Population genetic structure of serotine bats (*Eptesicus serotinus*) across Europe and implications for the potential spread of bat rabies (European Bat Lyssavirus EBLV-1).

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Running title: Genetic structure of *Eptesicus serotinus*

Word count: 6 847

Abstract

Understanding of the movements of species at multiple scales is essential to appreciate patterns of population connectivity and in some cases, the potential for pathogen transmission. The serotine bat (*Eptesicus serotinus)* is a common and widely distributed species in Europe where it frequently harbours European Bat Lyssavirus Type 1 (EBLV-1), a virus causing rabies and transmissible to humans. In the UK it is rare, with a distribution restricted to south of the country and so far the virus has never been found. We investigated the genetic structure and gene flow of *E. serotinus* across the England and continental Europe. Greater genetic structuring was found in England compared with continental Europe. Nuclear data suggest a single population on the continent, although further work with more intensive sampling is required to confirm this, while mitochondrial sequences indicate an east-west substructure. In contrast, three distinct populations were found in England using microsatellite markers, and mitochondrial diversity was very low. Evidence of nuclear admixture indicated strong male mediated gene flow among populations. Differences in connectivity could contribute to the high viral prevalence on the continent in contrast with the UK. While the English Channel was previously thought to restrict gene flow, our data indicate relatively frequent movement from the continent to England highlighting the potential for movement of EBLV-1 into the UK.

Key words: Chiroptera; gene flow; genetic structure; philopatry; dispersal; rabies

Introduction

Bats are widely distributed and vary enormously in their ecology, sociality and behaviour. Largely because of their small size and vagility, population genetics has been widely used to infer aspects of bat biology that are difficult to observe directly. Substantial variation in genetic structuring has been found, reflecting differences in bat movement, behaviour, mating and social systems (Burland and Wilmer, 2001; Moussy *et al.*, 2013). Additionally, molecular approaches have been used to assess many aspects of bat ecology including potential sex-biased dispersal and movements (Petit *et al.*, 2001), mating systems (Furmankiewicz and Altringham, 2007), effect of landscape barriers (García-Mudarra *et al.*, 2009), demography and history (Flanders *et al.*, 2009) and sociality (Metheny *et al.*, 2007). Gaining an understanding of these components of bat ecology and behaviour is fundamental in a conservation context (Racey and Entwistle, 2003), to improve our understanding of ecosystem services (Kunz *et al.*, 2011) and in disease epidemiology since bats are now thought to be the wildlife reservoirs for a range of important zoonoses and emerging diseases (Kuzmin *et al.*, 2011).

The serotine bat, *Eptesicus serotinus,* is a common species within its core range in continental Europe, but rare in the UK where it is largely restricted to southern England (Dietz *et al.*, 2009). The species is considered sedentary in the UK with recorded seasonal flights of under 100 kilometres, although ringing data is sparse, which restricts our understanding of any seasonal movements (Hutterer *et al.*, 2005). Adult females form maternity colonies in the summer, often in inhabited

68 buildings, and display some level of philopatry to their natal landscape, if not natal
69 colony (Harbusch and Racey, 2006). Due to the relative ease of locating these
70 maternity roosts, research has mainly focused on habitat and roost use, diet, and
71 activity patterns of adult females or of juveniles of both sexes (Catto *et al.*, 1996;
72 Robinson and Stebbings, 1997). In contrast, adult males are more difficult to find
73 and their ecology is very poorly understood. Similarly, little is known about the
74 winter ecology of the species and its mating system. Information on the social and
75 spatial organisation of this species, as well as on population and seasonal
76 connectivity is required for a more complete understanding of its ecology and
77 behaviour within its core range and at the edge of its distribution.

78 This basic information on serotine biology is particularly important as this bat is
79 the main reservoir for the most common European bat lyssavirus, EBLV-1 (Harris
80 *et al.*, 2006). In such a context, knowledge of the host's movement, mating
81 patterns and social organisation is crucial to gain further understanding of viral
82 dynamics. Currently the prevalence of EBLV-1 in continental Europe contrasts
83 strongly with its apparent absence from England (Schatz *et al*. 2013). Indeed,
84 both active and passive surveillance in the UK has failed to detect the virus.
85 However, passive surveillance is biased to common species with only few *E.*
86 *serotinus* submitted for testing. Furthermore, a juvenile bat was tested positive
87 for antibodies against EBLV-1 in a maternity roost of south of England (Harris *et al*.
88 2009). This case was surprising since the bat hence had not dispersed or mated,
89 and was therefore likely to have only been in contact with roost-mates, but no

90 other individuals from the same roost was found seropositive despite extensive
91 sampling over four years.

92 Here, we used ten microsatellites and a portion of the hypervariable region II
93 (HVII) of mitochondrial DNA (mtDNA) to investigate the genetic structure of *E.*
94 *serotinus* at multiple spatial scales. We initially describe in detail the genetic
95 structure of serotines in England and provide a first account of the level of genetic
96 differentiation on the continent to compare the core and edge populations. Based
97 on the bat's biology, two contrasting predictions were made. First, because the
98 English population is small and fragmented at the edge of the species range (Bat
99 Conservation Trust 2013), movement and gene flow could be reduced there,
100 resulting in genetic differentiation and structure. If the core continental
101 population were also large and continuous enough to result in low structure, then
102 these contrasting structure patterns could help explain the prevalence of EBLV-1
103 on the continent and its apparent absence in England – bats simply do not move
104 enough in the UK to spread the virus. Alternatively, because the geographic area
105 covered by the English population is small and there are few obvious barriers to
106 movement within the UK, it could display low structure and low genetic
107 differentiation. If this were so, then the apparent absence of EBLV-1 in the UK
108 could simply be due limited dispersal across the English Channel. We also tested
109 for possible sex-biased gene flow as male connectivity and female philopatry are
110 common in temperate bats (Moussy *et al.*, 2013).

Methods

Sample collection

A total of 593 individuals were captured at 28 maternity roosts in inhabited buildings across the English range in the summers of 2004-2006 and 2010-2011 (individuals per roosts: mean = 19.41; median = 14.50; range from 4 to 68; Table S1; Figure 1). No adult males were captured due to their absence from maternity roosts. A wing tissue sample was taken using a 3 mm biopsy punch (Stiefel Laboratories, Wooburn Green, UK) and stored in 70% ethanol at +4°C until DNA extraction. All bats were released back into the wild within a few minutes of the procedure at their location of capture. All work was done with the approval of ethical review bodies at FERA and University of Exeter and under license from the UK Home Office [Animals (Scientific Procedures) Act 1986] and Natural England (Habitats Regulations, 2010). Wing biopsies were also supplied for 106 individuals from eight locations in six countries across continental Europe (Table S1; Figure 1).

DNA extraction and Whole Genome Amplification

DNA was extracted following Sambrook and Russel (2001). Ammonium acetate (10 M; Sigma-Aldrich, Poole, UK) was used to precipitate proteins instead of potassium acetate. The DNA pellet was recovered in 1 x Tris–ethylenediaminetetraacetic acid buffer (TE) (Sigma-Aldrich) and stored at -20°C before further procedures. The whole genome of each individual was amplified using illustra GenomiPhi V2 DNA Amplification kit (GE Healthcare, Little Chalfont, UK). A subset of samples was genotyped for microsatellites before and after

133 whole genome amplification to ensure the procedure did not affect typing success
134 or quality.

135 *Microsatellite genotyping and mitochondrial DNA sequencing*

136 Samples were genotyped using a panel of 11 microsatellite markers (Smith *et al.*,
137 2011) as described in Table S2. PCR was carried out in 15 µl, with 7.5 µl 2x PCR
138 mastermix (ABgene, Epsom, UK) containing 1.5 mM $MgCl_2$, 1 µM of each primer
139 (Sigma-Aldrich/Applied Biosystems, Foster City, CA, USA), and 1.5 µl DNA
140 template (DNA extract or 1/10 dilution in 1 x TE of whole genome amplification
141 product). The remaining volume was made up with molecular grade water. PCR
142 reactions consisted of denaturation at 94°C for 2 min, followed by 35 cycles of
143 94°C for 1 min, 1 min at the annealing temperature (Table S2), 72°C for 1 min, and
144 final elongation at 72°C for 5 min and 60°C for 1 h. PCR products were diluted and
145 mixed into four sets (Table S2) and run on an ABI Prism 3130xl genetic analyser
146 (Applied Biosystems) with Genescan Rox 500 size standard (Applied Biosystems).
147 Microsatellite alleles were sized using GeneMapper 3.7 software (Applied
148 Biosystems). The genotyping error rate per loci was estimated by re-amplifying
149 and re-genotyping 11–24% of samples depending on the locus.

150 A 460 bp portion of the hypervariable domain II (HVII) of the mtDNA control
151 region was amplified in a subset of samples (Table S1) using primers for conserved
152 sequence block F on L-strand 5'-CTACCTCCGTGAAACCAGCAAC-3' (Wilkinson and
153 Chapman 1991) and for HVII on H-strand 5'-CGTACACGTATTCGTATGTATGTCCT-3'
154 (J. Juste , pers. comm.). PCR was carried out in 20 µl, with 10 µl 2x BioMix Red

(Bioline, London, UK) containing 1 mM $MgCl_2$, 0.5 µM of each primer (Sigma-Aldrich), and 1 µl of amplified genomic DNA. The remaining volume was made up with molecular grade water. PCR reactions consisted of denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1.5 min, 72°C for 2 min, and final elongation at 72°C for 5 min and 60°C for 1 h. PCR products were purified by an enzymatic reaction to remove leftover primers and dNTPs. A 10 µl mix of 0.5 U Exonuclease I (Fermentas, Vilnius, Lithuania) and 0.25 U Alkaline Phosphatase (Fermentas) in molecular grade water was added to each product before incubation at 37°C for 30 min and deactivation at 85°C for 15 min. The purified DNA fragment was sequenced from both directions using the BigDye Terminator v3.1 sequencing kit (Applied Biosystems) in a 20 µl reaction volume with 1.5 µl BigDye Terminator mix, 3.5 µl 5x sequencing buffer, 3.33 pmol of primer and 1 µl DNA template. The remaining volume was made up with molecular grade water. The sequencing reaction consisted of 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The sequencing reactions were cleaned by precipitation with 100% ethanol and 20 µg glycogen before a washing step with 70% ethanol. They were then reconstituted in 11 µl HiDi formamide before being run on an ABI Prism 3130xl genetic analyser (Applied Biosystems). The resulting sequences were assembled, aligned and trimmed into GENEIOUS 5.5.7 (Drummond *et al.*, 2011) to create a 424 bp consensus sequence for each individual.

Microsatellites analysis: Tests of assumptions, genetic diversity and basic statistics

176 The presence of scoring inconsistencies, null alleles, large allele dropout and
177 stuttering were tested in MICROCHECKER (Van Oosterhout *et al.*, 2004). Tests for
178 departure from Hardy-Weinberg (HW) equilibrium were performed for each roost
179 and each locus in GENEPOP 4.1.4 (Rousset, 2008) both as two-tailed tests with no
180 assumption as the direction of the deviation, and under the hypothesis of
181 heterozygote deficit. A sequential Bonferonni correction was applied to the p-
182 values (Rice, 1989).Tests of linkage disequilibrium were performed in FSTAT
183 2.9.3.2 (Goudet, 1995, 2001) and the significance levels were adjusted using
184 sequential Bonferonni correction. Genetic diversity indices were obtained for each
185 roost from FSTAT and GENETIX 4.05 (Belkhir *et al.*1996-2005). Estimated F_{ST}
186 averaged over loci (Weir and Cockerham, 1984) and the 95% confidence intervals
187 (CI) after 1000 bootstrap over loci were calculated in GENETIX. Finally, a log-
188 likelihood G-test for population differentiation (not assuming random mating
189 within roosts) was performed (Goudet *et al.*, 1996) in FSTAT based on 100,000
190 randomisations of complete multilocus genotypes.

191 *Nuclear population structure*

192 The Bayesian clustering program STRUCTURE was used to investigate genetic
193 structure (Pritchard *et al.*, 2000). We evaluated the number of assumed clusters K
194 from 1 to 6 using a burn-in of 5×10^6 iterations followed by 5×10^7 MCMC
195 iterations. The admixture model with correlated allele frequencies was run with
196 and without population information (roosts the individuals were sampled from) as
197 a prior. Each K value was run 12 times to ensure stability and convergence of the

198 chains. The optimal number of clusters K was obtained from ΔK, based on the rate
199 of change in the log probability of data in successive K values (Evanno *et al.*, 2005)
200 as implemented on STRUCTURE HARVESTER (Earl and VonHoldt, 2012).

201 Genetic structuring was further investigated using two multivariate methods: (1) a
202 discriminant analysis of principal components (DAPC) (Jombart *et al.*, 2010) and
203 (2) a spatial principal component analysis (sPCA) (Jombart *et al.*, 2008). In contrast
204 to Bayesian clustering, the strength of these approaches rests in their
205 independence from population genetic model, and inferences are therefore made
206 only on allelic similarity (Jombart *et al.*, 2009). The two methods were
207 implemented in the ADEGENET 1.3-4 package (Jombart, 2008) in R. DAPC has
208 been developed to summarise the overall genetic variability of individuals within
209 groups while optimising discrimination between groups. We used the roosts
210 individuals were sampled from as group prior to discriminant analysis. The first 30
211 principal components (PC) of PCA were retained in the data transformation step,
212 covering 90.5% of genetic variance. sPCA explicitly incorporates spatial
213 information in the investigation of genetic variability across landscapes (Jombart
214 *et al.*, 2008). We applied this method at the roost level. A connection network was
215 constructed using K-nearest neighbour (Cover and Hart, 1967), setting K to seven
216 to reflect the number of sites sampled on the continent. This network was used
217 for the calculation of Moran's *I* (Moran, 1948, 1950). sPCA optimises the product
218 of the variance of individual scores, based on roosts' allelic frequencies, and of
219 Moran's *I,* in order to summarise genetic variability in a spatial context.

AMOVA and genetic differentiation among clusters

Consensus on the population structure was reached based on results from the Bayesian and multivariate analyses. Roosts were assigned to one of the clusters defined (hereafter called populations) and this genetic structure was tested in an analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) in ARLEQUIN. Log-likelihood G tests for differentiation were performed between and within populations using 10,000 permutations in the HIERFSTAT package implemented in R. Pairwise F_{ST} between the populations were obtained from ARLEQUIN to assess the level of connectivity. Log-likelihood G tests were performed for all pairs of populations on 10,000 permutations by the HIERFSTAT package.

Isolation by distance

Mantel and partial Mantel tests were performed between matrices of linearised pairwise F_{ST} (Rousset, 1997) and log-transformed geographical distances among roosts. An indicator matrix was included to account for the presence of the most obvious geographical barriers including the English Channel (isolating the UK), the Solent (between the Isle of Wight and England), the Alps (isolating the Italian roost), the Carpathians (isolating the Slovakian roost) and the Caucasus range (isolating Georgia). Tests were carried out on the full dataset, the continent only dataset and on the English-only dataset.

Contemporary gene flow

240 The magnitude and direction of contemporary of gene flow occurring between
241 the consensus populations were estimated using the program BAYESASS 3.0.1
242 (Wilson and Rannala, 2003). The simulation was run with 10^7 iterations, with the
243 first 25 x 10^5 iterations discarded as burn-in. Samples were collected every 100
244 iterations. Allelic frequencies and inbreeding coefficients were set at 0.2 while
245 migration rate was left at its default value of 0.1. These mixing parameters
246 ensured the acceptance rates were between 0.2 and 0.4. The trace file was
247 examined in TRACER 1.5 to ensure mixing and convergence of the chains
248 (Rambaut and Drummond, 2009).

249 *Mitochondrial DNA analysis: Genetic diversity and differentiation*

250 Haplotype (*h*) and nucleotide diversities (π), number of polymorphic sites and
251 mean number of pairwise differences were calculated for each roost and each
252 population in ARLEQUIN. Differentiation index for haploid data ϕ_{ST} (Weir and
253 Cockerham, 1984) was estimated in ARLEQUIN and tested for significant
254 departure from panmixia after 10,000 permutations. Differentiation test among
255 roosts was also performed with 100,000 MC iterations after 10,000 burn-in
256 iterations in ARLEQUIN.

257 *Mitochondrial population structure*

258 Spatial structuring of genetic variation in the mtDNA sequences was investigated
259 by sPCA (Jombart *et al.*, 2008). All roosts were considered as neighbours in this

analysis, but their inter-connection was weighted by the inverse Euclidian distance separating the roosts.

Geographical structuring of mtDNA variation was also investigated in an AMOVA. Roosts were grouped in various clusters based on the sPCA results and on the structure observed at the nuclear level. Those structures were tested in order to optimise the among-groups variance component. The contribution of each hierarchical component (among groups, among roosts within groups and within roosts) was estimated and tested for significance after 10,000 permutations. Differentiation between identified clusters was inferred by pairwise ϕ_{ST} and tested by log-likelihood tests with 100,000 MC iterations after 10,000 burn-in iterations. All calculations were undertaken in ARLEQUIN.

Mantel and partial Mantel tests were performed between matrices of pairwise linearised ϕ_{ST} and log-transformed geographical distances among roosts to test for isolation by distance. The same indicator matrix used to infer presence of geographical barriers in the microsatellite dataset was included. Tests were carried out on the full dataset, the continent only dataset and on the English dataset.

Phylogenetic analysis

Phylogenetic reconstructions were undertaken on unique haplotypes by maximum-likelihood (ML) criteria (Beerli and Felsenstein, 2001) and a Bayesian approach. The K80+I model (Kimura, 1980) of DNA substitution

(transition/transversion ratio = 18.374, proportion of invariable sites = 0.817) was applied to the ML and the Bayesian analyses as determined by the program jMODELTEST 0.1.1 (Wu and Eisen, 2008; Posada, 2008) using Bayesian Information Criterion (BIC). The ML analysis was undertaken in PHYML 3.0 (Wu and Eisen, 2008) and the Bayesian inference in MRBAYES 3.1 (Ronquist and Huelsenbeck, 2003). For the Bayesian analysis, four chains of 10^7 iterations each were run, taking samples every 100 generations and the first 25% of trees were discarded as burn-in. The statistical support of the branching pattern obtained by ML approach was assessed by 1,000 bootstrap replicates. In addition, a haplotype network (Excoffier and Smouse, 1994) was constructed using ARLEQUIN and visualised in HAPSTAR (Teacher and Griffiths, 2011). Demographic history was inferred from Fu's F_S, a measure of marker neutrality sensitive to changes in population sizes, and mismatch distributions. Populations that have undergone recent expansion display significantly negative F_S value (Fu and Li, 1993) and a smooth or unimodal distribution of pairwise differences among haplotypes (Schneider and Excoffier, 1999). These analyses were run in ARLEQUIN with 10,000 bootstrap replicates to generate the simulated mismatch distribution in a population expansion model and 10,000 randomised samples to test for F_S significance.

Results

Microsatellites

Tests of assumption, genetic diversity and basic statistics

Of the 11 microsatellites genotyped, one (AF141650) demonstrated high level of estimated null alleles, genotyping inconsistencies and significant deviation from the assumption of HW equilibrium in a large number of sampled roosts. It was thus removed from subsequent analyses. Of the remaining loci, three deviated from HW equilibrium in one to four roosts out of 36 and six showed low to moderate levels of estimated null alleles. Deviation from HW expectations was due to heterozygote deficiency and since it was noticed in only a few roosts, it could be caused by factors other than by selection on the markers. No significant linkage disequilibrium between loci was detected after sequential Bonferonni correction. The ten remaining microsatellites were thus used for subsequent analyses. The mean genotyping error rate for the ten markers was 4.21% (± 2.50%). Mean observed and expected heterozygosity and within roost gene diversity were moderate and consistent across all roosts (H_o = 0.540 ± 0.06; H_E = 0.572 ± 0.04; H_S = 0.604 ± 0.04; Table S3), and allelic richness was low but still similar across all roosts (A_R = 2.9 ± 0.1; Table S3). The continent had significantly higher allelic richness than England ($p < 0.001$), but none of the other genetic diversity measures differed significantly between the two (H_O: $p = 0.594$; H_E : $p = 0.196$, H_S: $p = 0.127$, F_{IS}: $p = 0.721$)

Estimated F_{ST} averaged over loci was low at 0.048 but significant (0.039 – 0.059 95% confidence interval). Roosts were found to be significantly genetically differentiated (randomization test: $p<0.001$), which in combination with the significant structure, suggests the roosts sampled were not part of a single panmictic population.

Bayesian modelling

Despite high levels of admixture, clustering was consistent with the sampling geography. At K = 2, the sites on the continent and eastern England formed one group and those in western England and Isle of Wight (IOW) another. At K = 3, the continent was discriminated from eastern England. At K = 4 and 5, the IOW was distinct (Figure 2 and S1). The standardised second order rate of change ΔK indicated that the most likely number of clusters describing the data was three (Figure S1). Because this method only distinguished higher structuring level, each cluster was then run separately with the same settings. No obvious geographical sub-structure was supported for the continent or for eastern England. In contrast, sub-structure was revealed in the third cluster, with roosts on the IOW segregating from western England.

Multivariate analysis

The discrimination between roosts using DAPC was low, reflecting high within roost genetic variability and high admixture levels (91.72% of individuals have membership probabilities to the sampling roost lower than 90%, Jombart 2011). Despite this admixture, the first two PCs captured 42% of the genetic structure. PC-1 mostly delineated roosts from the continent and the IOW from those on mainland England, whilst PC-2 discriminated roosts from west of England and the IOW from those on the continent and in east of England, a pattern of clustering similar to the STRUCTURE analysis (Figure 2).

Population structure was also inferred by sPCA. The three first axes had the highest eigenvalues and were therefore retained. PC-1 mostly segregated England from the continent. PC-2 differentiated eastern England from western England and western continental sites from Eastern Europe. The continental sPCA scores were low compared to English ones, suggesting that the continental structuring was weak compared to that across England. PC-3 retrieved a final structure by clustering sites in France with the IOW and Central England (Figure S2). Thus, the UK was clearly differentiated from the continent and subdivided into three groups representing the east, the west and the IOW (Figure 3).

AMOVA and genetic differentiation among clusters

Four populations were retained through consensus from the previous analyses - continental Europe, eastern England, western England and the IOW (Table S2). Differentiation tests were significant both between and within populations (F_{CT} = 0.034, p = 0.0001; F_{SC} = 0.025, p = 0.0001), suggesting both the stratification of the genetic variation and the importance of roosts as structuring units within those clusters. Pairwise F_{ST} suggested a closer relationship between the continent and eastern England than with the other clusters, a very close relationship between eastern and western England, and a stronger connection between the IOW and western England than to the other clusters. The IOW was also more connected to the continent than to eastern England (Table 1). Differentiation was significant for all pairwise connections with the exception of east-west England (p = 0.510) and continent-IOW (p = 0.652).

Isolation by distance

Genetic and geographical distances were significantly correlated when controlling for the effects of physical barriers on the full dataset ($r = 0.214$, $p = 0.021$), on the continental dataset ($r = 0.463$, $p = 0.024$) and on the English dataset ($r = 0.302$, $p < 0.001$) (Figure S3). The physical barriers tested also had a significant effect on genetic differentiation when controlling for geographical distance at all scales (Europe: $r = 0.373$, $p < 0.001$; Continent: $r = 0.511$, $p = 0.024$; England: $r = 0.560$, $p < 0.001$).

Contemporary gene flow

Roosts were pooled into the four populations retained from previous analyses, and recent migration rates were estimated as the mean proportion of individuals moving between populations (Table 2). The continental population displayed the highest emigration rate and the lowest immigration rate, indicating that contemporary gene flow over the Channel is mostly biased toward England. Interestingly, the IOW seems to play an important role in mediating gene flow with immigration coming mostly from the continent (5.16%). Within England, most gene flow was to the west (from east 4.55% and from IOW 3.66%), whereas there was little flow to the east or to the IOW (Table 2). It is however worth noting that England has been more intensively sampled than the continent and the full extent of genetic diversity on mainland Europe has not been sampled. As a result, some finer sub-structuring might not have been recovered. This could affect the estimation of gene flow between the UK and mainland Europe and the

391 values quoted are therefore to be taken as a relative indication of the magnitude
392 of gene movement. Furthermore, since most continental samples are from central
393 Europe rather than bordering the English Channel, gene flow is likely to be
394 underestimated.

395 *Mitochondrial genetic diversity and differentiation*

396 A total of 26 haplotypes were identified among the 441 samples from 35 roosts.
397 Thirty polymorphic sites (7.79%) were recorded with a transition/transversion
398 ratio of 18.37. Higher diversity was observed in continental sites than in English
399 roosts (mean pairwise differences: $p < 0.01$; haplotype diversity: $p < 0.05$;
400 nucleotide diversity: $p < 0.01$; Table S2). Thus, all continental sites sequenced for
401 more than one individual displayed several haplotypes. No continental haplotypes
402 were shared among roosts, the exception being the French samples that shared
403 all five haplotypes with several samples from eastern England and the IOW (Figure
404 4). In contrast, in England, one haplotype was shared by 322 out of the 409
405 English samples, and all but one English roost contained this haplotype.
406 Furthermore, 13 out of the 28 English roosts were fixed for this haplotype. More
407 diversity was found in the roosts of eastern England and the IOW than in western
408 England, which displayed only six haplotypes, two of them being shared with the
409 rest of England. Estimated ϕ_{ST} was high at 0.405 and significant, indicating strong
410 differentiation among roosts at the mtDNA level.

411 *Mitochondrial population structure*

412 The sPCA investigated sequence variability in a spatial context. The two first axes
413 had the highest eigenvalues and were therefore retained. PC-1 separated France
414 and England from the rest of the continent, although the boundary was not sharp
415 and a slight cline across the whole of Europe could be noticed. PC-2 created two
416 clusters on the continent corresponding to eastern sites (Slovakia, Poland) and
417 western sites (Italy, Switzerland, France). It also segregated the IOW from the rest
418 of England, and eastern England from western England, although these last scores
419 were very low, suggesting this structure is weak (Figures 3 and S4).

420 AMOVA produced a simpler structure with the among groups variance
421 component optimised for three groups (ϕ_{CT} = 0.481): eastern continent, western
422 continent and England (Table 3). Pairwise ϕ_{ST} between the three groups (Table 4)
423 indicated strong differentiation between England and both the eastern and
424 western continental populations, and much weaker differentiation between the
425 two continental populations. All pairwise differentiation tests were significant (p =
426 0.008). The Mantel test revealed a significant correlation between genetic and
427 geographical distances when controlling for the potential effects of geographical
428 barriers ($r = 0.281$, $p < 0.05$; Figure S3). However, physical barriers did not
429 contribute in structuring genetic variation when controlling for geographical
430 distances ($r = 0.109$, $p = 0.170$). When splitting the full dataset into a continental
431 one and an English one, no cline in genetic differentiation or effect of
432 geographical barriers were detected on the continent or in England (continent:

geographical distance: r = 0.206, p = 0.209, barriers: r = 0.295, p = 0.074; UK: geographical distance: r = 0.079, p = 0.118, barriers: r = 0.019, p = 0.175).

Phylogenetic analysis

The haplotype network displayed a highly connected star-shaped structure, indicative of rapid population expansion (Figure 4). Some division within England was observed, although the two haplotypes responsible differed by only one base-pair, suggestive of the weakness of this structure. The topology of the ML and Bayesian analyses demonstrated a similar pattern of shallow genealogy among haplotypes (Figure 4). Overall there was a lack of obvious clustering or clear geographical patterns in the haplotype genealogies suggesting that all the haplotypes belong to the same clade and indicating rapid population expansion following a demographic bottleneck. This was confirmed by the mismatch analysis run on the three populations defined by the AMOVA and by F_S values. The English population displayed significantly negative F_S and non-significant sum of squared deviation (SSD) and raggedness index, indicating no significant difference from a model of sudden expansion (Table S4 and Figure S4). The signal of population expansion was less clear for eastern continent and western continent as the mismatch analysis also yielded non-significant SSD and raggedness indices (Table S4), despite mismatch distributions more consistent with those from stable populations (Figure S4). Furthermore, their F_S values, while negative, were non-significant (Table S4). Together those results indicate that the continental populations are likely stable while the English one recently expanded.

Discussion

Our analysis of the genetic structure of *E. serotinus* across Europe and within England revealed low but significant population genetic structure at the largest geographical scale, with English serotines being somewhat distinct from their European peers. There were different patterns of genetic partitioning within these populations, with stronger genetic homogeneity on the continent than in England, consistent with the hypothesis of reduced movement at edge population compared to core population. Despite this structuring, high admixture and assessment of gene flow among the differentiated populations indicated westward movement within England and a lesser role of the English Channel as physical barrier than initially thought. Stronger differentiation in mtDNA was detected, suggesting females are more philopatric than males, as we expected from a bat, and therefore gene movement is mostly mediated by male connectivity. We discuss each finding in turn, as well as the limitations of our study and direction for further research where appropriate.

Genetic differentiation among roosts across Europe was low (F_{ST} = 0.048) but significantly different from panmixia, with a pattern of isolation by distance indicating some limit to gene flow. Bats tend to be relatively mobile and levels of population structuring are often low (Moussy *et al.*, 2013). For example, the migratory bat *Nyctalus noctula* displays very low levels of genetic structuring across Europe (F_{ST} = 0.006) (Petit and Mayer, 1999). The fixation index we estimated for *E. serotinus,* while low, is relatively high for a bat and reflects values

477 reported for non-migratory species (Moussy *et al.*, 2013). Nonetheless, the
478 current evidence indicates *E. serotinus* is relatively sedentary.

479 Consistent with the departure from panmixia, bayesian and multivariate analyses
480 of nuclear data recovered four genetic populations, geographically represented as
481 continental Europe, east of England, west of England and the Isle of Wight. Some
482 evidence for physical barriers to gene flow was also found. Hence, while there was
483 weak evidence that mountains act as barriers, the sea appears to restrict gene
484 flow to a degree, even between England and the Isle of Wight - 6km. This distance
485 is less than the mean commuting-distance/night recorded for *E. serotinus* (Catto
486 *et al.*, 1996). However, open water restricts movement for many bats (García-
487 Mudarra *et al.*, 2009; Moussy *et al.*, 2013).

488 Despite the large geographical scale (> 3,000 km) and several mountain ranges,
489 individual-based analyses of the nuclear markers failed to detect any strong sub-
490 structuring within continental Europe. Similar findings have been reported in
491 other taxa (Demont *et al*. 2008) and suggest genetic homogenisation over a large
492 geographical scale and possibly high gene flow across the continent. Lack of
493 information on the species' mating system and movements restricts our
494 understanding of the mechanisms driving this gene flow. However, it is worth
495 noting that the sampling design to assess continental genetic diversity and
496 distribution thereof was not ideal since only eight locations across the continent
497 were sampled. The full extent of genetic diversity could thus not be recovered.
498 Finer genetic sub-structuring could have be missed and further work with more

499 intensive sampling across more locations is required to fully understand patterns
500 of genetic partitioning across the continent.

501 Within England, the fixation index was as high as within continental Europe, in
502 spite of the much smaller geographical scale, and smaller effective population
503 sizes could contribute to the high fixation index there. All analyses of nuclear
504 markers identified three UK populations: eastern England, western England and
505 the Isle of Wight. This pattern of stronger genetic structure in England than on the
506 continent had previously been reported in other bat species (Flanders *et al.*,
507 2009). The drivers for this relatively strong structuring are unknown, but the UK
508 represents the most northern limit of many bat species, including *E. serotinus*, and
509 this could affect individual behaviour (e.g. dispersal) and population dynamics,
510 and influencing genetic structure. Lower dispersal at range edges has indeed been
511 shown in other taxa (Travis and Dytham, 1999), primarily due to increased
512 dispersal costs and reduced habitat density (Dytham, 2009). The identification of
513 an Isle of Wight population can be explained by the sea restricting gene flow
514 between the island and mainland England. However, no obvious physical or
515 behavioural barriers, habitat fragmentation or lack of connectivity seem to explain
516 what appears to be two partially isolated populations on the English mainland,
517 although greater urbanisation in eastern England could potentially limit
518 movement. An alternative explanation is that the East/West structure in England
519 represents two catchments of gene circulation, possibly driven by limited and
520 geographically distinct swarming/hibernation sites with ongoing gene flow
521 through male, and to a lesser extent female, dispersal. Our finding of structure in

England starkly contrasts with a recent study of the genetic structure of *E. serotinus* in Poland (Bogdanowicz *et al.*, 2013). The geographical scale of our study and the Polish one are similar, and four out if the seven microsatellites they used were used here. However, Bogdanowicz *et al*. failed to detect any nuclear structure, recovering only one genetic population and very low differentiation among roosts (F_{ST} = 0.01). While these results differs from our English findings, they are consistent with our continental results, despite the sampling limits of our study.

Historical events, like the post-glacial recolonisation of Europe, influence the partitioning of genetic variation for some bats (Flanders *et al.*, 2009), and the significantly differentiated eastern and western continental clusters we detected at the mitochondrial level could be due to post-glacial recolonisation from separate refugia, with the low structure in the nuclear markers reflecting high contemporary gene flow. Additionally, lower mitochondrial diversity was found in England compared to mainland Europe, which, together with a unimodal mismatch distribution, could indicate past population bottlenecks before rapid population expansion in the UK. However, again, caution needs to be used in this interpretation due to the low continental sampling. Increasing the sample size and coverage on the continent and at the putative contact zone would be required to reconstruct the post-glacial history of the species and its contemporary influence on the distribution of genetic diversity.

While significant genetic structure was found for *E. serotinus*, there was nonetheless high admixture, as indicated by Bayesian and multivariate analyses of nuclear markers and the low F_{ST}. This indicates strong ongoing gene flow among populations rather than lack of drift because of recent population divergence, which in turn indicates regular movement of individuals between populations, even across the English Channel. Specifically immigration from continental Europe to the species' northern English range was inferred from nuclear data. Immigrants appear to be going mostly to the Isle of Wight and eastern England. Whilst eastern England is geographically close to continental Europe, the shortest distance to the Isle of Wight is ca. 150 km, so it is less clear why it appears so connected to mainland Europe. However interpretation here has to be tempered by the low number and the location of the continental samples and unfortunately no samples could be obtained from the continental borders of the English Channel. Gene flow analysis therefore only estimated movements from the overall continental population and might not reflect the true extent of gene movement, which are probably underestimated.

Within England the low biparental differentiation between populations and the recovery of eastern mitochondrial haplotypes in the west indicates most bat movements are from east to west. Similarly, movements between the Isle of Wight and mainland England are mostly directed toward western England. This could indicate a westward expansion of the species range, consistent with the lower haplotypic and nucleotide diversity in western England. *E. serotinus* has indeed been mostly recorded in south eastern England and its presumed absence

566 from far west has never been fully explained since habitat and roosting
567 opportunities seem favourable. However, since 2004, the National Bat Monitoring
568 Programme (NBMP) has reported a significant regional changes in bat
569 distributions, with a steep increases in the southwest (Bat Conservation Trust,
570 2013). This is consistent with reports from local bat workers and volunteers
571 registering *E. serotinus* calls in western counties on a more frequent basis,
572 prompting them to suspect the establishment of new communities in the west
573 (Marshall, pers. comm.).

574 Significant differentiation in the nuclear and the mitochondrial data occurred
575 among sites at the European scale. However, estimates of genetic differentiation
576 revealed that the level of population structure displayed by mtDNA haplotypes
577 was ten times higher than that inferred by nuclear markers. While the comparison
578 of differentiation between different markers is difficult due to different rates and
579 modes of mutations, with high migration, as expected for vagile species like bats,
580 mutation is likely to contribute little to differentiation (Balloux and Lugon-Moulin,
581 2002). Therefore the observed difference between biparental and maternal
582 fixation indices is probably the result of differences in effective population size for
583 the markers and sex-biased gene flow. Conservatively assuming that sexual
584 selection in *E. serotinus* is weak, the contrast in differentiation measured between
585 the two types of markers exceeds their expected four-fold difference in effective
586 population size. This suggests that gene flow in *E. serotinus* is mostly mediated by
587 males, while higher differentiation among roosts for mtDNA indicates female
588 philopatry (assuming the demography of this marker is a true reflection of

589 population demography (Balloux, 2010)), which correlates with the qualitative
590 experience of workers studying *E. serotinus* across Europe (Hutson, pers. comm.;
591 Harbusch and Racey 2006). The suggestion of male-biased gene movement and
592 female philopatry we infer is widely observed in temperate bats (Moussy *et al.*,
593 2013), but further work analysing adult male movements is required to confirm
594 this pattern.

595 Nonetheless, some female dispersal occurs in *E. serotinus* as several
596 mitochondrial haplotypes are shared among roosts. For example, all haplotypes
597 found in French specimens are shared with roosts in eastern England and the Isle
598 of Wight. The coexistence of these haplotypes within roosts could be the results
599 of past colonisation events, and possibly the contemporary recruitment of
600 dispersing females. Similarly, one haplotype common to eastern England was also
601 identified in a roost in the west, consistent with female dispersal and recruitment.
602 However, the pattern of isolation by distance found over Europe suggests limits to
603 female dispersal, although this could also reflect local selection for different
604 haplotypes (Balloux 2010; Arnqvist *et al.* 2010). Furthermore, no haplotypes are
605 shared among continental sites. The geographical scale involved there is much
606 larger than within England, and it therefore appears that female dispersal occurs
607 over sub-continental spatial scales (e.g. <500km). This is further supported by the
608 fact that with the exception of the main English haplotype that is widely
609 distributed, most shared haplotypes in the UK are found in neighbouring roosts.
610 Hence, whilst female dispersal can occur over longer distances, most females
611 apparently remain philopatric to their natal landscape if not to their natal roost.

Conclusion

Our findings indicated considerable gene flow on a large geographical scale in continental Europe despite the presence of physical barriers that could contribute to some level of sub-structuring, but further work with better sample coverage is required to confirm these patterns. Nonetheless putative movement could contribute to large scale circulation of EBLV-1 in Europe, maintaining the high viral prevalence recorded there (Schatz et al., 2013). This also seems to be concordant with the relatively low genetic diversity of EBLV-1 across Europe possibly promoted by its host's movement (McElhinney *et al.* 2013). We also suggest that the English Channel, while apparently restricting some gene flow, does not appear to be a substantial barrier, and similar inferences have been drawn for *M. daubentonii* (Atterby *et al.*, 2010), which is the host for EBLV-2, a virus found both on the continent and in the UK (Harris *et al.*, 2006). The relative fragmentation of serotine populations in England may sufficiently alter host-virus epidemiology to explain the apparent absence of EBLV-1 in England. This explanation is consistent with a previous small-scale comparison of the genetic structure of the EBLV-1 (*E. serotinus*) and EBLV-2 (*M. daubentonii*) hosts that revealed more structure within England in *E. serotinus* than in *M. daubentonii* (Smith *et al.*, 2011). The cause(s) of the reduced movement of serotines within the UK remains to be established.

Acknowledgements

The authors acknowledge DEFRA and University of Exeter for funding this study. We are grateful to the local bat groups for their help in locating and arranging

634 access to roosts, to all the home-owners for their hospitality and tolerance during
635 field work, and to the volunteers that assisted in the capture and sampling. We
636 also thank Laurent Arthur, Philippe Christé, Adrino Martinoli, Martina Spada,
637 Martin Celuch, Wieslaw Bogdanowicz, and Loseb Natradze for providing the
638 continental wing biopsies. Javier Juste shared his experience in sequencing
639 serotine mitochondrial DNA and provided primers sequences and conditions. We
640 also thank the Editor and Referee's for constructive comments which greatly
641 improved the manuscript.

642 **Conflict of Interest**

643 The authors declare no conflict of interest.

644 **Data archiving**

645 Mitochondrial haplotypes will be deposited in Genbank

646 Microsatellite genotypes and sample location records will be deposited in Dryad

647 Supplementary information is available at Heredity's website
648 (http://www.nature.com/hdy)

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