data meta_swallow_ind;
set meta;
if barn_swallow EQ 1 AND type_effect EQ 0;
run;

proc mixed data = meta_swallow_ind cl covtest method=REML;
title 'Barn swallow indirect effects';
class ID ID_paper;
model zr = / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
weight N3;
parms 1 1 1 / hold=3;
run;


proc mixed data = meta_swallow cl covtest method=REML;
title 'Barn swallow comparison direct indirect';
```

```
class type_effect ID ID_paper;
model zr = type_effect / solution ddfm=satterthwaite cl;
random intercept /sub= ID (ID_paper) group = type_effect;
random intercept /sub= ID_paper group = type_effect;
weight N3;
lsmeans type_effect / pdiff adjdfe=row cl;
parms 1 1 1 1 1 / hold=5;
run;

data meta_swallow_dist;
set meta;
if barn_swallow EQ 1 AND type_study EQ 'distribution';
run;

proc mixed data = meta_swallow_dist cl covtest method=REML;
title 'Barn swallow distribution';
class ID ID_paper;
model zr = / solution ddfm=satterthwaite cl;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
weight N3;
parms 1 1 1 / hold=3;
run;

data meta_swallow_repr;
set meta;
if barn_swallow EQ 1 AND type_study EQ 'reproduction';
run;

proc mixed data = meta_swallow_repr cl covtest method=REML;
title 'Barn swallow reproduction';
class ID ID_paper;
model zr = / solution ddfm=satterthwaite cl;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
weight N3;
parms 1 1 1 / hold=3;
run;

proc mixed data = meta_swallow cl covtest method=REML;
title 'Barn swallow comparison distribution reproduction';
class type_study ID ID_paper;
model zr = type_study / solution ddfm=satterthwaite cl;
random intercept / sub = ID(ID_paper) group = type_study;
```

```
random intercept / sub = ID_paper group = type_study;
weight N3;
lsmeans type_study / pdiff adjdfe=row cl;
parms 1 1 1 1 1 / hold=5;
run;

*analyses on other species;

data meta_other;
set meta;
if barn_swallow EQ 0;
run;

proc mixed data = meta_other cl covtest method=REML;
title 'other all';
class ID ID_paper species;
model zr = / solution ddfm=satterthwaite cl ;
random intercept / sub = ID(ID_paper) ;
random intercept / sub = ID_paper ;
random intercept /sub= species;
weight N3;
parms 1 1 1 1 / hold=4;
run;

data meta_other_dir;
set meta;
if barn_swallow EQ 0 AND type_effect EQ 1;
run;

proc mixed data = meta_other_dir cl covtest method=REML;
title 'other direct';
class ID ID_paper species;
model zr = / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
random intercept /sub= species;
weight N3;
parms 1 1 1 1/ hold=4;
run;

data meta_other_ind;
set meta;
if barn_swallow EQ 0 AND type_effect EQ 0;
run;
```

```
* one only paper, random effect for ID_paper removed ;
proc mixed data = meta_other_ind cl covtest method=REML;
title 'other indirect ';
class ID species;
model zr = / solution ddfm=satterthwaite cl ;
random intercept /sub= ID;
random intercept /sub= species;
weight N3;
parms 1 1 1/ hold=3;
run;


data meta_other_dist;
set meta;
if barn_swallow EQ 0 AND type_study EQ 'distribution';
run;

proc mixed data = meta_other_dist cl covtest method=REML;
title 'other distribution';
class ID ID_paper species;
model zr = / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
random intercept /sub= species;
weight N3;
parms 1 1 1 1 / hold=4;
run;

data meta_other_rep;
set meta;
if barn_swallow EQ 0 AND type_study EQ 'reproduction';
run;

proc mixed data = meta_other_rep cl covtest method=REML;
title 'other reproduction';
class ID ID_paper species;
model zr = / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
random intercept /sub= species;
weight N3;
parms 1 1 1 1 / hold=4;
run;
```

```
* variation in effect sizes with latitude;

proc mixed data = meta cl covtest method=REML;
title 'bias latitude all';
class ID ID_paper species;
model zr = latitude / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
random intercept /sub= species;
weight N3;
parms 1 1 1 1/ hold=4;
run;

proc mixed data = meta_swallow cl covtest method=REML;
title 'bias latitude swallow';
class ID ID_paper species;
model zr = latitude / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
weight N3;
parms 1 1 1 / hold=3;
run;

proc mixed data = meta_other cl covtest method=REML;
title 'bias latitude other';
class ID ID_paper species;
model zr = latitude / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
random intercept /sub= species;
weight N3;
parms 1 1 1 1/ hold=4;
run;

* variation in effect size with year;

proc mixed data = meta cl covtest method=REML;
title 'bias year of publication';
class ID ID_paper species;
model zr = year / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
random intercept /sub= species;
```

```
weight N3;
parms 1 1 1 1/ hold=4;
run;

proc mixed data = meta_swallow cl covtest method=REML;
title 'bias year of publication swallow';
class ID ID_paper species;
model zr = year / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
weight N3;
parms 1 1 1 / hold=3;
run;

proc mixed data = meta_other cl covtest method=REML;
title 'bias year of publication other';
class ID ID_paper species;
model zr = year / solution ddfm=satterthwaite cl ;
random intercept /sub= ID (ID_paper);
random intercept /sub= ID_paper;
random intercept /sub= species;
weight N3;
parms 1 1 1 1/ hold=4;
run;
```

Code for publication bias analyses run with R 3.0.1

We here provide commented code for all the analyses run with R 3.0.1 including steps for proper data selection from Table S1.

```
# load necessary libraries
library(metafor)
library(xlsx)


### data import----
# put your path to file Table S1.xlsx provided as
Supplementary Information

d <- read.xlsx("C:/…your path to file .../Table S1.xlsx",
"Data")

### Analyses on all effect sizes ----

# 1) select all effect sizes on Barn swallows ----
f <- subset(d, species == "Hirundo_rustica")
dim(f)

# Kendal Tau correlation test
cor.test(f$Zr, f$sample_size, method = "kendall")

# Fail-safe number
fsn(Zr, var_Zr, data = f, type = "Rosenthal", alpha = 0.05)

# Trim-fill
# A random effect model is required for trim-fill
ma <- rma.uni(Zr, var_Zr, data = f, method = "REML")
trimfill(ma, estimator = "L0")

# 2) select direct effects on Barn swallows ----
f <- subset(d, species == "Hirundo_rustica" & type_effect ==
1)
dim(f)

# Kendal Tau correlation test
cor.test(f$Zr, f$sample_size, method = "kendall")

# Fail-safe number
```

```
fsn(Zr, var_Zr, data = f, type = "Rosenthal", alpha = 0.05)

# Trim-fill
# A random effect model is required for trim-fill
ma <- rma.uni(Zr, var_Zr, data = f, method = "REML")
trimfill(ma, estimator = "L0")

# 3) select indirect effects on Barn swallows ----
f <- subset(d, species == "Hirundo_rustica" & type_effect ==
0)
dim(f)

# Kendal Tau correlation test
cor.test(f$Zr, f$sample_size, method = "kendall")

# Fail-safe number
fsn(Zr, var_Zr, data = f, type = "Rosenthal", alpha = 0.05)

# Trim-fill
# A random effect model is required for trim-fill
ma <- rma.uni(Zr, var_Zr, data = f, method = "REML")
trimfill(ma, estimator = "L0")

# 4) select effects on Barn swallow distribution ----
f <- subset(d, species == "Hirundo_rustica" & type_study ==
"distribution")
dim(f)

# Kendall Tau correlation test
cor.test(f$Zr, f$sample_size, method = "kendall")

# Fail-safe number
fsn(Zr, var_Zr, data = f, type = "Rosenthal", alpha = 0.05)

# Trim-fill
# A random effect model is required for trim-fill
ma <- rma.uni(Zr, var_Zr, data = f, method = "REML")
trimfill(ma, estimator = "L0")


#### Analyses on mean within study effect size ----

# 1) select all effect sizes on Barn swallows ----
f <- subset(d, species == "Hirundo_rustica")
```

```
dim(f)

# calcuale mean effect size within paper
Zrmean                                                   <-
data.frame(cbind(ID_paper=unique(f$ID_paper),Zr=NA,
se_Zr=NA,
     var_Zr=NA, avg_sample_size=NA, min_sample_size=NA,
          max_sample_size=NA))
dim(Zrmean)

for(i in 1:nrow(Zrmean)){
  IID <- as.numeric(Zrmean$ID_paper[i])
  Sub <- subset(f, ID_paper==IID)
  if(nrow(Sub)>0){

    mZr <- rma.uni(Zr, 1/N3, data = Sub, method = "REML")
    Zrmean$Zr[i] <- mZr$b
    Zrmean$se_Zr[i] <- mZr$se
    Zrmean$var_Zr[i] <- mZr$se^2
    Zrmean$avg_sample_size[i] <- mean(Sub$sample_size)
    Zrmean$min_sample_size[i] <- min(Sub$sample_size)
    Zrmean$max_sample_size[i] <- max(Sub$sample_size)
  }
}

# Kendal Tau correlation test
cor.test(Zrmean$Zr,   Zrmean$avg_sample_size,   method   =
"kendall")
cor.test(Zrmean$Zr,   Zrmean$min_sample_size,   method   =
"kendall")
cor.test(Zrmean$Zr,   Zrmean$max_sample_size,   method   =
"kendall")


# Fail-safe number
fsn(Zr, var_Zr, data = Zrmean, type = "Rosenthal", alpha =
0.05)

# Trim-fill
# A random effect model is required for trim-fill
ma <- rma.uni(Zr, var_Zr, data = Zrmean, method = "REML")
trimfill(ma, estimator = "L0")

# 2) select direct effects on Barn swallows ----
```

```
f <- subset(d, species == "Hirundo_rustica" & type_effect ==
1)
dim(f)

# calcuale mean effect size within paper
Zrmean                                                      <-
data.frame(cbind(ID_paper=unique(f$ID_paper),Zr=NA,
se_Zr=NA,
          var_Zr=NA,                    avg_sample_size=NA,
min_sample_size=NA,
          max_sample_size=NA))
dim(Zrmean)

for(i in 1:nrow(Zrmean)){
  IID <- as.numeric(Zrmean$ID_paper[i])
  Sub <- subset(f, ID_paper==IID)
  if(nrow(Sub)>0){

    mZr <- rma.uni(Zr, 1/N3, data = Sub, method = "REML")
    Zrmean$Zr[i] <- mZr$b
    Zrmean$se_Zr[i] <- mZr$se
    Zrmean$var_Zr[i] <- mZr$se^2
    Zrmean$avg_sample_size[i] <- mean(Sub$sample_size)
    Zrmean$min_sample_size[i] <- min(Sub$sample_size)
    Zrmean$max_sample_size[i] <- max(Sub$sample_size)
  }
}

# Kendal Tau correlation test
cor.test(Zrmean$Zr,   Zrmean$avg_sample_size,   method   =
"kendall")
cor.test(Zrmean$Zr,   Zrmean$min_sample_size,   method   =
"kendall")
cor.test(Zrmean$Zr,   Zrmean$max_sample_size,   method   =
"kendall")


# Fail-safe number
fsn(Zr, var_Zr, data = Zrmean, type = "Rosenthal", alpha =
0.05)

# Trim-fill
# A random effect model is required for trim-fill
```

```
ma <- rma.uni(Zr, var_Zr, data = Zrmean, method = "REML")
trimfill(ma, estimator = "L0")

# 3) select indirect effects on Barn swallows ----

f <- subset(d, species == "Hirundo_rustica" & type_effect ==
0)
dim(f)

# calcuale mean effect size within paper
Zrmean                                                    <-
data.frame(cbind(ID_paper=unique(f$ID_paper),Zr=NA,
se_Zr=NA,
          var_Zr=NA,                    avg_sample_size=NA,
min_sample_size=NA,
          max_sample_size=NA))
dim(Zrmean)

for(i in 1:nrow(Zrmean)){
  IID <- as.numeric(Zrmean$ID_paper[i])
  Sub <- subset(f, ID_paper==IID)
  if(nrow(Sub)>0){

    mZr <- rma.uni(Zr, 1/N3, data = Sub, method = "REML")
    Zrmean$Zr[i] <- mZr$b
    Zrmean$se_Zr[i] <- mZr$se
    Zrmean$var_Zr[i] <- mZr$se^2
    Zrmean$avg_sample_size[i] <- mean(Sub$sample_size)
    Zrmean$min_sample_size[i] <- min(Sub$sample_size)
    Zrmean$max_sample_size[i] <- max(Sub$sample_size)
  }
}


# Kendal Tau correlation test
cor.test(Zrmean$Zr,   Zrmean$avg_sample_size,   method   =
"kendall")
cor.test(Zrmean$Zr,   Zrmean$min_sample_size,   method   =
"kendall")
cor.test(Zrmean$Zr,   Zrmean$max_sample_size,   method   =
"kendall")


# Fail-safe number
```

```
fsn(Zr, var_Zr, data = Zrmean, type = "Rosenthal", alpha =
0.05)

# Trim-fill
# A random effect model is required for trim-fill
ma <- rma.uni(Zr, var_Zr, data = Zrmean, method = "REML")
trimfill(ma, estimator = "L0")

# 4) select effects on Barn swallow distribution ----

f <- subset(d, species == "Hirundo_rustica" & type_study ==
"distribution")
dim(f)

# calcuale mean effect size within paper
Zrmean                                                   <-
data.frame(cbind(ID_paper=unique(f$ID_paper),Zr=NA,
se_Zr=NA,
          var_Zr=NA,                    avg_sample_size=NA,
min_sample_size=NA,
          max_sample_size=NA))
dim(Zrmean)

for(i in 1:nrow(Zrmean)){
  IID <- as.numeric(Zrmean$ID_paper[i])
  Sub <- subset(f, ID_paper==IID)
  if(nrow(Sub)>0){

    mZr <- rma.uni(Zr, 1/N3, data = Sub, method = "REML")
    Zrmean$Zr[i] <- mZr$b
    Zrmean$se_Zr[i] <- mZr$se
    Zrmean$var_Zr[i] <- mZr$se^2
    Zrmean$avg_sample_size[i] <- mean(Sub$sample_size)
    Zrmean$min_sample_size[i] <- min(Sub$sample_size)
    Zrmean$max_sample_size[i] <- max(Sub$sample_size)
  }
}


# Kendal Tau correlation test
cor.test(Zrmean$Zr,   Zrmean$avg_sample_size,   method   =
"kendall")
cor.test(Zrmean$Zr,   Zrmean$min_sample_size,   method   =
"kendall")
```

```
cor.test(Zrmean$Zr,    Zrmean$max_sample_size,    method    =
"kendall")


# Fail-safe number
fsn(Zr, var_Zr, data = Zrmean, type = "Rosenthal", alpha =
0.05)

# Trim-fill
# A random effect model is required for trim-fill
ma <- rma.uni(Zr, var_Zr, data = Zrmean, method = "REML")
trimfill(ma, estimator = "L0")


### forest plot with subgrouping of studies (Figure 1)
o <- order(d$barn_swallow, d$Zr, decreasing = TRUE)

ma <- rma.uni(Zr, var_Zr, data = d, method = "REML")
dim(d)
dim(subset(d, barn_swallow==1))
dim(subset(d, barn_swallow==0))

windows(7,12)
forest(ma, slab = d$ID, xlim=c(-3, 2), ylim = c(1,82),
       xlab      =      expression(paste("Effect       size
(",italic(Z[r]),")", sep = "")),
       annotate = FALSE, addfit = FALSE,
       addcred = FALSE, order = o, pch = 19,
       rows=c(77:31, 25:1), psize = 1.5,
       cex.axis = 0.9, cex.lab = 0.9)
text(-3, 79, "Barn swallow", cex = 0.5, pos = 4, font = 2)
text(-3, 27, "Other bird species typical of rural habitats",
cex
     = 0.5, pos = 4, font = 2)
text(-3, c(29.2, -0.8), "OVERALL", cex = 0.5, pos = 4, font
=
       2)
points(c(0.272, 0.043), c(29.2, -0.8), pch=15, cex = 1)
arrows(0.170, 29.2, 0.374, 29.2, length = 0.03, angle = 90,
code =
         3)
arrows(-0.260, -0.8, 0.346, -0.8, length = 0.03, angle = 90,
code
       = 3)
```

List of reviewed papers

Ambrosini R, Bani L, Massimino D, Fornasari L, Saino N (2011) Large-scale spatial distribution of breeding Barn swallows *Hirundo rustica* in relation to cattle farming. Bird Study 58:495–505

Ambrosini R, Bolzen AM, Canova L, Saino N (2002b) Latency in the response of Barn swallow *Hirundo rustica* population to changes in breeding habitat conditions. Ecol Lett 5:640–647

Ambrosini R, Bolzern A, Canova L, Arieni S, Møller AP, Saino N (2002a) The distribution and colony size of Barn swallow in relation to agricultural land use. J Appl Ecol 39:524–534

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Báldi A., Batáry P, Erdős S (2005) Effects of grazing intensity on bird assemblages and populations of Hungarian grasslands. Agric Ecosyst Environ 108:251–263

Bazzi G, Foglini C, Brambilla M, Saino N, Rubolini D (2014) Habitat management effects on Pre-alpine grassland bird communities. Ital J Zool 82:251-261

Evans DM, Redpath SM, Evans SA, Elston DA, Dennis P (2005) Livestock grazing affects the egg size of an insectivorous passerine. Biol Lett 1:322–325

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Evans DM, Villar N, Littlewood NA, Pakeman RJ, Evans SA, Dennis P, Skartveit J, Redpath SM (2015) The cascading impacts of livestock grazing in upland ecosystems: a 10-year experiment. Ecosphere 6:42

Evans KL, Wilson JD, Bradbury RB (2007) Effects of crop type and aerial invertebrate abundance on foraging Barn swallows *Hirundo rustica*. Agric Ecosyst Environ 122:267–273

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Hart JD, Milsom TP, Baxter A, Kelly PF, Parkin WK (2002) The impact of livestock on Lapwing *Vanellus vanellus* breeding densities and performance on coastal grazing marsh: even at very low stocking densities, livestock reduced breeding densities of adult Lapwings and increase the risk of nest loss due to predation. Bird Study, 49: 67–78

Henderson I, Holt C, Vickery J (2007) National and regional patterns of habitat association with foraging Barn swallows *Hirundo rustica* in the UK. Bird Study 54:371–377

Herzon I, Marja R, Menshikova S, Kondratyev A (2014) Farmland bird communities in an agricultural landscape in Northwest Russia: Seasonal and spatial patterns. Agric Ecosyst Environ 183:78–85

Kragten S, Reinstra E, Gertenaar E (2009) Breeding Barn swallows *Hirundo rustica* on organic and conventional arable farms in the Netherlands. J Ornithol 150:515–518

Laiolo P, Dondero F, Ciliento E, Rolando A (2004) Consequences of pastoral abandonment for the structure and diversity of the alpine avifauna. J Appl Ecol 41:294–304

Loe LE, Mysterud A, Stien A, Steen H, Evans DM, Austrhelm G (2007) Positive short-term effects of sheep grazing on the alpine avifauna. Biol

Lett 3:109–111

Lubbe SK, de Snoo GR (2007) Effect of dairy farm management on Swallow *Hirundo rustica* abundance in The Netherlands. Bird Study 54:176–181

Lühr D, Gröschel M. (2006) Das Vorkommen der Rauchschwalbe *Hirundo rustica* im Norden Bielefelds und dessen Zusammenhang mit verschiedenen Umweltfaktoren. Vogelwarte 44:229–232

Møller AP (2001) The effect of dairy farming on Barn swallow *Hirundo rustica* abundance, distribution and reproduction. J Appl Ecol 6:378–389

Orłowski G, Karg, J (2013) Partitioning the effect of livestock farming on the diet of an aerial insectivorous passerine, the Barn swallow *Hirundo rustica*. Bird Study 60:111–123

Romano A, Ambrosini R. Caprioli M, Bonisoli Alquati A, Saino N (2011) Secondary sex ratio covaries with demographic trends and ecological conditions in the Barn swallow. Evol Ecol 26:1041–1053

Sicurella B, Caprioli M, Romano A, Romano M, Rubolini D, Saino N, Ambrosini R (2014) Hayfields enhance colony size of the Barn swallow *Hirundo rustica* in northern Italy. Bird Conserv Int 24:17–31

Tryjanowski P, Jerzak L, Radkiewicz J (2005) Effect of water level and livestock on the productivity and numbers of breeding white storks. Waterbirds 28:378–382

Van den Brink B (2003) Hygienemaatregelen op moderne boerenbedrijven en het lot van Boerenzwaluwen *Hirundo rustica*. Limosa 76:109–116

Willi T, Körner–Nievergelt F, Grüebler MU (2011) Rauchschwalben *Hirundo rustica* brauchen Nutztiere, Mehlschwalben *Delichon urbicum* Nisthilfen. Orn Beob 108:215–224

Supplementary materials to chapter 7:

Common swift feather microbiota described by high throughput DNA sequencing

Federica Musitelli • Roberto Ambrosini • Mario Caffi • Manuela Carpioli
• Diego Rubolini • Nicola Saino • Andrea Franzetti • Isabella Gandolfi

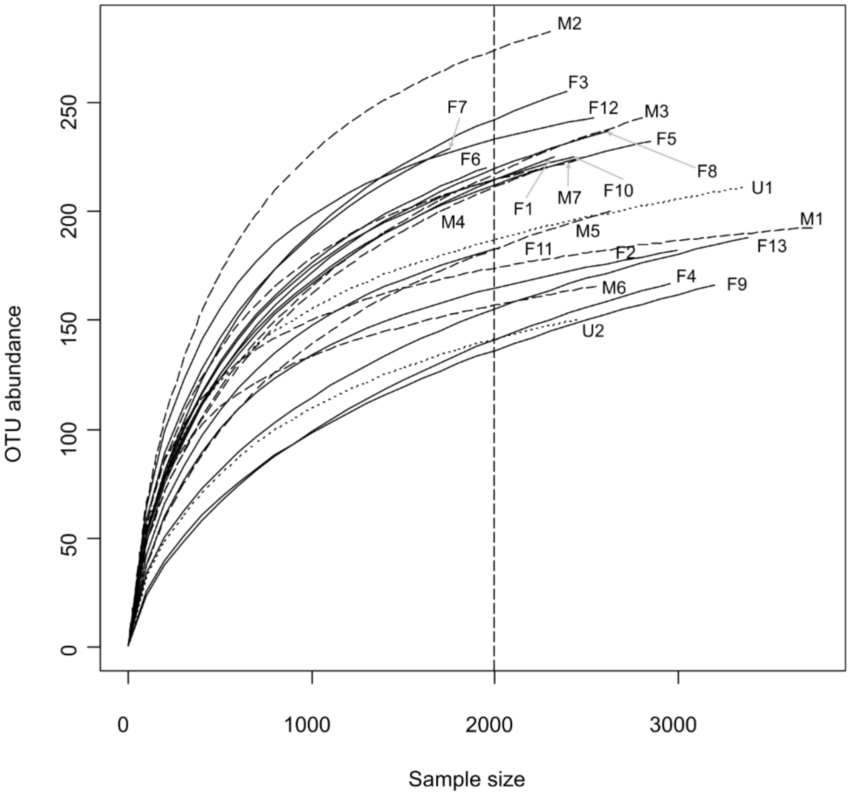

Figure S1. Rarefaction curves for the 22 feather samples. The dotted line indicates 2000 reads.

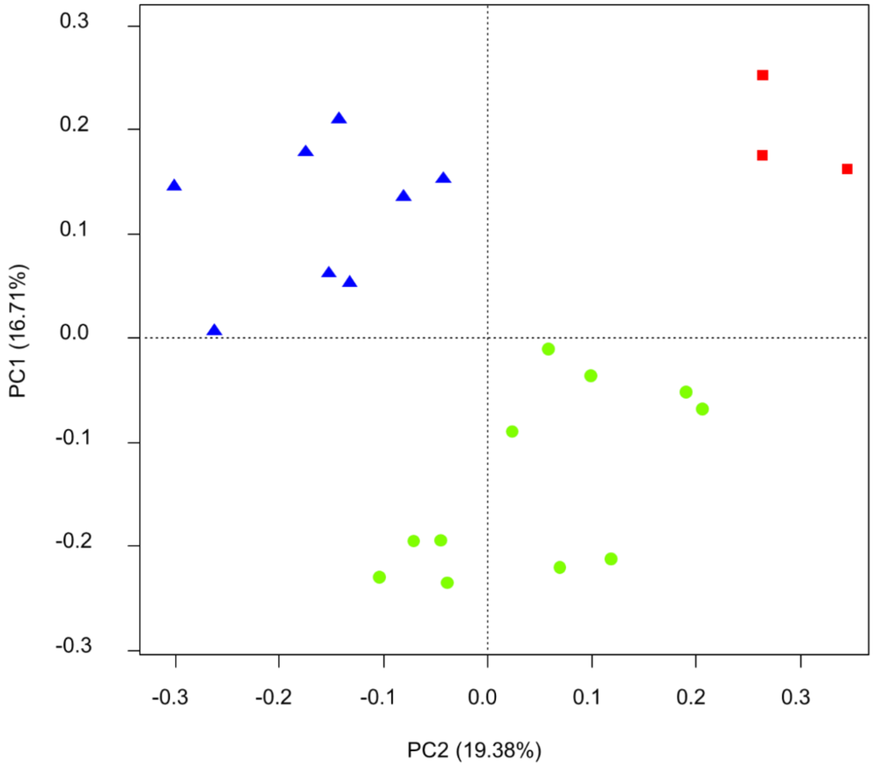

Figure S2. Biplot of the principal components from a PCA of the bacterial community of 22 feather samples. Each point represents the bacterial community of one bird. Blue triangles, red squares and green circles denote first, second and third cluster, respectively. The amount of variance explained by each axis is shown.

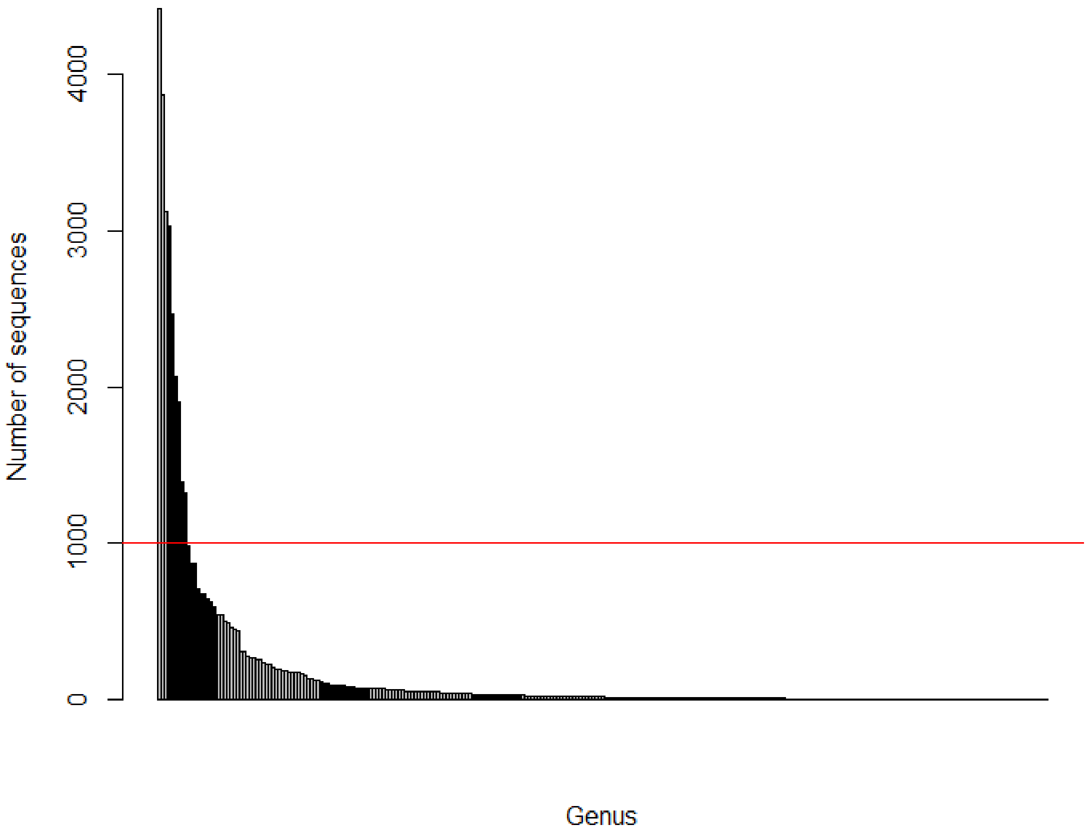

Figure S3. Barplot of the number of sequences that mapped onto each genus. The horizontal red line denote 1000 sequences.