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Population genetic structure and long-distance dispersal of a recently expanding migratory bird

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ABSTRACT

Long-distance dispersal events and their derivable increases of genetic diversity have been highlighted as important ecological and evolutionary determinants that improve performances of range-expanding species. In the context of global environmental change, specific dispersal strategies have to be understood and foreseen if we like to prevent general biodiversity impoverishment or the spread of allochthonous diseases. We explored the genetic structure and potential population mixing on the recently range-expanding European bee-eater *Merops apiaster*. In addition, the species is suspected of harbouring and disseminating the most relevant disease for bees and apiculture, *Nosema* microsporidia. In agreement with complementary ringing recovery data and morphometric measurements, genetic results on two mitochondrial genes and 12 microsatellites showed a reasonably well-structured population partitioning along its breeding distribution. Microsatellite results indicated that not only did a few birds recently disperse long distance during their return migrations and change their natal breeding areas, but also that a group of allochthonous birds together founded a new colony. Although we did not provide evidence on the direct implication of birds in the widespread of *Nosema* parasites, our finding on the long-distance dispersal of bird flocks between remote breeding colonies adds concern about the role of European bee-eaters in the spread of such disease at a large, inter-continental scale.

1. Introduction

Global warming is inducing changes in growth, recruitment, distribution, and migration patterns of many migratory species (e.g., Crick, 2004; Kullberg et al., 2015). In addition to local and global climatic fluctuations, both land use intensification and land abandonment due to rural depopulation also have an impact on the population dynamics of migratory wildlife by shaping the availability of suitable habitats for breeding, migrating, and wintering (Ostermann, 1998; Parody et al., 2001). In this regard, the bulk of research has focussed on human-induced impoverishment of biodiversity, and particularly on the negative effects of weather or habitat alterations on species' performance. However, much less attention has been given to those opportunistic species that take advantage of such anthropogenic alterations and expand their geographical range in the current context of global environmental change (e.g., Song et al., 2013). Understanding the impact of human-induced changes on the population dynamics of such colonising species, as well as their physical and genetic properties that make them successful colonizers, is a key issue in the field of conservation biology (Lodge, 1993; McKinney and Lockwood, 1999; Thomas et al., 2001).

The degree of admixture between breeding populations at the wintering sites is essential to migratory species, not only affecting their population structure and dynamics, but also increasing the epidemic risk of allochthonous diseases, which might threat several human interests (Altizer et al., 2011). In this regard, the dispersal ability of microorganisms is known to be limited (e.g., Green and Figuerola, 2005). However, migratory animals, and particularly long-distance migrants are usually able to mix remote breeding populations in common winter quarters, potentially enhancing the infection range of locally restricted diseases (Altizer et al., 2011). For instance, several insectivorous migratory bird species

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have been found to harbour pathogenic organisms, which may facilitate pathogen spread (Gulbahar et al., 2003; Reed et al., 2003). Therefore, when establishing the potential role played by long-distance migrants in infectious disease spread, the study of population structure and migratory connectivity of the vector species is mandatory to understand the worldwide spread of the disease.

The rapid technological growth in genetic techniques have allowed the study of dispersal pathways and population connectivity of many long-distance migrants over the last decade (e.g., Irwin et al., 2011). Studies comparing diverse genetic marker types such as mitochondrial and multiple nuclear markers are often reported as the most robust approaches in disentangling population dispersal patterns. For instance, the high rates of mutation experienced by microsatellites (MSAT) allow a more rapid accumulation of novel alleles in different populations than occur for maternally-inherited mitochondrial DNA (mtDNA; Estoup et al., 2002; Hedrick, 1999). Thus, the higher polymorphism rates of MSAT result in a better resolution when trying to differentiate populations or assign individuals to a given population. In addition, the high degree of resolution provided by multi-locus MSAT genotypes can better identify close relatives sampled in different areas that could presumably reflect dispersal processes among populations (Hedrick, 1999; Flagstad et al., 2003; Hansson et al., 2003). However, the direct comparison between diverse genetic markers (i.e., rapidly and slowly evolving genetic markers, as MSAT and mtDNA, respectively) is still required when trying to assess population structure of a given species and its potential dispersal events that occurred at different temporal scales.

The European bee-eater *Merops apiaster* is a non-passerine, long-distance migratory bird that has expanded its breeding range across Eurasia, particularly during the last century (Heneberg and Šimeček, 2004; Hoffmann, 1997; Joshua et al., 1997). In Europe, recovery began in the 1930s and, since then, European bee-eaters have continued to extend their breeding range northwards (Glutz von Blotzheim and Bauer, 1980). Kinzelbach et al. (1997) collated historical records of the occurrence of European bee-eaters in central Europe during the 16–20th centuries and found a strong correlation between range expansion and warmer temperature cycles. Although bee-eaters were typically abundant only in arid and semi-arid areas of southern Europe, northern Africa and western Asia (Fry, 1984), due to the climate change and desertification they have recently colonised several areas outside their traditional breeding range (Fig. 1), such as central Europe and Great Britain (Bernis, 1970; Fraser and Rogers, 2001). Under such expansion circumstances and due to their specialised diet (Fry, 2001), bee-eaters are often blamed for causing severe losses at apiaries (Al-ghzawi et al., 2009; Ali and Taha, 2012). Since ancient times, but also more recently due to the alarming reduction in size and production of most bee colonies (Oldroyd, 2007), bee-eaters have been considered as a significant pest by bee-keepers, thus suffering from direct human persecution throughout their distribution (e.g., Ali, 2012). In addition, bee-eaters sampled in Spain, Italy, and Slovakia have been found to carry spores of *Nosema* microsporidia (Higes et al., 2008; Valera et al., 2011), one of the most relevant threats for bees and apiculture in rural areas (Giersch et al., 2009; Gómez-Moracho et al., 2015; Klee et al., 2007). In fact, the rapid dispersal of *Nosema* parasites among vast, inter-continental areas has been suggested as a consequence of frequent exchanges of breeding individuals from remote populations of European bee-eaters (Valera et al., 2011). Therefore, in a scenario of bird range expansion related to human-induced changes and the potential microsporidia vector status of bee-eaters, the current population dynamics and migratory connectivity of the species is required.

To do so, we determined the population genetic structure of the European bee-eater by sampling nine breeding sites located in four remote areas across its vast breeding distribution (Iberian and Balkan peninsulas, Central Asia, and South Africa; Table 1). We used mtDNA sequence data of two genes and 12 polymorphic MSAT to investigate genetic structure and potential mixing of those sampled populations at different temporal scales. Ringing recovery data and morphometric measurements further complemented our molecular analyses. Using a multidisciplinary approach, we attempt to elucidate the relationship between a wide-ranging migratory bird and the implications it may have on agriculture through disease transmission.

2. Methods

2.1. Study species, breeding range and migration patterns

The European bee-eater is a locally abundant and widely distributed species, inhabiting semi-arid areas and dry lowland grasslands (Fig. 1; BirdLife International and NatureServe, 2013; Fry, 2001). It is an aerial insectivore that feeds on medium-sized flying insects, mainly bees and wasps, although its diet also includes beetles, butterflies, moths, and dragonflies (Inglisa et al., 1993; Kossenko and Fry, 1998; Krebs and Avery, 1984; Kristin, 1994; Martínez, 1984). The species is a long-distance migrant that breeds colonially and remains highly gregarious throughout the year, particularly during migration and in winter. European bee-eaters are monogamous, nest in long burrows that both pair members dig every breeding season, and they are assumed to pair life-long (Fry, 1984). Philopatry is assumed to be high (Dement'ev et al., 1951; Lessells and Krebs, 1989). Within colonies, collaborative breeding behaviour is common and failed breeders (named helpers) often feed the chicks of kin (Jones et al., 1991; Lessells, 1990; Lessells et al., 1994; Václav, 2000). The lifespan of this non-passerine bird is estimated at 6–7 years (Fry, 1984).

The European bee-eater has a vast breeding range. In the Palaearctic, it breeds from the Maghreb and the Iberian Peninsula through southern parts of Europe, ancient Mesopotamia, and East-Central Asia to the Altai mountain range (Fig. 1; Bernis, 1970; Dement'ev et al., 1951; Fry, 1984). The species displays a highly disjunct breeding range, with small populations breeding in Namibia and South Africa during the austral summer (Brooke and Herroelen, 1988). European bee-eaters undertake long and rapid migrations from its Palaearctic breeding grounds to its wintering sites in the African continent south of the Sahara. Ring recoveries suggest that western European and North African birds could move south-westwards probably to West Africa and the eastern European, and Asian ones could move south-east and south-westwards, respectively, and cross the east end of the Mediterranean and the Arabian Peninsula probably heading down the east of the continent, to southern Africa (Fig. 1; Bernis, 1970; Fry, 1984). On the other hand, South African breeding birds are presumed to winter in the African tropics during the austral winter, when their counterparts are breeding in the Palaearctic (Brooke and Herroelen, 1988).

2.2. Ringing recovery data, fieldwork and sampling design

We obtained recovery information on 1663 European bee-eaters spanning 61 years of ringing and recovery data (1952–2013) from the AFRING and EURING data bases (African and European Union Bird Ringing Schemes, respectively; www.afring.org and www.euring.org). Additionally, we added 6 recoveries that are mentioned in the literature (Bernis, 1970; Dement'ev et al., 1951). We excluded all records with less than 100 km between the ringing and recovery sites in order to eliminate local and pre-migratory movements.

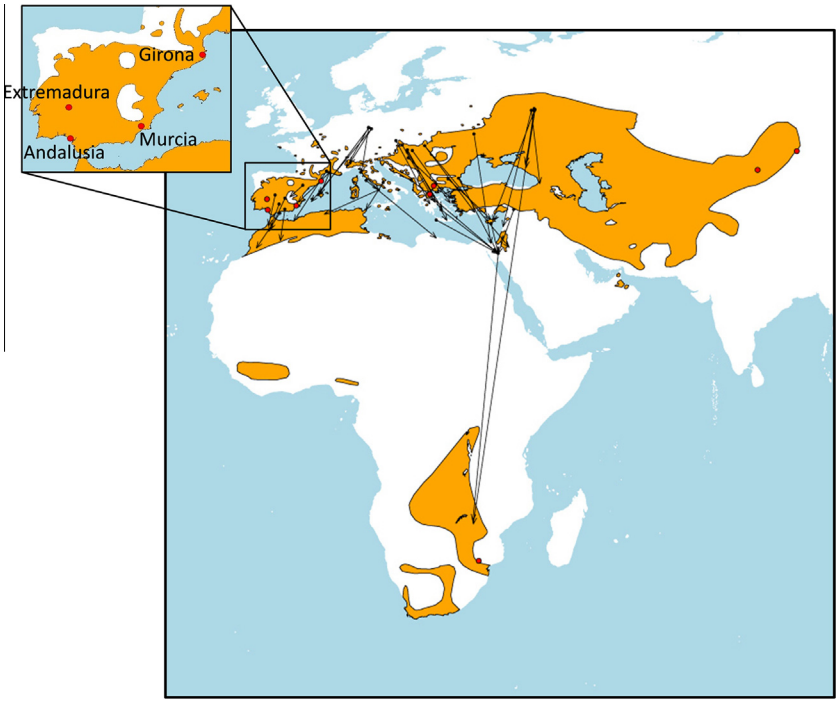

Fig. 1. Breeding and non-breeding range of the European bee-eater *Merops apiaster* (in orange; BirdLife International and NatureServe, 2013). Arrowed lines represent long-distance movements (more than 100 km) of adult birds between capture and recapture locations (ring recovery data offered by EURING and AFRING schemes). Sampled locations spread throughout its breeding range are depicted with red dots. Colony locations are detailed for the Iberian Peninsula (bottom right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Location of sampled individuals of European bee-eaters *Merops apiaster* around its breeding distribution.

Distribution area	Sampled locality (colony)	Longitude (°)	Latitude (°)	Year of sampling
Iberian Peninsula	Girona (Jafre)	42.08	3.01	2006 and 2008
	Murcia (Guadalentín)	38.19	−1.89	2009
	Extremadura (Piñuela)	38.92	−6.33	2009
	Andalusia (Doñana)	37.08	−6.46	2009
Balkan Peninsula	Central Macedonia (Kerkini)	41.21	23.13	2008
	Larissa (Ampelonas)	39.75	22.37	2008
Central Asia	Altay (Ulungur)	47.00	88.37	2007
	Ili Kazakh (Khorgas)	43.92	81.32	2010
South Africa	Johannesburg	−26.20	28.04	2009 and 2010

Fieldwork for data and tissue collection was conducted in eight breeding colonies located in three separated areas (Fig. 1), over seven years (2006–2012; see Table 1 for details). At each colony, breeding adults were captured either with mist-nets or spring-traps (specially designed for catching bee-eaters in the burrow; Moudry Traps, Říčany, Czech Republic). Every individual was sampled for 60 µl of blood either from the jugular (using insulin syringes) or the brachial vein (using a capillary tube), preserving it in ethanol. Birds were measured for culmen length (with digital callipers to 0.1 mm), length of the innermost tail feather (right 1st rectrix, hereafter *R*1) as well as wing length (with wing rule to 1.0 mm), weighed (with a Pesola spring balance to 0.1 g), photographed for the right wing completely extended on a squared paper, marked, and finally released. Additionally, five individuals where opportunistically trapped around Johannesburg (in Witwatersrand, South Africa) at the time when that remote population is assumed to breed (sampling ranges from November 2009 to January 2010). The wing area of all birds was calculated using digitalized pictures with ImageJ (www.imagej.nih.gov/ij/). Wing loading was then calculated as the ratio of bird's weight to wing area, expressed as $N\,m^{-2}$ (Pennycuick, 1989). Wing loading was preferred to body mass as it accounts for the effects of body size and feather wear on flight performance.

2.3. Molecular analyses

Genomic DNA was extracted using the Dneasy Blood & Tissue Kit (QIAGEN) following the manufacturer's protocol. The partial sequences of mitochondrial Cyt*b* and ND2 were amplified by PCR. The designed primer pairs BYF (5′-CTTCTCCTCCGTTGCC-3′)/BYR (5′-AGGGTGCTGATGATTGG-3′) and BNF (5′-CTTCTCCCTAAGCATACTCCT-3′)/BNR (5′-TGAAGGCCCTCGGTTT) were used for Cyt*b* and ND2 amplification, respectively. The thermocycling conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 40 cycles of 94 °C for 40 s, 53 °C for 40 s, and 72 °C for 60 s, plus a final extension at 72 °C for 5 min. The PCR products were purified using QIAquick™ PCR purification Kit (QIAGEN). Sequencing was carried out using an ABI PRISM 3730 automatic sequencer following the ABI PRISM BigDye Terminator Cycle Sequencing protocol. Both strands were sequenced using the same primers as in PCR.

Sequences were aligned and checked visually in the software Seqman II (DNASTAR). The presence of stop codons or indels, that suggests pseudogene sequences, was checked in MEGA3.1 (Tamura et al., 2007). All sequences produced in this study were deposited in GenBank (KU984741-KU984911 and KU957811-KU957981).

2.4. Microsatellites PCR and genotyping

PCR amplifications were carried out for 12 MSAT loci following Dasmahapatra et al. (2004). The PCR conditions were optimised with variable concentrations of $MgCl_2$, template DNA concentrations and annealing temperatures. The final MSAT fragments were amplified with fluorescently labelled forward primers (FAM, HEX, and TAMRA dyes) and were scanned by ABI Hitachi3730XL with the internal size marker GENESCAN-500 ROX (Applied Biosystems). The results were diagnosed and scored in the program GeneMapper 4.0.

2.5. Analyses for population structure and genetic parameters based on mtDNA

Haplotypes for the concatenated sequences containing Cyt*b* and ND2 genes were generated in DNASP 5.0 (Librado and Rozas, 2009). MrModeltest 2.3 was used to identify the appropriate model of sequence evolution (Nylander, 2004). Evolutionary relationships among haplotypes were reconstructed by Bayesian inference in MrBayes 3.2 (Ronquist et al., 2012) with the substitution model selected by the MrModeltest 2.3. We implemented MrBayes with two independent parallel runs, four incrementally heated Metropolis-coupled Monte Carlo Markov Chains in each run, ran for more than 5 million generations until the average standard deviation of split frequency was below 0.01. Maximum parsimony networks were constructed in TCS 1.21 (Clement et al., 2000), with a 95% connection limit. Loops were resolved following the criteria described by Pfenninger and Posada (2002).

Population structure was analysed by Analysis of Molecular Variance (AMOVA) in Arlequin 3.11 (Excoffier et al., 2005). *F*-statistic analogues, and Φ-statistics, were used to estimate the differentiation among groups (areas in our case, Φ_{CT}), among populations within groups (Φ_{SC}), and within populations (Φ_{ST}). The statistical significance of variance components in the AMOVA was tested with 1000 permutations.

Genetic parameters such as haplotype diversity (H_d), nucleotide diversity (π), Tajima's *D*, and Fu's *F* assessing evidence of population expansion were computed in DNASP 5.0. The genetic differentiation among populations and groups were estimated by the pairwise F_{ST} and the corrected average pairwise difference (PiXY − (PiX + PiY)/2) in Arlequin 3.11.

2.6. Summary statistics and population structure inference based on MSAT

We used Genepop 3.4 (Raymond and Rousset, 1995) and GENALEX 6.5 (Peakall and Smouse, 2012) to assess deviation from Hardy–Weinberg equilibrium (HWE). Average allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), and the exact tests of linkage disequilibrium (*LD*) between pairs of loci for each population were computed in Genepop 3.4 and FSTAT (Goudet, 2001).

The AMOVA was carried out in the GENALEX 6.5 (Peakall and Smouse, 2012) with the same grouping arrangement as for mtDNA dataset. To obtain a summarised view of genetic variation across populations as described by the occurrence of MSAT haplotypes, we conducted a Principal Coordinates Analysis (PCoA) using the standardised genetic distance matrix. The distance between individuals was calculated as the pairwise co-dominant genotypic distance. STRUCTURE 2.3.3 (Pritchard et al., 2000) was also used to test population structures based on the genotype distribution of each MSAT locus. The program started with a 'burn-in' of 10^5 iterations and followed by 10^5 MCMC iterations under the admixture model and the assumption of correlated allele frequencies among populations. As suggested by Hubisz et al. (2009), we set sampling locality as a prior to magnify potential signals of populations structuring. We conducted five replicated runs for each cluster value of *K* from 1 to 10. The criteria identifying the most likely number of clusters followed Pritchard et al. (2000) and Evanno et al. (2005), and the evaluation conducted in the website of STRUCTURE HARVESTER (Earl and VonHoldt, 2012). We used CLUMPP 1.1.2 (Jakobsson and Rosenberg, 2007) to merge results from replications of each *K*, and created bar plotting results with DISTRUCT 1.1 (Rosenberg, 2004).

Isolation By Distance (IBD) was tested based on pairwise geographical and genetic distances (F_{st} for mtDNA and G_{st} *Nei* for MSAT loci) with the IBD Web Service http://ibdws.sdsu.edu/~ibdws/ (Jensen et al., 2005). Significance levels were determined by conducting non-parametric procedures 1000 times.

2.7. Statistical analyses

After normality checks through Q–Q plots and using model information criteria, we evaluated the effect of locality on morphometric parameters by fitting a set of candidate generalised linear mixed models (GLMMs), where each of the five parameters (culmen length, *R*1 length, wing length, wing area, and wing loading) was the response variable and breeding locality was the main (fixed) explanatory variable (Table S1). To account for observer experience while measuring birds (Yezerinac et al., 1992), observer identity (among three researchers; RR, JN, and SG) was included in all the GLMMs as a random factor. Gaussian distribution of error terms and the identity link function were used in the modelling. The best-supported models were selected using the Akaike Information Criterion corrected for small sample sizes (AICc) and the corresponding AICc weights (Johnson and Omland, 2004). GLMMs were conducted in R with additional functions provided by the R packages 'lme4' (lmer; Bates et al., 2008) and 'MuMIn' (dredge; Bartoń, 2009).

3. Results

3.1. Ringing and morphometric analyses

Among 1669 recoveries of European bee-eaters, we retained 47 capture-recapture events that are considered as long-distance migratory movements (as they account for more than 100 km between capture and recapture locations). Birds ringed in Western and Central Europe (Portugal, Spain, southern France, Germany, and Italy) were mainly recovered in the Maghreb, Northwest Africa. On the other side, birds ringed in the Eastern Europe and Western Asia (from Poland, Czech Republic, Slovakia, Hungary, and Romania to Russia) were mainly recovered in the Eastern Mediterranean area (around Greece, Cyprus, and Israel) and as far as southern Africa. By their extreme long distances, two of these capture-recapture events stood out among others; these birds were ringed in Russia and Israel and both recoveries occurred in Zimbabwe, representing movements of 8179 and 5239 km, respectively.

After accounting for observer experience in measurement error, neither the measured lengths (for culmen, *R*1, and wing) nor wing area nor wing loading were found to be locality-dependent (Tables 2 and S1).

Table 2
Morphometric characteristics (mean ± SD, with the range in parentheses) of European bee-eaters sampled at eight breeding locations around the species breeding distribution. Additionally, similar data on five birds opportunistically captured in South Africa (i.e., southernmost distribution of the species) are shown. Total population characteristics are also shown in bold.

Sampled location	*N*	Culmen (mm)	*R*1 (mm)	Wing (mm)	Wing area (cm^2)	Wing loading (N/m^2)
Girona (Jafre)	18	35.3 ± 1.8 (31.4|38.5)	10.7 ± 0.8 (9.2|12.3)	14.9 ± 0.4 (14.1|15.6)	121.7 ± 11.1 (98.0|147.3)	18.3 ± 1.0 (16.9|21.1)
Murcia (Guadalentín)	32	35.2 ± 1.9 (31.3|38.8)	10.8 ± 0.5 (9.8|11.7)	14.6 ± 0.4 (13.8|15.3)	121.5 ± 14.8 (85.7|163.8)	18.2 ± 1.6 (15.9|25.6)
Extremadura (Piñuela)	19	35.5 ± 1.4 (31.6|37.6)	NA	14.6 ± 0.3 (13.8|15.1)	122.2 ± 7.3 (111.7|144.4)	18.3 ± 0.6 (17.3|19.4)
Andalusia (Doñana)	24	35.8 ± 2.1 (30.6|40.1)	10.7 ± 1.4 (1.8|12.1)	14.8 ± 0.4 (14.1|16.1)	121.2 ± 8.2 (103.9|139.4)	19.2 ± 2.1 (15.4|24.9)
Total Iberian Peninsula	**93**	**35.5 ± 1.9 (30.6|40.1)**	**10.8 ± 1.0 (1.8|12.3)**	**14.8 ± 0.4 (13.8|16.1)**	**121.6 ± 10.8 (85.7|163.8)**	**18.6 ± 1.6 (15.4|25.6)**
Central Macedonia (Kerkini)	20	35.8 ± 1.6 (33.1|38.8)	11.3 ± 2.1 (8.6|19.2)	15.0 ± 0.4 (14.2|15.7)	121.5 ± 9.4 (107.3|152.3)	18.4 ± 0.9 (17.5|20.2)
Larissa (Ampelonas)	22	35.7 ± 2.1 (32.5|40.2)	11.2 ± 0.6 (10.4|12.3)	15.1 ± 0.4 (14.3|15.8)	123.9 ± 7.3 (107.9|138.1)	17.9 ± 0.5 (17.0|18.8)
Total Balkan Peninsula	**42**	**35.8 ± 1.9 (32.5|40.2)**	**11.2 ± 1.5 (8.6|19.2)**	**15.0 ± 0.4 (14.2|15.8)**	**122.7 ± 8.4 (107.3|152.3)**	**18.2 ± 0.7 (17.0|20.2)**
Altay (Ulungur)	21	36.6 ± 1.9 (33.6|40.7)	NA	15.0 ± 0.3 (14.7|15.6)	121.2 ± 5.9 (109.5|133.8)	18.7 ± 1.1 (17.1|21.0)
Ili Kazakh (Khorgas)	10	37.1 ± 2.5 (34.2|41.2)	11.7 ± 0.3 (11.4|12)	14.9 ± 0.4 (14.3|15.6)	123.0 ± 7.2 (112.2|132.3)	18.2 ± 0.5 (17.6|18.7)
Total Central Asia	**31**	**36.7 ± 2.0 (33.6|41.2)**	**11.7 ± 0.3 (11.4|12)**	**15.0 ± 0.3 (14.3|15.6)**	**121.6 ± 6.1 (109.5|133.8)**	**18.6 ± 1.0 (17.1|21.0)**
Johannesburg	5	35.1 ± 1.8 (33.8|36.4)	12.1	14.6 ± 0.8 (14.0|15.1)	NA	NA
Total South Africa	**5**	**35.1 ± 1.8 (33.8|36.4)**	**12.1**	**14.6 ± 0.8 (14.0|15.1)**	**NA**	**NA**

Table 3
Genetic diversity analyses of two mitochondrial genes (Cyt*b* and ND2) for nine sampled localities of European bee-eaters.

	n	Haplotype diversity (*Hd*)	Nucleotide diversity (π)	Tajima's *D*	Fu and Li's *F*
Girona	18	0.974	0.0022	−1.67	−2.64*
Murcia	32	0.881	0.0018	−1.26	−2.08
Extremadura	19	0.988	0.0027	−1.94*	−2.72*
Andalusia	24	0.942	0.0023	−1.40	−2.42
Central Macedonia	20	0.989	0.0025	−1.79	−2.88*
Larissa	22	0.986	0.0028	−1.72	−2.50
Altay	21	0.984	0.0026	−1.55	−2.43
Ili Kazakh	10	0.945	0.0029	−0.21	−0.06
Johannesburg	5	1.000	0.0032	−0.51	−0.54
Total	171	0.972	0.0025	−2.40*	−5.27*

* *P*-value < 0.05.

3.2. Population genetics

The 898 base pair (bp) long fragment of Cyt*b* and 911 bp ND2 were successfully amplified and sequenced from 171 individuals. There were 58 variable sites in Cyt*b* sequences and 23 sites among them were parsimony-informative. The numbers of variable and parsimony -informative sites in ND2 were 50 and 23, respectively. The 1809 bp long combined sequences consisted of 108 variable sites, 46 of them were parsimony-informative. The total haplotype diversity (*Hd*) was 0.972 and nucleotide diversity (π) was 0.0025 (Table 3). Among the nine localities, Murcia showed the lowest *Hd* and π values (0.881 and 0.0018, respectively), whereas South Africa had the largest genetic diversity (*Hd* = 1.000 and π = 0.0032). The Tajima's *D* and Fu & Li's *F* were not significantly negative in most localities except for Extremadura and Central Macedonia. Significant negative values for the whole set of localities indicated a deviation from neutral molecular evolution of these mitochondrial genes in European bee-eaters (Table 3).

Ninety-six haplotypes were defined and the best evolutionary model selected by MrModeltest was GTR+I (AIC criteria). The phylogeny based on mitochondrial haplotypes was not well resolved, nor was there a clear signature of geographical diversification among populations (Fig. S1). The star-like network showed that individuals from the Iberian and Balkan peninsulas, Central Asia, and South Africa mixed together and shared common haplotypes (Fig. S2). AMOVA results suggested a weak genetic structuring among the four main areas (F_{CT} = −0.002, *P*-value = 0.493; Table 4). The greatest variance component was due to differences within localities (98.7%); whereas among localities and among areas, the variance component was very low (1.6% and 0.2%, respectively; Table S2). Population differentiation due to isolation by distance was not well supported by the IBD test (*P*-value = 0.457; Fig. S3).

Table 4
Pairwise F_{ST} values of microsatellite DNA, based on data from 12 loci (below the diagonal) and mitochondrial DNA based on 898 and 911 bp sequences belonging to two genes (above the diagonal).

	Girona	Murcia	Extremadura	Andalusia	Central Macedonia	Larissa	Ili Kazakh	Altay	Johannesburg
Girona	–	0.096	**0.337***	0.058	0.002	0.006	**0.308***	**0.180***	**0.495***
Murcia	0.012	–	0.072	−0.005	−0.038	0.026	0.096	−0.064	0.058
Extremadura	0.017	0.012	–	**0.180***	0.116	−0.016	0.103	0.061	0.005
Andalusia	**0.054****	**0.055****	**0.057****	-	−0.067	0.061	0.203	0.039	0.133
Central Macedonia	**0.062****	**0.065****	**0.065****	0.016	-	−0.016	0.097	0.002	0.019
Larissa	**0.052****	**0.055****	**0.059****	0.016	0.013	–	0.063	0.034	0.081
Ili Kazakh	**0.081****	**0.071****	**0.081****	0.026	0.046	0.039	–	0.050	−0.064
Altay	**0.063****	**0.063****	**0.063****	0.014	0.019	0.016	0.031	–	−0.072
Johannesburg	**0.070****	**0.070****	**0.080****	0.045	0.037	0.036	0.071	0.048	–

* *P*-value < 0.05.
** *P*-value < 0.001.

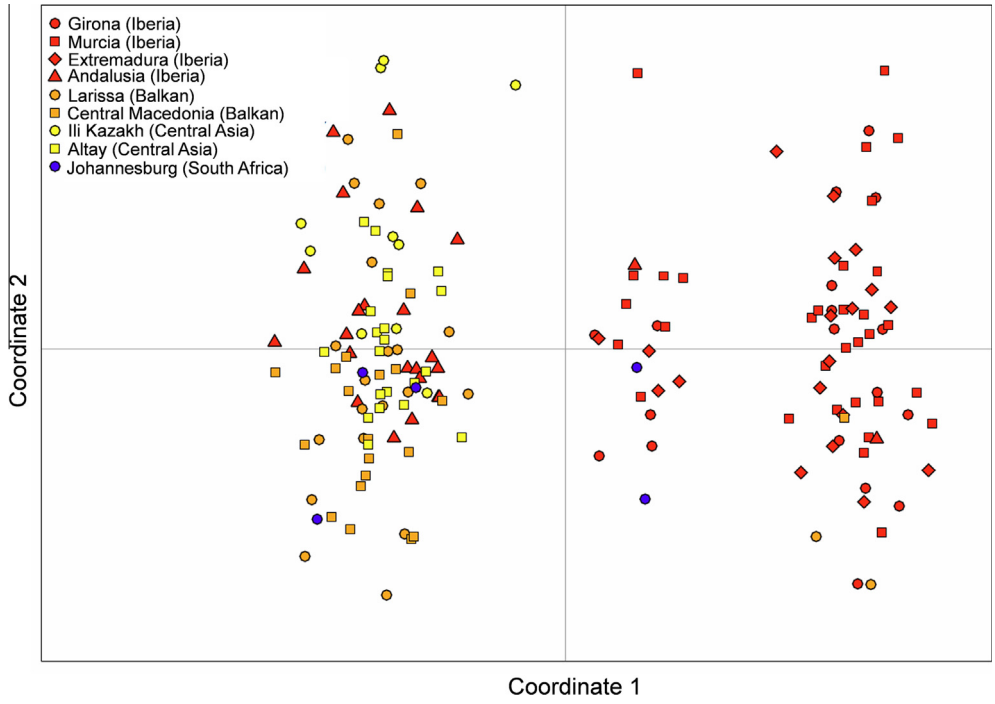

Fig. 2. Plot of the first two axes of Principal Coordinates Analysis (PCoA) based on standard genetic distance matrix calculated by variation at 12 microsatellite loci for 171 European bee-eaters. Individuals from the Iberian Peninsula are depicted in red, individuals from Balkan Peninsula in orange, individuals from Central Asia in yellow and individuals from South Africa in dark blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Among 12 MSAT loci, HWE was recorded in most localities, while the colony of Larissa showed deviation from HWE in 6 loci due to heterozygote deficiency (Table S3). There was no evidence of linkage disequilibrium between loci (Adjusted *P*-value for 5% nominal <0.0001).

A three-group structure was identified by the PCoA (Fig. 2). The result showed that all individuals from Central Asia (Ili Kazakh & Altay), most individuals from Balkan Peninsula (Larissa 20 out of 22, and Central Macedonia 19 out of 20), 22 out of 24 individuals from one single population of the Iberian Peninsula (Andalusia) and three out of five individuals from South Africa grouped together. A second middle group included mainly Iberian birds (from Extremadura, Murcia, and Girona localities, plus one bird from Andalusia) in addition to two birds from South Africa. Finally, the third group included three Balkan birds and the rest of individuals from the Iberian Peninsula (including one single bird from Andalusia; Fig. 2).

The STRUCTURE analyses showed that the best *K* value was 2 (Fig. 3). In one of the two cluster assignments, the localities of Murcia, Extremadura, and Girona grouped together. In the other defined cluster, a certain degree of differentiation between localities from Central Asia, Balkan Peninsula, and that of Andalusia (in the Iberian Peninsula) could be observed when trying with more grouping numbers. South African birds tended to show a mixture of components from the two major groups (Fig. 3). In addition, we performed STRUCTURE analyses within each cluster (excluding the South Africa population), and no further substructure was detected in any of the clusters (Figs. S4 and S5).

In agreement with these findings, results of the AMOVA based on 12 MSAT loci showed that most of the variance is due to among and within individuals (10.18% and 83.38%, respectively), indicating a poor genetic differentiation among the four geographic areas or localities (Table S2). Despite low pairwise F_{st} values, pairwise comparisons indicated significant differentiation between Iberian localities and those from Western Europe, Asia, and South Africa (Table 4). However, birds of Andalusia differentiated from their Iberian counterparts and they did not differ from the other sampled localities. Similar to the mtDNA, the IBD result based on MSAT showed no significant correlation between geographic and genetic distances (*P*-value = 0.357; Fig. S3).

4. Discussion

The evolution of long-distance migration routes in terrestrial animals is profoundly influenced by the distribution of vast land- and water-masses (Alerstam et al., 2003). In the case of the European bee-eater, our preliminary exploration based on population genetic data from nine localities distributed across four main regions revealed that the Mediterranean Sea and the presence of the inhospitable Sahara Desert have played a significant role on its population structuring at a relatively recent temporal scale. MSAT results showed that European bee-eaters have a relatively well-structured population partitioning, with two clear main clades: one with most birds from Iberia (excluding the colony of Andalusia, discussed in detail below) and another with birds from Eastern Europe and Central Asia. Such results agree with the presumed relatively high natal and breeding philopatry of the species (Fry, 1984; Lessells and Krebs, 1989). As it is also suggested by our ring recovery analysis (Fig. 1), birds from the Iberian Peninsula, and Western and Central Europe would recurrently migrate through the Straits of Gibraltar and Sicily to winter in a restricted area of West Africa. This migratory pattern is rather common in many other insectivorous trans-Saharan migrants breeding in Western and Central Europe, that are now known to exploit the subtropical grasslands and shrublands of West Africa during winter (Bächler et al., 2010; Bairlein et al., 2012; Lemke et al., 2013; Schmaljohann et al., 2012). On the other hand, Greek and Asian bee-eaters appeared genetically more closely related, presumably because they share common migratory corridors through the Sinai and Arabian Peninsula and wintering areas in southern Africa. Other small insectivorous long-distance migrants from Eastern

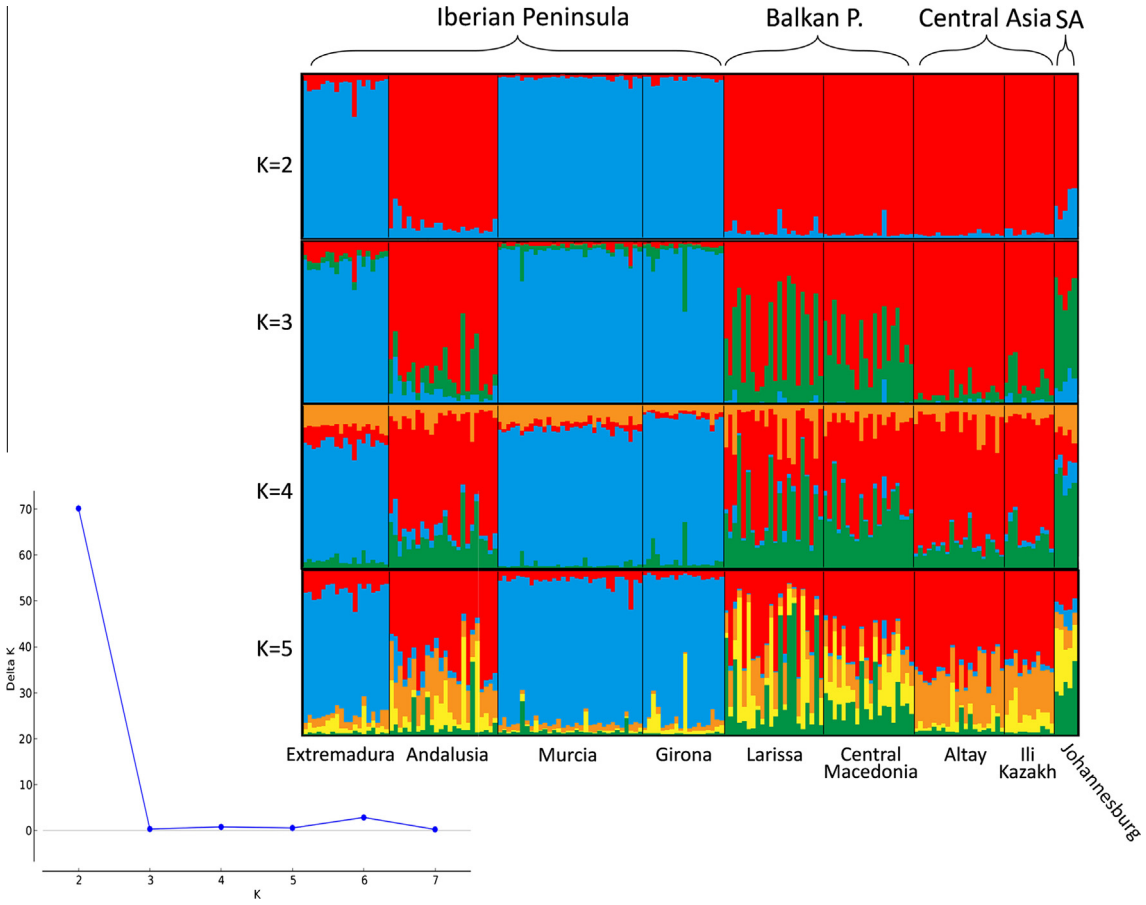

Fig. 3. Bayesian clustering of European bee-eater genotypes performed in STRUCTURE with $K = 2$, $K = 3$, $K = 4$, and $K = 5$. Each individual is represented by a vertical line, with the probability of assignment to different clusters along the *Y*-axis. Plots are sorted by sampled locality and distribution area (Iberian and Balkan peninsulas, Central Asia, and South Africa). SA refers to South Africa. Detection of the number of groups in the data set with STRUCTURE is shown in the lower part of the Figure, with ΔK [ΔK = mean ($|L''(K)|$)/sd($L(K)$)] as a function of K. ΔK indicates the most likely number of two genetic clusters.

Europe and Asia also use such corridors and remote areas for wintering (Bairlein et al., 2012; Stach et al., 2012; Tøttrup et al., 2012). Therefore, the observed population structure of European bee-eaters is likely due to the barrier that the Mediterranean Sea and the Sahara Desert represent for such terrestrial birds. European bee-eaters probably circumvent these barriers in their migration routes and gene flow among their populations seems to be conditioned by the distinct wintering areas they recurrently use in West and Southeast Africa.

Although a clear geographic structure was inferred based on MSAT data, the absence of any significant spatial structure in mtDNA (Fig. S2) suggested that a certain degree of dispersal and exchange between populations have existed. The absence of any trace of morphometric differentiation also supported the existence of mixing and dispersal among populations. Moreover, the weak IBD pattern (both in mtDNA and MSAT) indicated that geographic distance cannot constrain the genetic exchanges among populations. In fact, our finding on MSAT analysis that assigns most birds from a single colony of the Iberian Peninsula (that of Andalusia) to the group of Greek and Asian birds further proved the existence of long-distance dispersal events in the species. Therefore, our results suggested that long-distance dispersal events might be relatively frequent in European bee-eaters throughout their distribution range.

Long-distance dispersal events, understood here as those long-distance movements with breeding purposes and away from their natal source, are typically rare in nature (Nathan et al., 2003). Most research on the topic has been conducted on plant and invertebrate species considering that seed and propagule dispersal over long distances is passively conducted through external actors (e.g., wind and water flows, birds; Green and Figuerola, 2005; Kinlan et al., 2005; Soons and Ozinga, 2005). However, fewer examples on long-distance dispersal of vertebrates can be found in the literature, in spite of the high relevance of such events when determining population dynamics and genetic composition of their populations (Nathan et al., 2003). In this regard, we reported here on robust genetic evidence for an active long-distance dispersal event that is certainly going to condition the genetic composition of those Iberian populations of European bee-eaters, and likely their population dynamics in the recent future.

Interestingly, most birds from the colony of Andalusia (22 out of 24) were clustered together with birds from Greece and Central Asia. This indicated that not only did a few birds recently disperse long distance during their return migrations and change their natal breeding areas, but also that a group of allochthonous birds together founded a new colony (or colonised an abandoned one). European bee-eaters are known to be highly gregarious both while breeding and during their long-distance migrations. Thus, it might not be surprising that in the event of losing the given migratory route an entire flock of birds followed or joined another group of birds in either their back or forth migrations. In the present case, individuals sampled in Andalusia were captured in a new breeding colony recently founded in a flat sandy terrain within the Doñana National Park (Fernando Ibáñez personal communication).

Founding events are characterised by a loss of genetic variation in the new population as genetic diversity only represents a small proportion of that found in the native range (Cornuet and Luikartt, 1996; Leberg, 1992). In addition to that, higher inbreeding rate is predicted through the small number of individuals in introduced populations further reducing its genetic diversity and their potential abilities to adapt to novel environments (Dlugosch and Parker, 2008; Hoffmann and Blows, 1994). However, the colony of Andalusia did not differ from the other colonies in its genetic diversity indices (Tables 3, and S3 and Fig. S4). Other examples reported little effect of the founding event on the genetic diversity of the new populations due to a diverse set of extrinsic and intrinsic factors (Berthouly-Salazar et al., 2013; Dlugosch and Parker, 2008; Song et al., 2013). European bee-eaters are well-known to breed in close proximity to relatives (i.e., both brothers and parents; Jones et al., 1991; Lessells et al., 1994). Thus, the already-reduced genetic diversity produced by the cooperative breeding and the high kinship among colony members of the species could mask the quantifiable consequences of a founding event (e.g., an excess of the observed heterozygosis; Cornuet and Luikartt, 1996; Parreira and Chikhi, 2015).

Long-distance dispersal events promote increases of genetic diversity and decreases of genetic differentiation. Although we did not detect evidence of mixing between native and non-native individuals around the recent founding site, an increase of genetic diversity can be seen as a powerful advantage of maximising adaptive potential when populations face new environmental conditions (Guarino and Lobell, 2011; Hoffmann and Blows, 1994). Therefore, long-distance dispersal strategies should be highlighted here as ecological and evolutionary determinants that improve performances of expanding species.

Finally, we report here on an example of long-distance dispersal and colony founding event by birds from the Eastern range of the European bee-eater settling in the Iberian Peninsula (i.e., the westernmost distribution range of the species). However, similar dispersal events between remote populations may also occur in other non-sampled localities within the vast range of the species. Such long-distance dispersals have important consequences not only for the population dynamics of this recently expanding bird, but also for the epidemiology of emerging nosemosis. Although we did not provide robust evidence on the direct implication of birds in the spread of such disease, our finding on the long-distance dispersal of bird flocks between remote breeding colonies adds concern about the potential vector status of the European bee-eater in the recent expansion of *Nosema* microsporidia. Strong evidence on bee-eaters acting as efficient vectors of *Nosema* parasites would require specific epidemiological surveys in strategic ringing stations or tracking the migratory movements of birds known to feed on infected bees (e.g., Arbeiter et al., 2012). At the present, (1) the long-distance migrations of the species, (2) its dispersal potential among remote breeding populations, (3) its foraging habits linked to man-made apiaries (Al-ghzawi et al., 2009; Ali and Taha, 2012), and (4) its ability of carrying viable *Nosema* spores (Higes et al., 2008), highlight the potential of the European bee-eater of spreading nosemosis throughout its vast breeding range, including Eurasia and Africa.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.03.015.

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