

# Population Genetics of the Eurasian Bat (*Myotis brandtii*)

Megan Forster<sup>1</sup>, Janine Coombes<sup>1</sup>, Steve Paterson<sup>2</sup> and Thomas Lilley<sup>2</sup>.

<sup>1</sup>3<sup>rd</sup> year Bioveterinary Science BSc, School of Life Sciences, Liverpool, UK, L69 7ZB. <sup>2</sup>The Institute of Integrative Biology, Liverpool, UK, L69 7ZB.

Population structure defines relatedness between individuals that live in the same place or in different geographical locations. Individuals may be more related when they live in small groups or are isolated from other populations, due to inbreeding. Individuals from populations that are less related tend to be separated by long distances or geographical barriers such as mountains. An understanding of a species' population structure can help conserve species and understand how diseases spread between populations. This is particularly important in bats as they carry a lot of viruses that can infect humans, such as rabies. This study investigated population structure within the Eurasian Brandt's bat (*Myotis brandtii*) and subspecies found in Mongolia (*Myotis brandtii gracilis*). This study found a lack of population structure in the European populations but found the Mongolian population to be isolated from the others. Populations with increased distances between them were less related. However, the European populations still appear to be breeding with one another, suggesting a lack of geographical barriers. Male bats tend to travel further distances than female bats and how this affects population structure is an interesting focus for further study.

## Abstract

Genetic studies of bat populations are important due to the wider implications within conservation and the control of transmissible diseases. Brandt's bats (*Myotis brandtii*) are widely distributed throughout Europe and Asia and exhibit swarming; a promiscuous mating system that reduces the risk of inbreeding. The aim of this study is to use seven microsatellite *loci*, polymorphic in *Myotis brandtii*, to determine the level of large-scale population structure across eight populations in Europe and Asia, including a population of the cryptic subspecies *Myotis brandtii gracilis*. The distance between sites is large and highly variable with multiple potential dispersal barriers. Genetic diversity between and within populations was measured using Hardy-Weinberg exact tests, F-statistics and the Mantel test assessed isolation by distance (IBD). The results of this study indicated a low level of population structure between the individuals sampled in Europe, with high levels of heterozygosity. The Mongolian population of suspected subspecies *M. b. gracilis*, was the most genetically differentiated from the other populations. Mantel tests found significant IBD between each population, with a gradual increase in genetic differentiation with geographical distance. Despite high IBD, evidence of high gene flow and heterozygosity suggests IBD is not considered a conservation issue in the Brandt's bat. This study stimulates the need for further investigation into sex-biased dispersal.

## Introduction

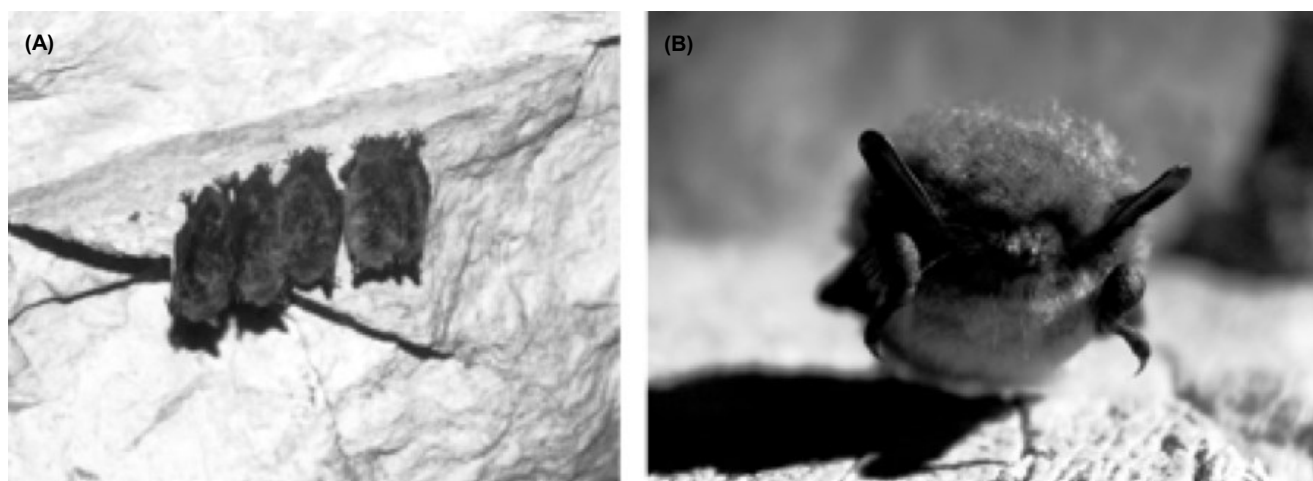
Data obtained through population studies has implications within conservation biology. Knowledge of migration between sub-populations can help to predict the likely impacts of habitat destruction and fragmentation. *M. brandtii* is currently listed as 'least concern' on the IUCN red list due to high abundance (1) and widespread distribution throughout Europe and Asia (2). However, in the event that a species becomes threatened or endangered, population studies can help make informed decisions regarding breeding programmes and reintroductions (3-4). Identifying the lack of population structure in the closely related Natterer's bats (*Myotis nattereri*) highlighted the importance of the identification and conservation of swarming sites, as they are invaluable in reducing inbreeding by increasing gene flow between sub-populations (5).

Population genetics also give an insight into movement patterns, aiding the development of accurate analytical models of the spread of disease (7). This is particularly important within bats as they are major natural reservoirs of several multi-host viruses (SARS-Like Coronaviruses, MERS-Like Coronaviruses, Nipah virus, Ebola virus, Rabies virus and Hendra viruses) (8). European Bat Lyssaviruses type 1 (EBLV-1), type 2 (EBLV-2) and Bokeloh Bat Lyssavirus (BBLV), are all causative agents of rabies in European bats, and have been isolated from several *Myotis* bat species (*M. daubentonii*, *M. dasycneme* and *M. nattereri*) (9). As a result of habitat disturbance, bats are living in closer proximity to humans and the infection of new host species is becoming more frequent, posing a

higher risk for human infection (10). Studies also provide an insight into the epidemiology of diseases affecting bats. White-nose syndrome (WNS) is a fungal disease of North American bats and causes death in susceptible species (11). Population studies of little brown bats (*Myotis lucifugus*) suggested that population structure, derived from female migration patterns, influenced the spread of WNS across Pennsylvania and West Virginia (12).

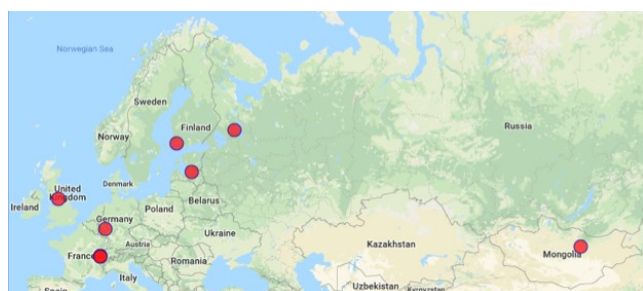
In this study, seven microsatellite *loci*, polymorphic in *M. brandtii* (13-15) are analysed to identify genetic variation between and within sub-populations of *M. brandtii* and subspecies *M. brandtii gracilis*. This study will provide evidence to assess the level of population structure and isolation by distance in *M. brandtii* and discuss the implications. Ecological data of the Brandt's bat is limited due to cryptic morphology, nocturnality and ability of flight. *M. brandtii* morphologically resembles three other species (*Myotis alcaethoe*, *Myotis mystacinus* and *Myotis ikonnikovi*) (16). *M. brandtii* is considered a small bat species with an adult body weight of 4-8 g and can live upwards of 40 years (2). They inhabit temperate broadleaf, mixed or coniferous woodland, typically near water (17), and feed on non-aquatic small insects and spiders (18). Summer roosts are being found more frequently near human habitation; in roofs and bird boxes, but more commonly in hollow trees. Hibernation locations tend to be in caves, cellars, mines and tunnels (19).

The Brandt's bat could show population substructure. A previous study found that Schreibers' long-fingered bat,



**Figure 1.** A small group of Brandt's bats roosting in a cave (A) and ventral photograph of a single Brandt's bat (B); reproduced from (55).

*Miniopterus schreibersii natalensis*, demonstrated strong population substructure in the South, West and North-East regions of South Africa. Without obvious geographical barriers, the study concluded that the genetic diversity between the three sub-populations resulted from morphological differences and local biomes (20). However, due to the bats' flight ability, promiscuity and presence at swarming events, we predict *M.brandtii* will show little to no population structure, similar to that of Daubenton's bats (*Myotis daubentonii*) (21). Many studies have found a lack of population structure to be universal in seasonally migratory bats (22).



**Figure 2.** Red dots represent sampling locations across Europe. Map created for this study using mapping software [online] Available from: <https://www.darrinward.com/lat-long/>

## Methods

A total of 135 bats were sampled from eight locations including England (Easegill Caverns), Germany (Mayener Grubenfeld), Switzerland (Gouffre de la Pleine Lune and Couffre Cathy within Parc Jurassien Vaudois), Finland (Turku), Latvia (Sikspārņu Cave), Russia (Petrozavodsk) and Mongolia (Ulan Bator) (Fig. 2). DNA was extracted and purified from 3 mm wing punches (stored in 70% ethanol at  $-80^{\circ}\text{C}$ ) using the Qiagen DNeasy Blood and Tissue Kit. Wing punches (3 mm) do not disrupt flight and heal completely in 2-3 weeks (23). The yield of genomic DNA was measured using the Thermo Fisher Invitrogen Qubit Fluorometer. Single template multiplex PCR was carried out with a final volume of 10  $\mu\text{l}$ , containing: Qiagen Multiplex PCR Master Mix, forward and reverse primers (fluorescently labelled) (Table 1), Genomic DNA, and

$\text{dH}_2\text{O}$ . Thermal cycling programme:  $95^{\circ}\text{C}$  for 5 minutes followed by 28 cycles ( $95^{\circ}\text{C}$  for 30 seconds,  $59^{\circ}\text{C}$  for 90 seconds and  $72^{\circ}\text{C}$  for 30 seconds and lastly,  $60^{\circ}\text{C}$  for 30 minutes). The PCR products were diluted with water (1:100) and amplicons were separated by capillary electrophoresis on the Applied Biosciences 3500xL Genetic Analyzer. Genemapper V software (24) was used to size and assign alleles.

For each sample site, observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), allelic richness and effective number of alleles ( $A_e$ ) was calculated using GENALEX 6.5 (25) add-in for Microsoft Excel. Guo and Thompson's (26) exact Hardy-Weinberg test was used to assess compliance with Hardy-Weinberg Equilibrium (HWE) for each locus in each population using the population genetics program GENEPOP 4.6 (27). Hardy-Weinberg exact tests are performed frequently in population genetics as deviations from HWE may highlight problems such as selection bias (28) and genotyping errors (29). The Markov Chain Method is used to estimate the exact  $P$ -values for each locus in individual populations and Fisher's method was used to calculate the overall  $P$ -value of all  $loci$  in each population. Pairwise  $F_{ST}$  values were calculated using GENEPOP 4.6 (27).  $F_{ST}$  (the fixation index) is the value for genetic distance. It is proportional to the level of inbreeding within subpopulations relative to the metapopulation (30). The inbreeding coefficient ( $F_{IS}$ ) of an individual relative to the subpopulation were calculated using GENALEX using the Weir & Cockerham (31) method. A negative  $F_{IS}$  value suggests individuals are less related to each other and a positive  $F_{IS}$  value suggests individuals are less related. Isolation by distance is assessed on GENALEX using the Mantel test, which compares genetic distance and geographical distance. Statistical significance of  $R$  (mantel coefficient) was attained by forming 999 permutations.

Ethical approval for this study was obtained by my supervisor, Professor Steve Paterson.

## Results

The yield of genomic DNA, extracted from 3 mm wing punches, ranged from 1.9–220  $\text{ng}/\mu\text{l}$ , with a mean final concentration of 20.2  $\text{ng}/\mu\text{l}$ .

Locus	Sequences (5'-3') Forward (F) and Reverse (R) Primer	Array	Fluorescent Tag	Allele Size Range (bp)	Marker concentration (mM)
A24-Mluc <sup>(13)</sup>	F: GTGGTATGAAATAACCAGTTCACCTTTG R: GTTTCAGACTGCATTACTGAAGAAATTATGG	(AC)n	FAM	473-491	0.2
Clone A2-Mluc <sup>(13)</sup>	F: TGGCCCATGCTCATCATC R: GTTCTGGTCTCAACTGGGTGCTC	(CA)n	VIC	91-135	0.05
D9 <sup>(14)</sup>	F: GTTCTTTCTCCCTGTGCTC R: TCTGGACCCAAAATGCAGG	(CT)n	NED	120-150	0.2
E24 <sup>(14)</sup>	F: GTTTCAGGTTCAATCCCTGACC R: AAAGCCAGACTCCAAATTTCTG	(TC)n	FAM	215-253	0.2
ES43-Mluc <sup>(13)</sup>	F: GTTTAAGGGGAGAGGAGTGG R: GCTGCGTCCAGAGG	(AC)n	FAM	377-403	0.2
H29 <sup>(15)</sup>	F: GTTTCAGGTGAGGATTGAAAACAC R: GCTTATTTAGCATTTGGAGAGC	(CA)n	FAM	170-202	0.4
Paur 6 <sup>(16)</sup>	F: GATCAGATTTCCAAACAGAG R: GTTTAGGTTCTTCTTCAGCTATG	(AC)n(AG)n	PET	156-186	0.2

**Table 1.** Adapted from (22). Forward and reverse primer sequences, arrays, fluorescent tag, allele size range (bp) and marker concentration (mM) for the microsatellite *loci* used in this study. Allele sizes adapted for *M. brandtii*.

Sample site	n	Observed heterozygosity	Expected heterozygosity	Allelic Richness	Effective Number of Alleles	HWE <i>p</i>
England	21	0.649	0.703	7.14	4.67	0.3638
Germany	33	0.686	0.694	10.00	5.05	0.0540
Switzerland	23	0.621	0.641	7.71	4.73	0.2198
Finland	25	0.584	0.661	9.14	4.42	< 0.001
Latvia	20	0.634	0.652	8.57	4.96	0.2613
Russia	13	0.702	0.670	6.29	4.41	0.4213
Mongolia	29	0.842	0.803	10.00	5.96	0.9192

**Table 2.** Genetic diversity indices averaged across microsatellite *loci* for each population and the HWE *P*-values for exact tests. n= sample size.

Sample Site	A24-Mluc	Clone A2-Mluc	D9	E24	ES43-Mluc	H29	Paur6
England	0.4286	0.3708	0.9683	0.2280	<b>0.0226</b>	1.0000	0.6281
Germany	<b>0.0388</b>	0.1466	0.1358	0.0656	0.4036	0.6770	0.5965
Switzerland	-	<b>0.0386</b>	0.5689	0.0945	0.5664	0.5725	0.6693
Finland	<b>0.0005</b>	<b>0.0035</b>	0.0608	0.1211	0.1792	<b>0.0116</b>	0.7078
Latvia	1.0000	0.1308	0.8831	0.6416	<b>0.0051</b>	1.0000	0.5691
Russia	0.5046	<b>0.0060</b>	0.8908	0.3585	0.7923	1.0000	0.9758
Mongolia	0.1413	0.8094	0.7102	<b>0.0000</b>	<b>0.04041</b>	0.8657	0.8804

**Table 3.** HWE *p* values for each locus in each individual population calculated using Markov chain method. The significant values are underlined ( $P < 0.01$ ) and in boldface ( $P < 0.05$ ).

### Population Diversity

The observed heterozygosity in each population ranged from 0.584-0.842 (Table 2). One population (Finnish) out of six deviated significantly from HWE for  $P < 0.05$ . Three *loci* deviated significantly from HWE in the Finland population ( $P < 0.05$ ) as opposed to a maximum of two *loci* in other populations (Table 3). Russia and Mongolia had higher observed heterozygosity (0.702 and 0.842, respectively) than expected (0.670 and 0.803) whilst the other populations had lower observed heterozygosity than expected (Table 3). Mongolia and Russia also had the least number of positive  $F_{IS}$  values (two *loci*) inferring higher levels of inbreeding than expected as opposed to a minimum of three *loci* in the other populations. Positive inbreeding coefficients ( $F_{IS}$ ) were found in all populations at a maximum of six *loci* (Finland). The highest inbreeding coefficient (1.000) was found at the Finnish site for the locus A24-Mluc (Table 4), where all individuals are homozygous. The lowest inbreeding coefficient (-0.3333) was found at the Russian site (Table 4).

### Population Differentiation

Pairwise genetic distance ( $F_{ST}$ ) and geographical distance are presented in Table 5.  $F_{ST}$  values range from 0.0055 (Latvia and Germany) to 0.1662 (Finland and Mongolia). The largest linear geographical distance (7018 km) exists between Switzerland and Mongolia. The Mantel test found

a positive correlation between genetic distance and geographical distance (Mantel test:  $R = 0.416$ ,  $P < 0.001$ ) showing significant isolation by distance.

## Discussion

### Population Diversity

Each population had high heterozygosity across *loci*. One of the possible explanations for high individual population heterozygosity values in *M. brandtii* is that they are one of many bat species present during swarming (32-34). Swarming is a phenomenon that reduces the risk of inbreeding, taking place in late Summer and early Autumn, where large numbers of bats visit hibernacula (34). Currently, there is no consensus as to why bats swarm but there are multiple hypotheses. Three principal theories include opportunities to mate (35), information transfer from parental bats to offspring and, to assess suitable hibernacula (34). Opportunity to mate as a hypothesis has the most supporting evidence. Bats display significant social vocalisation, chasing and copulatory behaviour during this period (36). There is significant evidence to suggest swarming events increase gene flow between sub-populations, increasing genetic diversity in progeny (32).

Bats also demonstrate reproductive strategies known to increase genetic diversity within other animal species. The behaviours demonstrated by *M. brandtii* during mating periods are described as indiscriminate and promiscuous

Sample Site	A24-Mluc	Clone A2-Mluc	D9	E24	ES43-Mluc	H29	Paur6
England	0.5714	0.1765	-0.0026	0.2139	0.1504	-0.1799	-0.0696
Germany	0.1754	0.1384	-0.0999	0.1537	-0.0613	-0.0604	0.0000
Switzerland	-	0.2636	-0.0649	0.0686	0.1304	0.1493	-0.1020
Finland	1.0000	0.3070	-0.0360	0.1273	0.0523	0.2222	0.0213
Latvia	-0.1053	0.0788	-0.1362	0.0917	0.1268	-0.1347	0.0710
Russia	-0.3333	0.2809	-0.0602	0.0000	-0.0614	-0.0588	-0.0254
Mongolia	-0.0162	-0.0859	-0.1027	0.0403	0.0949	-0.0897	-0.0335

**Table 4.**  $F_{IS}$  values at each *loci* for each population. Results that infer higher levels of inbreeding than expected are in boldface (31).

Sample Site	England	Germany	Switzerland	Finland	Latvia	Russia	Mongolia
England		790.3	1057	1628	1764	2292	6880
Germany	0.0214		436.4	1465	1413	2085	6678
Switzerland	0.0272	0.0204		1873	1773	2468	7018
Finland	0.0195	0.0153	0.0161		391.6	665.5	5276
Latvia	0.0123	0.0055	0.0139	0.0083		709.4	5265
Russia	0.0241	0.0200	0.0212	0.0198	0.0100		4616
Mongolia	0.1488	0.1622	0.1645	0.1662	0.1590	0.1554	

**Table 5.** Pairwise  $F_{ST}$  values and distance between populations (km). Distances (km) are above the diagonal.  $F_{ST}$  values are below the diagonal.

(35). After copulation, females store spermatozoa within their reproductive tract, delaying ovulation and fertilisation until Spring (35, 37). Studies on dissimilar species have demonstrated that sperm storage and promiscuity increase genetic diversity, for example, in the *Anolis sagrei* lizard (38) and passerine birds (39).

Despite populations showing high observed heterozygosity and not deviating significantly from HWE, each population had positive inbreeding coefficients ( $F_{IS}$ ) at various *loci*, particularly in three *loci* (Clone A2-Mluc, E24 and ES43 Mluc). This contradiction could be attributed to sampling bias, small sample sizes and null alleles (40).

### Population Differentiation

Pairwise European  $F_{ST}$  values indicated little genetic differentiation between populations, using guidelines for interpretation (41), indicating low population structure in the Brandt's bat. The results suggested there are no significant barriers to gene flow in Europe. These findings are in agreement with other population genetic studies on seasonally migratory bats such as the Daubenton's bat (21) and wide ranging species such as the Regent Honeyeater bird, *Anthochaera phrygia* (42). The Mongolian population had high pairwise  $F_{ST}$  values with all other populations, suggesting they are isolated from the others and not interbreeding.

Low  $F_{ST}$  values suggest populations are not significantly genetically distinct. However significant IBD presents a barrier to gene flow. As distance increases, populations become more genetically diverse. Geographical barriers of movement in the Brandt's bats may include mountain ranges and large expanses of different climates (deserts). Bright lights and disruption of vision can affect bat migration and tracking long-range movements (43) suggesting brightly lit cities and roads have the potential to restrict geneflow. However, migratory behaviour which can reach up to 618 km (43-45), and flight abilities may be the reason for the apparent lack of barriers to gene flow between sub-populations.

Similarly to the apparent lack of geographic barriers found in this study, Castella *et al.* (14) also identified a lack of population structure between two populations of *Myotis myotis* on either side of the Gibraltar Strait (14 km wide stretch of water separating peninsular Spain and Gibraltar from Morocco and Ceuta in Africa). However, further analysis of a mitochondrial gene (cytochrome b) confirmed the two populations were genetically distinct with no interbreeding over the Strait of Gibraltar. This suggests other methods of molecular analysis on the European populations of *M. brandtii* are required to definitively state whether they are interbreeding. *Myotis* bats also demonstrate a lack of site fidelity, changing roosts frequently depending on their reproductive status (35, 46), encouraging interbreeding between sub-populations. Male bats are recorded to disperse further than females. Females tend to be more philopatric as they have to exploit resources and provide parental care to immobile progeny (21, 47). Population structures generally fall into three categories – populations divided by age, sex or social/geographic factors (48). This study analysed geographic factors suggesting further investigation into age and gender diversity indices is necessary to determine sex barriers on population structure.

### Implications within conservation and Disease transmission

Low genetic distance between populations suggests a lack of geographical barriers within Europe which is promising for bat conservation. This study highlights the importance of protecting swarming sites as swarming species have higher gene flow (49). A major feature currently concerning the conservation of many species is climate change. Studies have indicated that European bats may face a serious threat with climate change (50) and may respond with a population shift northwards, breeding earlier (51) or changing migration times (52). This study confirms the widespread distribution of *M. brandtii* in temperate regions and tropical climates (Mongolia) suggesting they are able to adapt to changing climates, providing there is available habitat northwards.

Migratory bats are known to migrate over open sea as they are frequently found on remote islands, ships and oil rigs (53). This study indicates that British bat populations are interbreeding with continental European populations; presenting an interesting insight into the spread of diseases, such as the rabies virus. Many studies focus on the control of dogs and foxes to control the spread of rabies, and highlight rabies-free status in some European countries. However, rabies is a trans-boundary disease due to the migratory nature of bats, and the importation of infected animals and reinfections have occurred in Italy, Greece and Slovakia (54). This highlights the need for trans-boundary and species-wide approaches to controlling the spread of rabies.

## Conclusions

In summary, European populations of *M. brandtii* show low population structure but significant isolation by distance. The high  $F_{ST}$  values between the Mongolian population of *M. b. gracilis* suggest they are isolated and not breeding with the other populations. Similar to the Gibraltar Strait study (14), further molecular analyses could confirm or refute the lack of population structure in Europe. There are a number of possible reasons for high heterozygosity and low  $F_{ST}$  values in the European populations, despite significant isolation by distance, such as: a lack of physical boundaries, ability of flight, migratory behaviour, swarming, lack of site fidelity, sex-biased dispersal and promiscuity. Similar literature suggests a study on sex-biased dispersal would provide further insight into the population structure of Brandt's bats. These findings highlight the importance of conserving swarming sites to reduce inbreeding, and the possible consequences to climate change. They also support the idea of trans-boundary disease control strategies due to the migratory nature of bats.

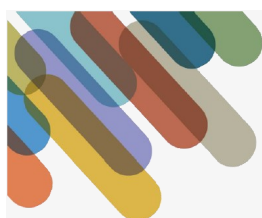
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## Driving is a risky business!

Dr Laura Bonnett (2020 William Guy Lecturer)

**ROYAL  
STATISTICAL  
SOCIETY**  
DATA | EVIDENCE | DECISIONS

Wednesday 2nd December 2020 15.30-17.00

Leggate Theatre, Victoria Gallery and Museum  
(Building 421 in grid square D7 on campus map)



Named in honour of William Augustus Guy, an early medical statistician and past RSS president, this prestigious volunteer role recognises fellows with a successful track record in undertaking school outreach activities. Over 2020, Laura (pictured) will be delivering lectures to statisticians and students across the UK on the topic of 'Driving is a risky business!' which covers various aspects of road safety where statistics can illuminate people's decisions. The lecture will include a discussion regarding conditional probabilities, together with ethical considerations, which will appeal across the curricula.

For talk abstract, and registration details please go to the RSS Merseyside website  
<https://sites.google.com/site/rssmerseyside/research-meetings/william-guy-lecturer>

**FREE  
TO ATTEND**