Bat population genetics and *Lyssavirus* presence in Great Britain

G. C. SMITH[1]*, J. N. AEGERTER[1], T. R. ALLNUTT[1], A. D. MacNICOLL[1],
J. LEARMOUNT[1], A. M. HUTSON[2] AND H. ATTERBY[1]

[1] *The Food and Environment Research Agency, Sand Hutton, York, UK*
[2] *Winkfield, Station Road, Plumpton Green, East Sussex, UK*

SUMMARY

Most lyssaviruses appear to have bat species as reservoir hosts. In Europe, of around 800 reported cases in bats, most were of European bat lyssavirus type 1 (EBLV-1) in *Eptesicus serotinus* (where the bat species was identified). About 20 cases of EBLV-2 were recorded, and these were in *Myotis daubentonii* and *M. dasycneme*. Through a passive surveillance scheme, Britain reports about one case a year of EBLV-2, but no cases of the more prevalent EBLV-1. An analysis of *E. serotinus* and *M. daubentonii* bat genetics in Britain reveals more structure in the former population than in the latter. Here we briefly review these differences, ask if this correlates with dispersal and movement patterns and use the results to suggest an hypothesis that EBLV-2 is more common than EBLV-1 in the UK, as genetic data suggest greater movement and regular immigration from Europe of *M. daubentonii*. We further suggest that this genetic approach is useful to anticipate the spread of exotic diseases in bats in any region of the world.

Key words: Animal pathogens, genetics, lyssavirus, rabies (animal).

INTRODUCTION

European bats are host to two lyssaviruses: European bat lyssavirus type 1 (EBLV-1), which is identified in most of the >800 European cases [1] and European bat lyssavirus type 2 (EBLV-2), which has only been recorded about 20 times [2]. Both cause fatal rabies in humans, although cases are extremely rare. However, contact with bats results in an unspecified number of post-exposure prophylaxis (PEPs) in humans within Europe, e.g. Britain (H. Kirkbride, personal communication) and France [3] both record over 100 per year. EBLV-1, is most closely related to Duvenhage virus [4], and is generally found in serotine bats (*Eptesicus serotinus*), which account for more than 95% of cases where the bat species is identified [5]. *E. serotinus* is found across most of Europe, but is restricted to southern parts of Sweden and England [6]. The *E. serotinus* population size in Britain has been estimated by extrapolation at 15 000 [7]. In The Netherlands alone, one-third of recovered bats that had bitten humans tested positive for EBLV-1, and 12% of bats recorded in contact with cats were EBLV-1 positive [8]. EBLV-2 is most closely related to Australian bat lyssavirus, and classical rabies virus [4], and has been recorded in Daubenton's bat (*Myotis daubentonii*) and the Pond bat (*M. dasycneme*). *M. daubentonii* occurs commonly across Europe, including southern Sweden, Norway, Finland and most of Britain and Ireland. In Britain the population

* Author for correspondence: Dr G. C. Smith, The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, North Yorkshire, UK.
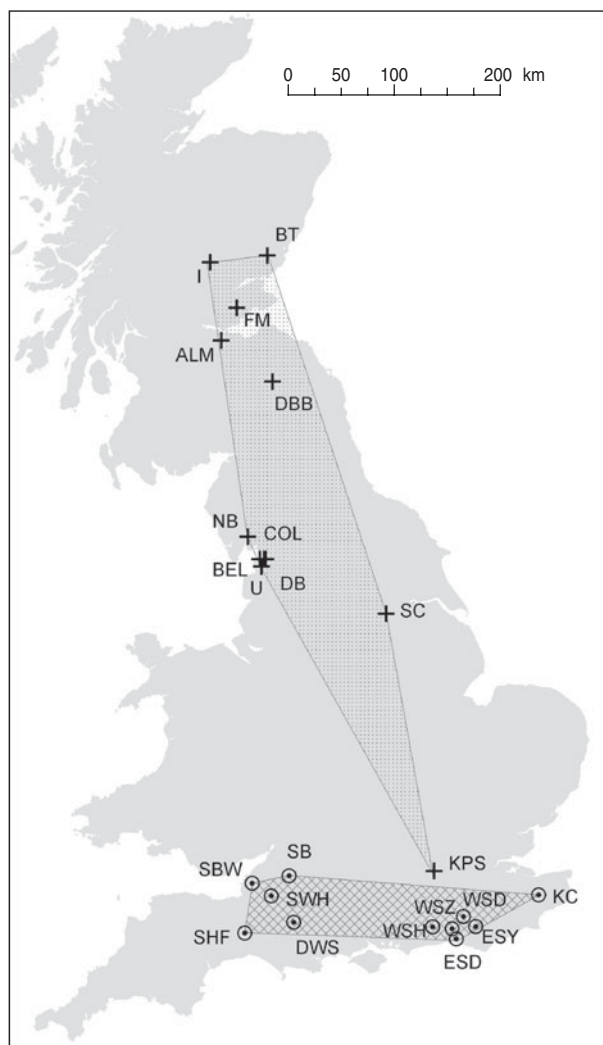(Email: graham.smith@fera.gsi.gov.uk)

Fig. 1. Distribution of *M. daubentonii* and *E. serotinus* colonies sampled in the UK. The number of samples taken at each colony are given in Table 1. +, Location of *M. daubentonii* colonies sampled; ⊙, location of *E. serotinus* colonies sampled. The shaded areas show the extent of the sampled range within each species.

Table 1. *The number of bats sampled at each colony. The geographical locations of the colonies are given in Figure 1*

M. daubentonii colony	No. of bats sampled	*E. serotinus* colony	No. of bats sampled
BT	70	SBW	21
I	10	SWH	33
FM	27	SB	45
ALM	18	SHF	15
DBB	7	DWS	25
NB	38	WSH	16
COL	72	WSZ	8
BEL	28	WSD	14
U	53	ESD	45
DB	47	ESY	9
SC	33	KC	33
KPS	40		

while also confirming the ongoing maintenance of an extremely rare lyssavirus (EBLV-2) in *M. daubentonii*. The occurrence of bat rabies in Britain depends on two factors: (i) introduction – it must be carried across the English Channel by infected bats and (ii) persistence – it must be maintained in the recipient population. These factors depend on the movement or migration of individual bats, and this can be inferred by analysis of genetic variation of colonies and local populations. Here, we contrast the genetic variations of *E. serotinus* and *M. daubentonii* within Britain and use this to produce a hypothesis for the current EBLV occurrence in Britain. We propose that further work is undertaken to examine this hypothesis, as greater understanding of genetic variation may provide new information on movement patterns related to mating and thus help our understanding of lyssavirus transmission.

METHODS

E. serotinus wing puncture samples were collected from maternity roosts in Sussex, Dorset and Somerset in England, from 2004 to 2006 (Fig. 1). These wing punctures had no short- or long-term deleterious effect on the bats' ability to fly. Analysis of these samples were compared with those collected from *M. daubentonii* caught at summer roosts in central and southern Scotland, and Cumbria, Lancashire, Yorkshire and Middlesex in England, between 2003 and 2006 (Fig. 1, Table 1) [13]. The geographic area covered by the *M. daubentonii* colonies studied was about 150 km west to east and 600 km north to south,

size is estimated by extrapolation at 150 000 [7]. *M. dasycneme* is one of the rarer European bats occurring in northern continental Europe, but generally restricted in the west, and absent from Britain.

Although most of EBLV-1 cases have occurred in *E. serotinus*, there are no cases in Britain. However, of the ~20 confirmed cases of EBLV-2, eight cases have occurred in *M. daubentonii* in England [9] and one *M. daubentonii* [10] and one human case [11] in Scotland. Active sampling of *M. daubentonii* in England has revealed a seroprevalence of 1–4% [12]. It seems unlikely that sampling artefacts alone have resulted in the common European lyssavirus (EBLV-1) appearing to be absent from English *E. serotinus*,

Table 2. *Details of microsatellite loci optimized for* E. serotinus

Locus	Forward and reverse sequence 5′ to 3′*	Microsatellite repeat	Magnesium concentration (mM)	Annealing temperature (°C)	No. of alleles	Allele size range (bp)	Ref.
Paur05	F: 6-FAM-GGACAGTATGCCATGTTATGCTG R: GCACTTTCACAAACCTAGATGG	$(GT)_{10}$	3·0	66–56†	11	235–255	[24]
AF141650	F: HEX-ACAGGAACCCTCAGAAGTGG R: TGGTCTCCTTTTCTTCACTTTGT	$(TATC)_9$	3·0	52	11	267–307	[25]
NN8	F: NED-TTGTGTTTTAAAGAAAATCC R: ATAGGTGATTTCCATTCCCA	$(GT/CA)_{21}$	1·5	44	2	141–143	[26]
EF1	F: 6-FAM-ATCTGGGCAATGATACCTTT R: GCAGGCTGGGCTGAG	$(GT)_{22}CT(GT)_{17}$	1·5	50	2	177–183	[27]
EF4	F: HEX-ATAGGCTCCCAGAAATAGC R: GATCACCACAAAATGTGC	$(CT)_4(GT)_{17}$	1·5	48	6	215–229	[27]
EF6	F: HEX-ATCACATTTTTGAAGCAT R: ATCTGTTTTTCTCTCCTTAT	$(GT)_{20}$	1·5	41	16	159–195	[27]
EF14	F: HEX-ATCATATATTTGTGTTCTGG R: AAAATCAGCTATGTAGCAC	$(GT)_{19}$	1·5	43	9	113–129	[27]
EF15	F: NED-AGCAGCAAAGGGGACTCAGA R: GAGAAGCAGGGAGGGCATTT	$(CA)_3GA(CA)_{20}$	1·5	55	11	109–131	[27]

* ABI dyes HEX, NED, 6-FAM are indicated where present on the forward primer.
† Touchdown programme consisting of 20 cycles of 66–56 °C falling by 0·5 °C per cycle followed by 30 cycles at 56 °C.

whereas the region covering *E. serotinus* colonies sampled was about 280 km west to east and 70 km north to south. All procedures were in adherence to UK Home Office guidelines [Animals (Scientific Procedures) Act 1986] and under licence from the appropriate nature conservation bodies. Upon collection all biopsy samples were stored in 70% ethanol at 4 °C, until analysis.

Simple sequence repeat (SSR) markers from other closely related species were optimized for *E. serotinus* as detailed in Table 2. A more detailed, within-population, analysis of the serotine data will be published in a later paper. Here we concentrate on population summary level statistics to compare between these species. Different SSR markers were used for *M. daubentonii*, so the full dataset from both species cannot be analysed together. DNA extraction and genotyping methods for both species have been described earlier along with statistical analysis [13]. Common population genetic statistics were calculated: F_{ST}, the proportion of variation in populations [14, 15] was calculated using the AMOVA procedure of Arlequin [16], and used to produce dendrograms, drawn by Phylip [17], to illustrate the structure and degree of genetic variation in the two species in the UK. H_E, gene diversity, is calculated within populations only; F_{IS}, the proportion of alleles expected to be identical in an individual; and H_T, total heterozygosity, were calculated with Genetix [18]. F_{IS} P values were calculated using a 1000 replicate bootstrap. Arlequin [16] was used to estimate linkage disequilibrium (LD) and Hardy–Weinberg equilibrium (HWE). Mantel tests were performed between pairwise geographic distance and F_{ST} with 1000 replicates using the Microsoft Excel add-in, PopTools (http://www.cse.csiro.au/poptools/).

RESULTS

A total of 264 *E. serotinus* and 443 *M. daubentonii* bats were included in the analysis. For this analysis, each roost was treated as a separate population. Within all *M. daubentonii* colonies, the average proportion of pair-wise loci in significant LD was 11·6% ($P \leqslant 0.05$) and 9·4% of loci were not in HWE (all heterozygote deficiency). Within all *E. serotinus* colonies, the average proportion of pair-wise loci in significant LD was 6·5% ($P = 0.05$) and 13·6% of loci were not in HWE (all heterozygote deficiency). In colonies F_{ST} estimates (Table 3) were low for both species, but greater for *E. serotinus* than

Table 3. *A comparison of F statistics and genetic diversity for* E. serotinus *and* M. daubentonii *populations*

Statistic	*E. serotinus*	*M. daubentonii*
F_{ST}	0·034	0·019
F_{IS}	1 colony >0*	No colonies >0*
H_E range	0·6–0·7	0·7–0·8
H_T	0·68	0·84

See Methods section for a definition of each statistic.
* $P < 0.05$.

M. daubentonii between the UK colonies sampled. There was little or no inbreeding (F_{IS}) observed within colonies of either species [only 1 colony (KC) had a significant result; $F_{IS} = 0.1$, $P(F_{IS} > 0) < 0.05$]. Within-population genetic diversity (H_E) was high in both species, but slightly higher in *M. daubentonii* (Table 3).

Pair-wise F_{ST} values between all *E. serotinus* and *M. daubentonii* colonies sampled are shown in Tables 4 and 5, respectively. Comparative pair-wise F_{ST} dendrograms of *M. daubentonii* and *E. serotinus* colonies show clearly the different scale of genetic structure between the two species (Fig. 2). There is a significant east–west substructure within *E. serotinus* populations studied ($F_{ST} = 2.85\%$, $P = 0.002$), with the eastern populations in Sussex (WSH, WSZ, WSD, ESD, ESY) and Kent (KC) clustering separately from those in Somerset (SHF, SBW, SB, SWH) and Dorset (DWS). The *M. daubentonii* colonies studied show a degree of genetic separation by distance with two northerly clusters [13]; however, there is much less genetic differentiation between colonies compared to the differentiation evident between *E. serotinus* colonies.

When the distribution map of *E. serotinus* and *M. daubentonii* colonies sampled in this study (Fig. 1) is compared with the scaled dendrograms for each species (Fig. 2), it is apparent that in the UK the population genetic structure of *E. serotinus* colonies is on a smaller geographic scale than the structure of *M. daubentonii* colonies. The geographic scale of the *M. daubentonii* colonies studied covers about three times the area of the *E. serotinus* colonies sampled; however, roughly twice the amount of genetic differentiation is found within the smaller area from which *E. serotinus* colonies were sampled.

A Mantel test between geographic distance and pair-wise F_{ST} showed a significant correlation in *E. serotinus* ($\rho = 0.602$, $P = 0.004$) but was not significant in *M. daubentonii* ($\rho = 0.369$, $P = 0.058$).

Table 4. *Pair-wise F_{ST} values (below the diagonal) and their significance (as P values) for* E. serotinus *colonies sampled. The geographical locations of the colonies are given in Figure 1*

	KC	ESD	ESY	WSH	WSD	WSZ	SD	SBW	SWH	SHF	DWS
KC	0	0·000	0·108	0·027	0·000	0·333	0·000	0·000	0·000	0·000	0·000
ESD	0·026	0	0·135	0·000	0·000	0·171	0·000	0·000	0·000	0·000	0·000
ESY	0·018	0·007	0	0·162	0·000	0·757	0·009	0·036	0·000	0·000	0·000
WSH	0·018	0·024	0·015	0	0·054	0·405	0·000	0·000	0·000	0·000	0·000
WSD	0·041	0·057	0·067	0·020	0	0·000	0·000	0·000	0·000	0·000	0·000
WSZ	0·007	0·009	−0·013	0·007	0·030	0	0·036	0·153	0·000	0·000	0·009
SD	0·033	0·038	0·027	0·032	0·070	0·021	0	0·496	0·045	0·027	0·054
SBW	0·022	0·041	0·022	0·033	0·058	0·016	0·000	0	0·081	0·153	0·036
SWH	0·043	0·043	0·055	0·035	0·073	0·040	0·007	0·010	0	0·000	0·009
SHF	0·052	0·067	0·055	0·049	0·085	0·059	0·019	0·010	0·027	0	0·009
DWS	0·059	0·062	0·064	0·056	0·107	0·047	0·008	0·018	0·012	0·033	0

Significant F_{ST} values are represented by P value (above the diagonal) of <0.05.

Table 5. *Pair-wise F_{ST} values (below the diagonal) and their significance (as P values) for* M. daubentonii *colonies sampled. The geographical locations of the colonies are given in Figure 1*

	BT	I	FM	ALM	DBB	U	DB	BEL	COL	NB	SC	KPS
BT	0	0·005	0·006	0·000	0·005	0·000	0·000	0·000	0·000	0·000	0·000	0·000
I	0·021	0	0·124	0·016	0·006	0·000	0·000	0·000	0·000	0·000	0·007	0·002
FM	0·008	0·009	0	0·072	0·008	0·000	0·000	0·000	0·000	0·000	0·000	0·000
ALM	0·016	0·026	0·007	0	0·384	0·003	0·014	0·060	0·001	0·057	0·000	0·000
DBB	0·021	0·055	0·021	0·002	0	0·002	0·003	0·012	0·000	0·062	0·002	0·000
U	0·035	0·041	0·022	0·016	0·030	0	0·010	0·627	0·000	0·000	0·000	0·000
DB	0·028	0·029	0·018	0·009	0·026	0·006	0	0·674	0·039	0·000	0·099	0·009
BEL	0·029	0·048	0·021	0·007	0·020	−0·002	−0·002	0	0·118	0·006	0·000	0·000
COL	0·034	0·040	0·021	0·016	0·037	0·014	0·003	0·003	0	0·000	0·001	0·000
NB	0·035	0·041	0·024	0·006	0·013	0·021	0·009	0·011	0·022	0	0·000	0·000
SC	0·035	0·021	0·019	0·015	0·031	0·016	0·003	0·013	0·011	0·016	0	0·390
KPS	0·029	0·021	0·015	0·023	0·040	0·022	0·006	0·017	0·011	0·023	0·000	0

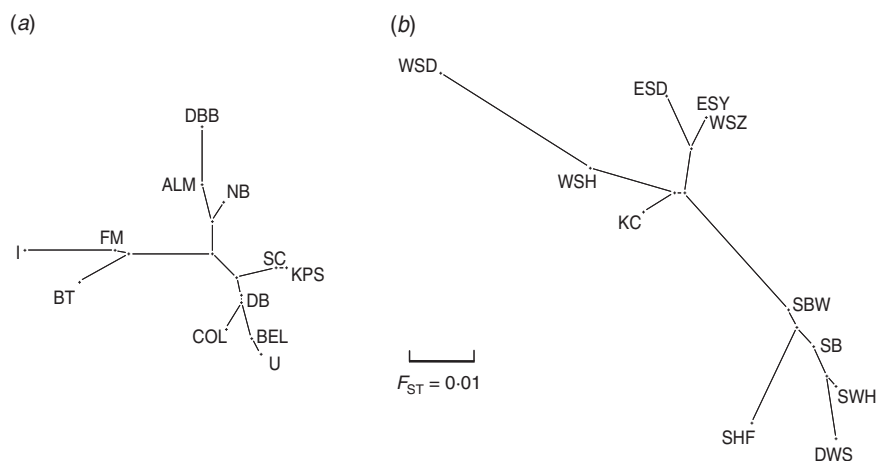Significant F_{ST} values are represented by P value (above the diagonal) of <0.05.

Fig. 2. Pair-wise F_{ST} dendrogram of (*a*) *M. daubentonii* and (*b*) *E. serotinus* colonies sampled in the UK. The letters represent the colony locations as shown in Figure 1.

DISCUSSION

There was no extreme divergence from HWE in either species' colonies, as shown by the observed low F_{IS} values and no major differences in genetic diversity in colonies within species (H_E). The greater genetic differences observed between populations of *E. serotinus* compared to *M. daubentonii* within the UK are likely to be the result of lower migration/mixing for mating between *E. serotinus* colonies in the UK compared to *M. daubentonii* colonies. The lack of inbreeding in colonies indicates that none of the colonies sampled in either species are genetically isolated; however, the lower genetic diversity (H_E) in *E. serotinus* compared to *M. daubentonii* is concordant with the greater genetic differentiation found between *E. serotinus* colonies compared to *M. daubentonii* colonies. More extensive sampling of *M. daubentonii* in Scotland [19] demonstrates very low microsatellite F_{ST} values, in line with the differences reported here.

M. daubentonii is among the species that performs 'autumnal swarming' (mating) behaviour, where individuals from many local colonies gather in large numbers to mate [20] and individuals are known to move >30 km between maternity roost and swarming sites [21]. There is currently no published evidence to suggest that *E. serotinus* exhibit this same behaviour; female *E. serotinus* are noted to disappear from their maternity colonies in the autumn [22] but there are no records of autumn gatherings for mating in the UK, and their mating system appears yet to be described. However, as a large bat, it is a strong flyer, with mean daily movement distances to foraging sites of 8 km, and maximum nightly distances flown exceeding 40 km [23]. It is possible that they may still swarm within a small geographic area, but the mating strategies would have to be significantly more confined than in *M. daubentonii* to explain the results of this current study.

M. daubentonii genetic structure in Western Europe is relatively homogeneous [13], suggestive of recent expansion, or a relatively well mixed population, with some similarity to English populations. Whereas preliminary analysis of a few *E. serotinus* roosts in northern Europe suggests a more marked differentiation with English roosts (H. Atterby, unpublished data). The genetic data are suggestive that there is more movement between mainland Europe and the UK for *M. daubentonii* than for *E. serotinus*. If this is correct, then the introduction of a disease into the UK is more likely if carried by *M. daubentonii*. In Europe,

EBLV-1 in *E. serotinus* is much more common and widespread than EBLV-2 in *M. daubentonii*. However, the risk of introduction to the UK would not be solely proportional to the prevalence of disease, so the risk of EBLV-2 introduction may be higher than expected by the rate of occurrence of this disease in continental Europe.

Assuming that EBLVs arrive in the UK, then *M. daubentonii*, with higher gene flow, will be more likely to establish and spread EBLV-2. The more isolated *E. serotinus* colonies indicate that the geographic spread of EBLV-1 is less likely. Thus, the genetic data suggests that, following arrival, EBLV-2 would spread across Britain, whereas EBLV-1 may remain more geographically constrained, and if it did not spread successfully, may actually go extinct due to stochastic chance in a small population. Based on the available genetic data, we therefore hypothesize that, over time, EBLV-1 may exist for short periods in a few *E. serotinus* colonies in the UK but does not transfer to sufficient individuals to remain within the population on a long-term basis, and as a result remains undetected. However, it is still possible that given sufficient time, EBLV-1 could both arrive and spread successfully in the UK. In order to test this hypothesis it is suggested that more precise comparisons of the population genetic structure of continental and UK serotine (and other species) colonies and the genetic relationship between them are made. Alternatively, it is also possible that the geographical restriction of EBLV-2 could be due to factors other than the gene flow of the host; such as climatic restrictions limiting the maintenance to roosts in particular climatic regions, or different local behavioural adaptations of the host.

A general understanding of the movement patterns of European bats, inferred through genetic data, may be useful in understanding the potential for exotic lyssavirus spread into new areas. Such analysis is not limited to EBLVs, but may also help our predictions for the spread of the new species of *Lyssavirus* recorded in insectivorous bats in Asia and Africa.

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DECLARATION OF INTEREST

None.

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