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Roberto Ambrosini, Anders Pape Møller, Nicola Saino. A quantitative measure of migratory connectivity. *Journal of Theoretical Biology*, 2009, 257 (2), pp.203. 10.1016/j.jtbi.2008.11.019 . hal-00554539

HAL Id: hal-00554539

<https://hal.science/hal-00554539>

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Author's Accepted Manuscript

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PII: S0022-5193(08)00616-4
DOI: doi:10.1016/j.jtbi.2008.11.019
Reference: YJTBI5381

To appear in: *Journal of Theoretical Biology*

Received date: 12 February 2008
Revised date: 18 October 2008
Accepted date: 26 November 2008

Cite this article as: Roberto Ambrosini, Anders Pape Møller and Nicola Saino, A quantitative measure of migratory connectivity, *Journal of Theoretical Biology* (2008), doi:10.1016/j.jtbi.2008.11.019

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A quantitative measure of migratory connectivity

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Total word count: 7681

Abstract

The study of the extent of the connection between areas where populations of birds breed and areas where they winter has flourished in recent years mainly thanks to the development of new techniques, but also due to traditional ringing and recovery schemes, which allow tracking of individuals or populations linking wintering and breeding distributions. Currently, studies on migratory connectivity focus on retention of breeding population spatial structure on the non-breeding grounds and vice versa.

Here we propose a method to quantify migratory connectivity based on Mantel correlation coefficients and to statistically test for deviations of the observed connectivity from a random mix of individuals. In addition, we propose a procedure, based on clustering algorithms, to identify whether observed connectivity depends on aggregation of individuals or on rigid transference of distribution patterns between areas.

We applied this method to a large dataset of ringing recoveries of barn swallows (*Hirundo rustica* L.) migrating from their Western Palearctic breeding areas to sub-Saharan winter quarters. We show that migration of barn swallow populations connects specific breeding and wintering areas, and that the 'sub-populations' quantitatively identified by our method are consistent with qualitative patterns of migratory connectivity identified by studies of individual geographical populations based on other methods.

Finally, we tested the performance of the method by running simulations under different scenarios. Such simulations showed that the method is robust and able to correctly detect migratory connectivity even with smaller datasets and when a strong geographical pattern is not present in the population. Our method provides a quantitative measure of migratory

connectivity and allows for the identification of populations showing high connectivity between the breeding and wintering areas. This method is suitable for a generalized application to diverse animal taxa as well as to large scale analyses of connectivity for conservation purposes.

Keywords: barn swallow; *Hirundo rustica*; k-means clustering; Mantel test; migration;

Introduction

Migratory connectivity has been defined as the links between breeding and non-breeding areas of migratory animals due to migration (Webster et al 2002). Several different approaches have been proposed for the study of migratory connectivity, such as ringing recoveries (Anderson et al 2000), satellite radio telemetry (Cohn 1999), stable isotope analysis of feathers (e.g. Hobson and Wassenaar 1997; Marra et al 1998; Møller and Hobson 2003; Hobson 2005), genetic analysis (Webster et al 2002; Lopes et al 2006), and correlation analysis between point estimates of annual survival rates and proxies of ecological conditions putatively affecting survival (e.g. the Normalised Difference Vegetation Index; Szép et al 2006).

A qualitative measure of migratory connectivity has been proposed by Webster et al (2002), who argued that “strong” connectivity occurs when most individuals from one breeding population move to the same non-breeding location, while a “weak” or “diffuse” connectivity occurs when individuals from a single breeding population spread through several non-breeding grounds. Statistical approaches that have been proposed to quantify migratory connectivity are based on the same procedure that is commonly used to solve the problem of the “derivation of harvest” in waterfowl management (Marra et al 2006). They imply the definition of transition probabilities that describe how individuals from a breeding population move to each wintering location and the calculation of conditional probabilities of derivation of an individual from a given site (“origin probabilities”) using Bayes’ Rule (Marra et al 2006). This procedure, however, requires an *a priori* identification of ‘sub-ranges’ (see Webster et al 2002; Marra et al 2006), which may be a

difficult and somewhat arbitrary task, at least in species with continuous breeding and/or non-breeding ranges. In addition, estimating conditional probabilities in such Bayesian framework requires prior knowledge of population sizes, at least relative to each other, among the various origin sub-ranges (Marra et al 2006) that may be difficult to acquire unless information from intensive, large-scale population survey programs is available. Finally, recovery probabilities must be assumed to be proportional to the relative abundance on the origin grounds. If this assumption is unwarranted, true connectivity among sub-populations may be overlooked.

Currently, studies on migratory connectivity focus on the retention of breeding population structure on the non-breeding grounds (and vice versa) (Marra et al 2006). If individuals that breed close to each other also winter close to each other (*allohiemy* sensu Salomonsen 1955) then a strong connectivity occurs, while if they mix (*sinhiemy*) connectivity is weak. Theoretically, if the positions of individuals, both in the breeding and the wintering grounds are known, then two distance matrices can be calculated, representing the distance within each pair of individuals in the breeding or the wintering grounds, respectively. Besag and Diggle (1977) proposed to test for the significance of the maintenance of reciprocal distribution in breeding and wintering grounds in a blackbird (*Turdus merula* L) population by testing for the significance of the correlation between the matrix of distances in the breeding grounds and that in the wintering grounds. If individuals that breed close together also winter close together (i.e. the population shows strong connectivity), then a strong positive correlation is expected between the two matrices. Conversely, if individuals mix (weak connectivity) a weak correlation is expected. A measure of the correlation between the two distance matrices

can therefore be used to quantify migratory connectivity and to statistically test for the probability of observing a given pattern, under the null hypothesis of a random mix among individuals at the breeding and/or the non-breeding grounds, a condition for which a correlation coefficient of zero is expected. Such a test, known as the Mantel test (Sokal and Rohlf 1995), does not imply an a priori identification of separated sub-ranges in the breeding and wintering grounds. We emphasize that, in this approach, the length of the migration journey is not taken into account as the only measures involved in the analysis are distances between individuals in the breeding and in the wintering grounds.

A significant correlation between the distance matrices, however, can result from two different processes. The first consists of individuals that maintain the same reciprocal position in the breeding and wintering grounds. Following Besag and Diggle (1977) we refer to this process as to “distribution pattern transfer”. We note that the correlation between the distance matrices will not vary under translation, rotation and scale change of the pattern of distribution of individuals. Alternatively, if the population under study consists of groups of individuals that stay close together both in the breeding and in the wintering grounds, then a significant correlation between the distance matrices is also expected, as within-group distances will, on average, be smaller than distances between individuals of different groups.

A procedure for the quantitative study of migratory connectivity should ideally allow disentangling these two processes and, if necessary, to identify the clusters of individuals that breed and winter together. These goals can be achieved by applying a clustering algorithm to a distance matrix obtained from the combination of the two matrices of distances between individuals at the breeding grounds and in the winter

quarters. A significant connectivity in a non-structured (i.e. non-clustered) population indicates that migratory connectivity arises from a process of distribution pattern transference. Conversely, if cluster analysis reveals a structure in the population, then grouping of individuals that always stay together contributes to migratory connectivity. Within (main) clusters, individuals may then show pattern transference or further grouping, resulting in sub-clusters.

In this study, we thus propose a novel approach to quantify migratory connectivity. The method is based on Mantel test to assess the strength of migratory connectivity and cluster analysis to investigate the process that generates it and to unravel the clustered structure of the population.

We first assessed the performances of the method by running simulations under three different scenarios. In the first scenario, the connectivity was due to a process of distribution pattern transference. In the second, connectivity arose from the clustered structure of the simulated population, but no pattern transference occurred for the individuals of the same cluster. In the third, we simulate a condition where both processes occur simultaneously. In addition, to exemplify the use of this method, we apply it to a large dataset of recoveries of barn swallows (*Hirundo rustica* L) ringed in their breeding range in Europe and recovered in their sub-Saharan wintering quarters or vice versa.

Data from recoveries of ringed birds as a tool in the study of connectivity have been criticized on several bases, such as the large biases in the probability of recovery of individuals between different parts of the world (Webster et al 2002). Still, they represent the largest (and largely under-utilised) datasets currently available on numerous migratory species. The results obtained from the analysis of the present dataset should

therefore be considered with this *caveat* in mind, and they will be compared to other available information about the migration of barn swallows, including stability of the genetic variance-covariance matrix of different populations (Roff et al 2004) and stable isotope analyses of feathers of different populations (Evans et al 2003; Møller and Hobson 2003).

The barn swallow is a small passerine bird that migrates each year from Europe to Africa south of the Sahara desert. A considerable effort has been spent in the last decades to capture and ring barn swallows both in Africa and Europe mainly within the EURING and SAFRING ringing schemes. Recoveries and recaptures of barn swallows are available from the entire breeding and wintering range of the species, encompassing an extremely wide geographical area and a very long time span. To the best of our knowledge, this is one of the largest available data sets of ringing recoveries and we are convinced that the amount of information it contains largely overcomes the ‘noise’ due to differences in ringing and capture efforts in different locations and years.

Materials and Methods

The method we are proposing is based on the coordinates of individuals recorded in the areas occupied in different phases of the annual cycle. We assume that such grounds are geographically separated and individuals move annually between them during migrations. Since the large majority of the data about migrants come from ornithological studies, we will refer to these areas as the breeding and the wintering grounds, albeit these definitions may not be strictly applicable to other migrants (e.g. whales, for which ‘breeding’ and ‘feeding’ grounds may be more appropriate). In all the analysis, including the simulations, we always started from datasets where the position of individuals was expressed by geographical coordinates, since in real datasets position of individuals is usually recorded in this way. Nevertheless, this method is applicable to any other coordinate system as it basically requires only distances between individuals.

Analysis of migratory connectivity

From geographical coordinates of individuals both in the breeding and wintering grounds, we calculated two matrices of orthodromic distances (i.e. the minimum path on the surface of the Earth), between all pairs of barn swallows both in the breeding and the wintering quarters (see Fig. 1 for further details).

Mantel correlation coefficient (r_M) was used to test for the correlation between orthodromic distance matrices. It corresponds to a simple Pearson product moment correlation coefficient between the two matrices, whose significance is assessed with a

randomization procedure because distances between individuals within each distance matrix are not statistically independent (Sokal and Rohlf 1995). We randomly permuted the position of individuals at the breeding grounds 999 times; for each permutation, a distance matrix was calculated and its correlation coefficient with the actual distance matrix of individuals at the wintering grounds was calculated. The significance of the observed Mantel coefficient was determined based on its rank in the set of the coefficients from the randomization procedure as $P = [(1000 - \text{rank})/1000]$.

The analysis of the spatial structure of the dataset was based on a cluster detection approach. First, we combined the two distance matrices into one by computing, for each pair of individuals, the square root of the sum of their squared orthodromic distances at the breeding and wintering grounds. This measure can be considered an overall distance index similar to the Euclidean distance between two points that also takes the round shape of the Earth into account as it is based on orthodromic distances.

Cluster detection was then performed with the *pam* algorithm in S-Plus 4.5 applied to the overall distance indices matrix. The number of clusters was identified as the number that maximized the *overall average silhouette width* (hereafter *oasw* for brevity) (Rousseeuw 1987). The *pam* procedure (fully described in Kaufman and Rousseeuw 1990) is a partitioning clustering algorithm where the number of clusters must be indicated *a priori*. The *oasw* is a measure of the goodness of the overall classification of points in a given number of clusters. It is a dimensionless coefficient of the extent of structure of clustering that has been identified. It is defined as the average value of the *silhouettes* $s(i)$ computed over all objects in the dataset where

$$s(i) = \frac{b(i) - a(i)}{\max\{a(i); b(i)\}}$$

206
 207
 208 $a(i)$ being the average dissimilarity of i to all other objects of the cluster to which i
 209 belongs and $b(i)$ the average dissimilarity of i to all objects of the nearest cluster to which
 210 i does not belong. $s(i)$ values range between -1 and 1 and the larger the value the better
 211 the classification of an object in a given cluster (Rousseeuw 1987). The best number of
 212 clusters corresponds to the number that maximizes the *oasw*. *Oasw* values lower than
 213 0.25 indicate that no substantial structure has been found in the data, values between 0.26
 214 and 0.50 indicate a “weak” structure, between 0.51 and 0.70 a “reasonable” structure and
 215 values larger than 0.71 a “strong” structure (Rousseeuw 1987). Each cluster detected in
 216 the first step was investigated further with the same procedure, and, if a significant
 217 structure was recognized, it was divided into sub-clusters, numbered as (dataset).(main
 218 cluster).(sub-cluster).

219 220 *Simulations*

221
 222 In all simulations we assumed a uniform spherical surface with no limits to the movement
 223 of individuals. The centre of the cluster(s) of individuals in the breeding grounds were
 224 always set at 30° N, while at 30° S in the wintering grounds. Longitude of the cluster
 225 centre(s) varied during simulations (see below). Positions of individuals were generated
 226 as a random determination from a bivariate Gaussian distribution with unit standard
 227 deviation (SD) centred on the centre(s) of the cluster(s). The distance between cluster
 228 centres and the random noise added to individual positions (see below) were expressed as

standard deviations (SD) of the bivariate Gaussian used to generate individual position within clusters. At each step of the simulation, all the analyses were run according to the method described above, and the significance of the Mantel correlation coefficient, the *oasw* value, the number of clusters identified and the proportion of individuals correctly classified were recorded.

The first simulation aimed at testing the ability of the method we propose to detect migratory connectivity arising from a process of distribution pattern transference (Besag and Diggle 1977). One cluster with 200 individuals was generated in the breeding ground. In a process of pure pattern transference, the reciprocal position of individuals in the wintering grounds should strictly reflect that in the breeding grounds except for random variation. This process was simulated by generating positions in the wintering grounds corresponding to those in the breeding grounds plus a bivariate normal random noise added to the position of each individual. At each step of the simulation, the standard deviation of the noise increased by 0.1 SD from 0 to 4 SD. Hence, this simulation was run 41 times.

The second simulation aimed at testing the ability of the method to detect migratory connectivity arising from a clustered structure in the population. Two clusters of 100 individuals were generated. The position of individuals within each cluster both in the breeding and in the wintering grounds was randomly determined. Hence, no pattern transference existed within each cluster. At each run of the simulation, the distance between cluster centres in the wintering and/or in the breeding grounds increased by 1 SD from 0 (i.e. clusters superimposed both in the breeding and in the wintering grounds) to 10 SD, so that this simulation was run 121 times.

The third simulation investigated a situation when both clustering and pattern transference occurred. This third simulation followed the general scheme of the second, involving two clusters of individuals with increasing distance between cluster centres, but, as in the first simulation, the reciprocal position of individuals within clusters in the wintering grounds reflected that in the breeding grounds plus a random Gaussian noise with $SD = 0.2$. This value was arbitrarily chosen as the first simulation demonstrated that the amount of noise does not influence the performance of the method (see below).

The barn swallow dataset

The whole dataset consisted of recoveries of individually marked barn swallows between 1911 and 1998. Only first recoveries were selected in order to avoid pseudo-replication. Individuals breeding east of longitude 60° E (approximately corresponding to the Ural Mountains) were excluded. A first dataset (hereafter called “All” for brevity) of 1103 barn swallows included all individuals that were within the breeding range of the species between April and September and within the wintering range between October and March. We then quantitatively assessed the potential noise arising from recoveries of individuals during migration by selecting a second dataset (“FP”) of 225 barn swallows that were within the breeding range in May-June and within the winter quarters in December-February, i.e. in the focal periods of reproduction and wintering, respectively. Natal dispersal in the barn swallow is larger than breeding dispersal (Turner 1994). This may bias the results if the proportion of ringed adults and young varies geographically. In addition, in Africa a large number of barn swallows was ringed in a rather small number

of sites so that cluster detection could be biased by the small distance between ringing sites of many individuals. Hence, a third dataset (“AE”) was selected, only including barn swallows ringed in Europe as adults. Finally, the two previous selection criteria were combined to obtain a fourth restricted dataset (“R”) of 71 barn swallows ringed as adults in Europe in May-June and recovered in Africa in December-February. We then compared the results of the analyses based on the four datasets by investigating consistency of classification of individuals into clusters.

Results

Simulations

In all the 41 runs of the first simulations, a highly significant Mantel correlation coefficient was found ($r_M < 0.001$ in all cases). No cluster structure was present in the data and therefore classification of individuals into clusters was not relevant.

Fig. 2 summarizes the results of the second simulation. In 14 cases, the method failed to detect connectivity, i.e. both the Mantel correlation coefficient was not significant and the *oasw* value was lower than 0.5. In all these cases, the distance between cluster centres in one area was 0 or 1 SD. Clusters generated as random determination from a bivariate Gaussian are roughly circular and about 90% of individuals lie within 2 SD from the centre. Hence, a distance of 1 SD between cluster centres implies that at least 60% of individuals lie in the common area. We note that no within-cluster pattern transference occurred in this simulation, so that non-significant Mantel coefficients were expected when clusters were close together. In another 14 cases the *oasw* value did not indicate a reasonable structure in the data, but the Mantel coefficient was significant. In all these cases the two clusters partly overlapped at least in one area. However, also in these cases more than 90% of individuals were correctly classified in two clusters.

Mantel correlation coefficient was significant in all the 121 runs of the third simulation. In 24 cases, always with partly overlapping clusters, *oasw* values did not reveal a reasonable structure in the data albeit only in 10 cases cluster analysis correctly classified less than 90% of individuals.

Barn swallow dataset

The Mantel correlation coefficient for the “All” dataset was 0.0247 (Fig. 3a). In the randomization procedure we obtained 54 times a correlation coefficient larger than the observed one. Migratory connectivity should therefore be considered marginally non-significant ($P = 0.054$). However, the *oasw* value suggested a “reasonable” structure with two clusters that also emerged from the analysis of the data subsets (see below). For this reason, we identified two clusters that are shown in Fig. 4a and pictorially in Fig. 5a. The corresponding silhouette plot is shown in Fig. 6. Mean orthodromic distance between individuals in Europe was 1358.16 (941.673 SD) km (range 0-5400.34 km) while in Africa it was 1493.67 (1553.880 SD) km (range 0-6192.57 km). Hence, distances in both ranges were similar, and the pattern of distribution in the two ranges had similar effects on cluster detection and composition. Cluster All.1 mainly included barn swallows breeding in South-Western Europe and wintering from Liberia to Uganda, while cluster All.2 was mainly constituted of barn swallows from Northern Europe that winter south of the Equator, from Zaire to South Africa. The clusters partly overlap at the breeding grounds while they are well separated in the wintering grounds.

Mantel tests showed a significant migratory connectivity for cluster All.2 and a marginally non-significant connectivity for cluster All.1 that, however, showed an *oasw* of 0.433 (i.e. reasonably high) (Fig. 3). We therefore analyzed the structure of both clusters (see also Discussion) that could be divided into two sub-clusters each (All.1.1, All.1.2, All.2.1 and All.2.2, pictorially shown in Fig. 5b). In the next step of the analysis, only clusters All.1.2 and All.2.1 could be further divided in two and six third-level sub-clusters, respectively. However, the results at this fine level were inconsistent between data subsets (see below), and they are therefore not presented in detail.

The “FP” subset showed a significant connectivity ($n = 255$, $r_M = 0.0701$, $P = 0.005$). The *oasw* reached its maximum value ($= 0.582$) for two clusters, thus indicating a reasonable structure in the data. At the second step of analysis, cluster FP.1 could be divided into two second-level sub-clusters based on *oasw* value ($n = 32$, $r_M = 0.0768$, $P = 0.140$, $oasw = 0.484$), while cluster FP.2 was significantly structured with two sub-clusters ($n = 223$, $r_M = 0.0670$, $P = 0.002$, $oasw = 0.510$), none of which could be further subdivided (details not shown). These results are consistent with the results of the analysis of the “All” set of data. Indeed, all individuals classified in cluster FP.1 had been classified in cluster All.1 and all individuals in FP.2 had been classified in All.2. At the second level of analysis, only 3 individuals were misclassified.

The details of the results of the analysis of the “AE” set of barn swallows are shown in Fig. 3b. The individuals showed significant connectivity and could be grouped into two clusters that, in turn, were both structured in two sub-clusters. Sub-clusters AE.1.1 and AE.1.2 showed non-significant connectivity, while AE.2.1 and AE.2.2 were structured and could be divided into two and four third-level sub-clusters, respectively (details not shown). However, as we did for the “All” dataset, we cautiously considered the results only to the second step of the analysis. The results obtained from this selection criterion were highly consistent with the results of the analysis of the “All” dataset at the first level of analysis, and at the second level for cluster AE.1. Indeed only 3 out of the 86 individuals classified in AE.1 were classified in All.2, and 3 individuals of AE.1.2 were classified in All.2.1. The partitioning of cluster AE.2 was not consistent with that obtained from the “All” dataset. This was due to the small number of barn swallows (32) in cluster All.2.1 that were ringed in Europe as adults and thus included in the “AE”

subset.

The most restricted subset of data (“R”) only included 71 barn swallows. The Mantel test indicated a significant connectivity ($r_M = 0.2243$, $P < 0.001$), and the *oasw* value (= 0.646) showed that it could be divided in two sub-clusters. The first cluster could be divided in two sub-clusters according to *oasw* value (R.1: $n = 32$, $r_M = 0.0768$, $P = 0.155$, $oasw = 0.484$) while the second was not structured (R.2: $n = 39$, $r_M = -0.0371$, $P = 0.555$, $oasw \leq 0.367$). All individuals in R.1 had previously been classified in All.1 and all individuals in R.2 in All.2. Consistent results emerged also at the second level with no individual being misclassified.

Thus, the four levels of selection of the dataset, despite differing in composition and number of individuals, led to highly consistent clustering of individual barn swallows, whereby the large majority of individuals were assigned to corresponding clusters in the different analyses.

These largely consistent results could arise because of the geographical structure that the barn swallow population showed in the winter quarters, with two latitudinally well-separated main clusters. To further investigate the robustness and the generality of our method we then re-ran the first level analysis on a partly simulated dataset. We assigned to barn swallows classified in cluster All.2 a new position in the wintering ground by adding to their actual latitude the difference in latitude between the centres of clusters All.1 and All.2, while leaving unchanged longitude in the wintering grounds as well as position in the breeding area (see Fig. 5). This partly de-structured our dataset. Mantel correlation coefficient for this partly simulated dataset increased to 0.0519, $P = 0.002$. The cluster algorithm identified three rather than two first level clusters (*oasw* =

0.5067), which however corresponded to cluster All.1, All.2.1 and All.2.2, respectively, with only 31 out of 1103 individuals being misclassified. Hence, despite the weaker geographical structure, our method was able to detect the cluster structure present in the data.

Accepted manuscript

Discussion

In this study, we propose a novel approach to quantitatively and objectively investigate migratory connectivity, which can be applied to any organism where clearly distinct geographical ranges, corresponding to different phases of the life cycle, can be identified. We also propose a method to quantify migratory connectivity based on the Mantel correlation coefficient and a method to distinguish between two processes that can generate the observed connectivity, *i.e.* distribution pattern transfer *versus* clustering of individuals. In the event of clustering, we also propose a method to identify ‘sub-populations’ of individuals that tend to associate during the two phases of their life cycle (e.g. breeding and wintering).

The number of studies focusing on ‘migratory connectivity’ has been increasing rapidly in recent years, mainly due to an expanding set of techniques, but also due to new analyses of data from ‘traditional’ mark-recapture methods, allowing identification of breeding and wintering areas of several bird species. To date, however, quantitative methods to estimate migratory connectivity have been based on the calculation of origin probability of individuals from *a priori* identified geographical populations or areas. These methods do not test statistically for the deviation of the observed patterns of reciprocal distribution of individuals from a random mix. The method we propose, which is based on relatively simple calculations and statistics, may thus contribute to fill this gap.

A prominent feature of our approach is that the investigation of migratory connectivity is not based on an *a priori* identification of breeding and wintering ‘sub-

ranges', which may be difficult because several species show continuous distributions both in the breeding and the wintering quarters. We propose that the identification of highly connected ranges should be based on the actual distribution pattern of individuals. This has the double advantage of reducing the subjectivity of the analysis and increasing its biological realism by relying on inherent patterns present in the data.

In addition, we emphasize that our approach subtly differs from that based on the *a priori* identification of separate sub-ranges. In fact, following that approach, the level of connectivity would appear to differ depending on which of the different breeding and wintering populations is currently under focus. Imagine, for example, a single group (population) of individuals that breeds in an area (B) and winter in two well separated areas (W1 and W2). B would be defined as moderately connected to either W1 or W2, whereas both W1 and W2 would be defined as strongly connected to B. Thus, an asymmetry is implicit in that approach, with a larger relevance usually given to the breeding areas. Our approach solves this problem as equal relevance is given to each distance matrix, and number and composition of groups of individuals is assessed *a posteriori* by means of cluster analysis. In our approach, identification of sub-ranges and highly connected areas follows from the identification of clusters of individuals that connect regions by means of their migration.

Some of the features of our method deserve close consideration. First, the method we used to combine the two distance matrices into the overall Euclidean distance matrix implies that, for a given distance between two data-points in one range, their chances to be classified in the same cluster declines as their distance in the other range increases. This is desirable because, according to the symmetric approach that we are proposing

(see above), only individuals that tend to both breed and winter together should be classified into the same group. Second, the clustering procedure we chose involves the comparison of the clustering efficiency, based on the *oasw*, when data are forced into 2, 3, ..., n clusters and the univocal identification of the best number of clusters. As a drawback of the method, however, if the pattern of distribution of sampling locations varies markedly between the breeding and the wintering areas, some clusters may be obscured and forced by the clustering algorithm to join with other clusters. Finally, high connectivity does not imply, *per se*, a highly clustered population. If, for example, uniformly dispersed individuals in the breeding areas tend to maintain the same position, relative to the other individuals, in the wintering areas (i.e. in the case of distribution pattern transfer), the connectivity will be high even in the absence of a clear grouping of individuals. Thus, our approach allows inferring connectivity arising from distribution pattern transfer when the Mantel correlation coefficient is significant, but the whole population cannot be partitioned into well-defined clusters.

The simulations we run under three different scenarios give further insights into the interpretation of the results and the robustness of this method. First of all, the Mantel correlation coefficient is able to detect connectivity due to distribution pattern transference even when a large random noise is added to the data. This clearly emerges from the results of the first and the third simulations. In the second and third simulation, *oasw* values lower than the suggested threshold of 0.5 were recorded in several runs where a cluster structure could then be identified, as indicated by the fact that the same clustering algorithm was able to detect the correct number of clusters and to correctly classify more than 95% of individuals in all but 18 simulations of the second and third

type. In all cases where the method failed to detect connectivity, the distance between cluster centres was not larger than 3 SD in one area and not larger than 2 SD in the other, implying that the grouping structure was obscured by the fact that about 13% of individuals mixed in one ground and about 60% in the other (details of this calculations are not shown for brevity). On the other hand, Mantel correlation coefficients seemed rather sensitive in detecting a structure in a population also when it arose from grouping of individuals. Based on these results, we suggest to always investigate the cluster structure of a population when the Mantel test is significant, and to use the value of the Mantel correlation coefficient as a measure of migratory connectivity. In addition, we tentatively suggest to investigate the structure of a group of individuals when the *oasw* is larger than 0.4, since, based on our simulations, the suggested threshold of 0.5 may be too high. We admit, however, that further investigation is needed to identify an optimal threshold for the *oasw* value.

Our method can be widely applied to different kinds of data about migratory birds (and other migratory animals). Indeed, this method of analysis is applicable to any pair of matrices of distance indices between individuals and not to geographical distances only. For example, a large number of studies about migratory connectivity is based on the analysis of the isotopic composition of feathers (see e.g. Hobson 2005). Differences in the isotopic composition are considered to be related to the distance in the locations where individuals moult their feathers at least in areas where specific geographical gradients in isotopic abundance exist (Hobson 2005). Hence, a measure of the migratory connectivity for a population can be obtained by correlating the matrix of geographic distances between places where individuals were captured and the matrix of differences

in the isotopic composition of their feathers. During moult, birds simultaneously wear both recently moulted and old feathers when in the wintering or in the breeding areas. The two matrices derived from isotopic profiles of newly moulted and old feathers should be readily suitable for the analyses proposed here. Likewise, our method could be applied to a matrix of isotopic profiles produced in winter and a second matrix of genetic markers for the same individuals reflecting the population genetic structure of individuals at the breeding grounds. We emphasize, however, that our method could be applied to distances in the isotopic profiles and/or genetic distances provided that they are univocally related to geographic distances between individuals. For example, deuterium values in North America vary along a latitudinal gradient and were therefore used to study migration of birds moving in a north-south direction (Hobson 2005), but this may not be the case for other areas or other isotopes, like ^{13}C and ^{15}N in Africa, which provide habitat-specific, rather than geographic area-specific markers (Møller and Hobson 2003), so that distance in isotopic composition may not reflect true geographic distance between individuals. As concerns genetic distances, these usually fulfil the criterion because isolation by distance is a common feature of such distances.

We applied our method to a large ringing and recovery dataset of a Palearctic migratory bird species that has been subject to intensive ringing programmes. We found that the Western Palearctic breeding population of barn swallows is structured into two main 'sub-populations'. The first population breeds in South-Western Europe and winters in an elongated belt from Liberia to Uganda, while the second population breeds in Northern Europe and winters south of the Equator. A second level of analysis showed that both main groups could be split into two sub-groups. Barn swallows breeding in

South-Western Europe could be divided according to segregation in the wintering quarters with a first sub-group including barn swallows wintering from Liberia to Cameroon and a second including barn swallows that winter from Cameroon to Uganda. Conversely, the second main group can be divided according to segregation in the breeding grounds, with a first sub-cluster that includes barn swallows mainly breeding in North-Eastern Europe and a second group including barn swallows that breed in North-Western Europe (Fig. 5b).

This pattern was identified based on the entire dataset (1103 individuals), although the analyses were possibly confounded by the fact that either breeding and wintering ringing or recovery could partly refer to migrating individuals. In addition, differences in ringing effort at each ringing location may skew the results as the number of ringed birds recovered at each location can be considered proportional to capture effort. This problem could be exacerbated by the fact that larger ringing effort may be devoted in areas with higher bird densities. Unfortunately, no quantitative measure of ringing effort was available so it was impossible to correct for this potential bias. However, the analyses based on different subsets of data identified according to increasingly conservative criteria gave largely consistent results to those based on the entire dataset, suggesting that the analysis was robust to the interference of different potential sources of bias and even sample size. However, for several bird species less than 200 recoveries may be available and small sample size may reduce the power of the tests. Further theoretical as well as simulation studies are therefore needed to assess the effect of sample size on the detection of migratory connectivity. However, some preliminary results indicate that this method is robust when at least 35 recaptures are

517 available (R. Ambrosini, unpublished results). In addition, we emphasize that our main
518 goal was to propose a method to quantify migratory connectivity, and that this large set of
519 real data was only used to exemplify its application.

520 Despite these potential drawbacks, the results of the present analysis of
521 connectivity can be qualitatively compared to the results obtained using different
522 approaches. The subdivision of the Western Palearctic barn swallow population in two
523 main sub-populations is roughly consistent with the observation of differences in the
524 quantitative genetic variance-covariance matrix (G-matrix) between barn swallows from
525 Northern and Southern Europe (Roff et al 2004). In addition, in an analysis of the isotopic
526 composition of feathers, Evans et al (2003) identified segregation in the wintering
527 quarters of barn swallows breeding in England and Switzerland. This is consistent with
528 our results, as British barn swallows are mainly classified in a group that winters in
529 Southern Africa, while Swiss barn swallows were included in the cluster wintering north
530 of the Equator. Moreover, stable isotope composition of feathers of barn swallows
531 indicates that birds that breed in Denmark winter in at least two different areas with
532 different levels of $\delta^{15}\text{N}$ (Møller and Hobson 2003). This is consistent with our results,
533 since barn swallows breeding in Denmark were classified partly in the first and partly in
534 the second first-level clusters, whose African wintering grounds are separated. High $\delta^{15}\text{N}$
535 values in willow warbler (*Phylloscopus trochilus* L) feathers sampled in South Africa
536 was reported by Bensch et al. (2006). If we assume that isotopic profiles in barn swallow
537 feathers are not dissimilar to those in the willow warbler, we can go further and
538 tentatively suggest that $\delta^{15}\text{N}$ enriched cluster of Møller and Hobson should correspond to
539 cluster All.2 and the $\delta^{15}\text{N}$ depleted one to cluster All.1.

These results suggest that analyses of stable isotopes or other markers of group identity of barn swallows should provide evidence of clusters similar to those reported in Fig. 4, both when based on captures in the winter quarters in Africa or at the breeding grounds in Europe. Hence, the connectivity pattern we found based on ringing recoveries is largely consistent with the picture provided by studies of individual breeding populations based on different approaches. In addition, although the identification of a structure of the barn swallow population under focus is not novel, our analysis provides a first quantitative measure of migratory connectivity.

In conclusion, we have proposed a quantitative measure of migratory connectivity and a method for identification of ‘sub-populations’ that are amenable to inferential statistical analysis and open the possibility of quantitatively investigating the relationships between different areas due to the movement of migrants between them. This measure can be quickly calculated for several species as large sets of data from recoveries are already available at least for the most common species, and datasets on genetic and isotopic distances between populations are rapidly growing. In addition, a quantitative measure of migratory connectivity allows the comparison of migration strategies of different species and/or populations and thus allows further investigation of the ecological bases of the evolution of migratory systems. Finally, this method allows estimates of migratory connectivity between geographic regions based on assemblages of species that migrate between them. Such a measure may provide a substantial contribution in the planning of effective conservation strategies for migratory species.

Acknowledgments

EURING, SAFRING and numerous ringing centres in Europe kindly provided access to their recoveries of ringed barn swallows. We are grateful to Dr. Riccardo Borgoni and to two anonymous referees whose comments greatly improved the quality of the manuscript.

Accepted manuscript

References

- Anderson, Å., Follestad, A., Nilsson, L., Persson, H., 2001. Migration patterns of Nordic Greylag Geese *Anser anser*. *Ornis Sve.* 11, 19-58.
- Besag, J., Diggle, P. J., 1977. Simple Monte Carlo tests for spatial pattern. *Appl. Stat.* 26, 327-333.
- Bensch, S., Bengtsson, G., Åkesson, S., 2006. Patterns of stable isotope signatures in willow warbler feathers collected in Africa. *J Avian Biol*, 37, 323-330., doi: [10.1111/j.2006.0908-8857.03628.x](https://doi.org/10.1111/j.2006.0908-8857.03628.x)
- Cohn, J. P., 1999. Tracking wildlife: high-tech devices help biologists trace the movement of animals though sky and sea. *BioScience* 49, 12-17.
- Evans, K. L., Waldron, S., Bradbury, R. B., 2003. Segregation in African wintering ranges of England and Swiss Swallow *Hirundo rustica* populations: a stable isotope study. *Bird Study* 50, 294-299.
- Hobson, K. A., Wassenaar, L. I., 1997. Linking breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia* 109, 142-148., doi: 10.1007/s004420050068

- Hobson, K. A., 2005. Stable isotopes and the determination of avian migratory connectivity and seasonal interactions. *Auk* 122, 1037-1048.
- Hooge, P. N., Eichenlaub, B., 2000. Animal movement extension to Arcview. Ver. 2.0. - Alaska Science Centre - Biological Science Office, U.S. Geological Survey, Anchorage, AK, USA. Available from: <http://www.absc.usgs.gov/glba/gistools/index.htm> (accessed July 2007)
- Jennrich, R. I., Turner, F. B., 1969. Measurement of non-circular home range. *J. Theor. Biol.* 22, 227-237.
- Kaufman, L., Rousseeuw, P. J., 1990. Finding Groups in Data: An Introduction to Cluster Analysis. Wiley, New York.
- Lopes, R. J., Marques, J. C., Wennerberg, L., 2006. Migratory connectivity and temporal segregation of dunlin (*Calidris alpina*) in Portugal: evidence from morphology, ringing recoveries and mtDNA. *J. Ornithol.* 147, 85-94. 10.007/s10336-005-0048-y.
- Marra, P. P., Hobson, K.A., Holmes, R.T., 1998. Linking winter and summer events in a migratory bird by using a stable-carbon isotopes. *Science* 288, 2040-2042., doi: 10.1126/science.282.5395.1884
- Marra, P.P., Norris, D.R., Haig, S.M., Webster, M., Royle, J.A., 2006. Migratory

- connectivity. In: Crooks, K. R., Sanjan, M. (Eds) Connectivity Conservation. Cambridge University Press, Cambridge, pp 157-183.
- Marshak, S., 2001. Earth: Portrait of a Planet. W. W. Norton & Company, New York.
- Møller, A. P., Hobson, K.A., 2003. Heterogeneity in stable isotopes profiles predicts coexistence of populations of barn swallows *Hirundo rustica* differing in morphology and reproductive performance. Proc. R. Soc. B. 71, 1355-1362., doi: 10.1098/rspb.2003.2565.
- Roff, D. A., Mousseau, T., Møller, A. P., de Lope, F., Saino, N., 2004. Geographic variation in the G matrices of wild populations of barn swallow. Heredity 93, 8-14., doi: 10.1038/sj.hdy.6800404
- Rousseeuw, P. J., 1987. Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. J. Comput. App. Math. 20, 53-65.
- Salomonsen, F., 1955. The evolutionary significance of bird migration. Biologiske Meddelelser 22, 1-62.
- Sokal, R. R., Rohlf, F. J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edn. Freeman, New York
- Szép, T., Møller, A. P., Piper, S., Nuttall, R., Szabó, Z. D., Pap, P. L., 2006. Searching for

636 potential wintering and migration areas of a Danish Barn Swallow population in South

637 Africa by correlating NDVI with survival estimates. *J. Ornithol.* 147, 245-253., doi:

638 10.1007/s10336-006-0060-x

639
640 Turner, A. K., 1994. *The swallow*. Hamlyn, London.

641
642 Webster, M. S., Marra, P. P., Haig, S. M., Bensch, S., Holmes, R. T., 2002. Links

643 between worlds: unravelling migratory connectivity. *Trends Ecol. Evol.* 17, 76-83., doi:

644 10.106/S0169-5347(01)02380-1.

Figure legends

Figure 1. Calculation of orthodromic distance between points A and B along a spherical surface. Geographical coordinates (latitude = ϕ and longitude = λ) are first converted into three-dimensional Cartesian coordinates (x, y, z) with Eq. 1-3 in figure. r is the average radius of the Earth (6371 km; Marshak 2001). Euclidean distance (d) between the points is easily calculated from Cartesian coordinates by means of Pythagoras' theorem and then converted into orthodromic distance (l) with Eq. 4.

Figure 2. Results from the second simulation. *Oasw*: overall average silhouette width. Gray surface colour indicates a simulation with a significant ($P \leq 0.05$) Mantel correlation coefficient. Black surface colour represents non-significant simulations. Plans represent *oasw* values of 0.5 and 0.7 respectively. Distances between cluster centres are expressed as standard deviations (SD) of the bivariate Gaussian used to generate individual position within clusters.

Figure 3. Dendrogram of the analysis (a) of the whole set of recoveries and recaptures of barn swallows (All) and (b) of the subset of data only including barn swallows ringed in Europe as adults (AE). r_M : Mantel correlation coefficient; P : significance of the Mantel test as assessed with the randomization procedure; *oasw*: overall average silhouette width.

Figure 4. First level clusters for different selection criteria. a) All barn swallows . b)

Barn swallows recorded in the breeding and wintering grounds in the focal periods of reproduction and wintering respectively. c) Barn swallows ringed in Europe as adults. d) Barn swallows ringed in Europe as adults in the focal period of reproduction and recovered in Africa in the focal period of wintering.

Figure 5. Pictorial representation of first (a) and second (b) level clusters. Ellipses represent the Jennrich and Turner (1969) Bivariate Normal Home Range calculated with the Animal Movement extension to ArcView Gis (Hooge and Eichenlaub 2000). Dashed ellipses in (a) represents the simulated position of cluster All.2 used to assess the robustness of the method with a less geographically structured dataset.

Figure 6. Silhouette plot showing the classification of the All dataset in two first-level clusters. Each bar represents the silhouette values $s(i)$ for a single barn swallow (see also Statistical methods). Within each cluster, bars are drawn in decreasing length order. Large values indicate good classification.

Figure 2

a

b

Figure 5

Figure 6