

**Further evidence for cryptic north-western refugia in Europe?
Mitochondrial phylogeography of the sibling species *Pipistrellus pipistrellus*
and *Pipistrellus pygmaeus***

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The geographic ranges of European plants and animals underwent periods of contraction and re-colonisation during the climatic oscillations of the Pleistocene. The southern Mediterranean peninsulas (Iberian, Italian and Balkan) have been considered the most likely refugia for temperate/warm adapted species. Recent studies however have revealed the existence of extra-Mediterranean refugia, including the existence of cryptic north-west European refugia during the Last Glacial Maxima (24–14.6 kyr BP). In this study we elucidated the phylogeographic history of two sibling bat species, *Pipistrellus pipistrellus* and *P. pygmaeus* in their western European range. We sequenced the highly variable mtDNA D-loop for 167 samples of *P. pipistrellus* ($n = 99$) and *P. pygmaeus* ($n = 68$) and combined our data with published sequences from 331 individuals. Using phylogenetic methodologies we assessed their biogeographic history. Our data support a single eastern European origin for populations of *P. pygmaeus* s.str., yet multiple splits and origins for populations of *P. pipistrellus* s.str., including evidence for refugia within refugia and potential cryptic refugia in north western Europe and in the Caucasus. This complex pattern in the distribution of mtDNA haplotypes supports a long history for *P. pipistrellus* s.str. in Europe, and the hypothesis that species with a broad ecological niche may have adapted and survived outside southern peninsula throughout the LGM.

Key words: novel refugia, western Europe, last glacial maxima, mammal, Chiroptera

A deep divergence time between sister species of *Eidolon* (Pteropodidae) with evidence for widespread panmixia

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The pteropodid fruit bat genus *Eidolon* is comprised of two extant species: *E. dupreanum* on Madagascar and *E. helvum* on the African mainland and offshore islands. Recent population genetic studies of *E. helvum* indicate widespread panmixia across the continent, although island populations off western Africa show genetic structure. Little is known about the genetic connectivity of *E. dupreanum* or the divergence time between these two sister species. We examine sequence data for one mitochondrial (*cyt-b*) and three nuclear regions (*β -fib*, *RAG1*, and *RAG2*) to assess population genetic structure within *E. dupreanum* and divergence between the two *Eidolon* spp. In addition, we characterize the demographic history of both taxa using coalescent-based methods. We find little evidence for population structure within *E. dupreanum*, and suggest that this reflects dispersal based on seasonal fruit availability and a preference for roosting sites in exposed rock outcrops. However, despite apparent panmixia in both *Eidolon* spp. and large dispersal distances reported in previous studies for *E. helvum*, these two taxa diverged in the mid-to-late Miocene. Both species are also characterized by population expansion and young, Pleistocene clade ages, although slower population growth in *E. dupreanum* is likely explained by its divergence via colonization from the mainland. Finally, we discuss the implications of population connectivity in *E. dupreanum* in the context of its potential role as a reservoir host for pathogens capable of infecting humans.

Key words: phylogeography, divergence time, Africa, Madagascar, *Eidolon*

Population genetics of the Mauritian flying fox, *Pteropus niger*

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The Mauritius flying fox *Pteropus niger* is distributed on the islands of Mauritius and La Réunion in the western Indian Ocean. Although recent studies have examined the phylogenetics and systematics of this genus, relatively few have assessed the population genetics of species distributed on oceanic islands and no study has focused on the demographics of *P. niger*. Here, we present mitochondrial DNA sequence data from 39 individuals of *P. niger* collected from four main colonies distributed throughout Mauritius. Our results indicate that the Mauritian population of *P. niger* is likely panmictic, with moderate to high levels of gene flow occurring among colonies distributed across the island. Collectively, our sequence data suggest moderate levels of genetic variation within the population. These findings will help to inform ongoing conservation and disease surveillance initiatives.

Key words: genetic structure, Mauritius, phylogeography, *Pteropus niger*

MHC-DRB exon 2 diversity of the Jamaican fruit-eating bat (*Artibeus jamaicensis*) from Mexico

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Major Histocompatibility Complex (MHC) encodes a group of closely linked genes that play a central role in the vertebrate immune system, those are crucial for understanding the influence of natural selection on genetic diversity in wild populations. We examined genetic variation at the MHC class II DRB gene in 15 sampled localities of the Jamaican fruit-eating bat (*Artibeus jamaicensis*) in Mexico. *Artibeus jamaicensis* is one of the most abundant and widely distributed species in the Neotropics, and is therefore an excellent species in which to examine immunological gene variation. Using PCR amplifications, cloning and sequencing, we assessed individual DRB allelic diversity. Sequences from 193 individuals were analyzed and no deletions or insertions were detected, thus likely representing functional alleles. We identified 161 alleles (allele diversity = $0.9789 + 0.0022$), with three to five alleles per individual, suggesting gene duplication events. Our results suggest the presence of recombination involved with generating DRB diversity in *A. jamaicensis*; we detected one recombination breakpoint and one recombination event. In the antigen-binding site (ABS), the average number of nonsynonymous substitutions per site is greater than the synonymous substitutions per site (0.7033 versus 0.2966, respectively) providing evidence for positive selection acting above the evolutionary history of the species in shaping MHC diversity.

Key words: antigen-binding site, immunogenetics, positive selection

Stable isotopes reveal that little brown bats have a broader dietary niche than northern long-eared bats

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Stable isotope analysis (SIA) was used to quantify intra- and inter-specific variation in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in plagiopatagium tissue of little brown (*Myotis lucifugus*) and northern long-eared (*M. septentrionalis*) bats in several regions of the Canadian Maritimes where they occur in sympatry. There was large intraspecific variation in the $\delta^{13}\text{C}$ of *M. lucifugus*, with the range exceeding 30‰, whereas the range of $\delta^{13}\text{C}$ values observed in *M. septentrionalis* was less than 7‰. Similarly, the standard ellipse area of *M. lucifugus* was larger than that of *M. septentrionalis* at all sites and together, these data support the contention that *M. lucifugus* has broader dietary niche breadth than *M. septentrionalis*. Some *M. lucifugus* from Fundy National Park, New Brunswick exhibited very low $\delta^{13}\text{C}$ values, suggesting an energy input from an unknown source, possibly with carbon assimilated from biogenic methane. High $\delta^{13}\text{C}$ values for *M. lucifugus* from Brier Island, Nova Scotia are consistent with a diet that is at least partially derived from marine sources. Finally, $\delta^{15}\text{N}$ values for both species from Prince Edward Island were high relative to New Brunswick or Nova Scotia, suggesting inputs of anthropogenically-derived nitrogen from a more agriculturally-intensive landscape.

Key words: bats, diet, *Myotis lucifugus*, *M. septentrionalis*, niche, stable isotopes, sympatry

Winter and summer torpor in a free-ranging subtropical desert bat: the fishing myotis (*Myotis vivesi*)

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Use of torpor likely favors the survival of subtropical bats in harsh environments. The fishing myotis (*Myotis vivesi*) is a species endemic to desert islands in the Gulf of California, where summers are extremely hot and winters are cold and windy. We explored thermoregulating abilities of *M. vivesi* measuring skin temperature (T_{skin}) on free-ranging individuals in winter 2010 and 2011, and in summer 2010. We also measured ambient (T_a) and roost (T_{roost}) temperatures during the study, and we obtained data for wind speed at night time during winter periods. We found that all bats entered torpor in both winters and that at least three individuals hibernated for several days, which had not been reported previously for bats in subtropical deserts. In summer, three individuals entered short bouts of shallow torpor in early mornings. Roosts were slightly warmer than T_a in winter at nighttime, and in summer they never reached temperatures $> 38.7^\circ\text{C}$, even at $T_a \approx 45^\circ\text{C}$. Roost occupancy in winter was higher during windy nights in 2010 but no pattern was found in 2011. Therefore, in winter fishing myotis were more likely to remain in their night roosts and enter torpor when ambient conditions (e.g., strong winds) limit fishing on marine waters. In summer, roosts provide good insulation against high T_a , and bats might not need to resort to torpor to lower their metabolic rate except for a brief period during early mornings. When resources are limited the use of torpor may increase this insular species' chances of survival.

Key words: daily torpor, deserts, fishing bats, Gulf of California, heterothermy, hibernation, Vespertilionidae

Roosting habits of Daubenton's bat (*Myotis daubentonii*) during reproduction differs between adjacent river valleys

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The use of different roost types by Daubenton's bats (*Myotis daubentonii*) during reproduction was investigated in two adjacent river valleys in northeastern Scotland. Forty-six individuals from six colonies were radiotracked during the summers of 2004–2006. The frequency of roost switching varied with reproductive status, and was lowest in lactating females and highest in non-reproductive females, which changed roosts on average once every 5.0 and 1.5 days, respectively. Although Daubenton's bats regularly switched roosts, strong faithfulness to the roosting area was apparent regardless of whether they formed maternity colonies in trees or buildings. Although most roosts found at both study areas were in trees, lactating females and juveniles in one valley roosted only in trees whereas in the other they roosted exclusively in buildings, in which ambient temperatures were significantly higher. The implications of roosting habits for the transmission of European bat lyssavirus are discussed.

Key words: Daubenton's bat, *Myotis daubentonii*, reproduction, roosting behaviour, roost switching, EBLV2

**Roost site selection in pregnant and lactating soprano pipistrelles
(*Pipistrellus pygmaeus* Leach, 1825) at the species northern extreme:
the importance of warm and safe roosts**

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In summer reproducing female bats prefer roosts with temperatures in their thermo neutral zone, but in northern Europe, such roosts may be difficult to find. In this study, summer roost site selection of pregnant and lactating soprano pipistrelles were investigated using radio telemetry (2004–2006) in a fiord landscape with complex topography at the species northern extreme. The soprano pipistrelles' common roost sites were in hollow trees ($n = 14$), but they also used crevices in rock walls ($n = 2$) and the attic of one building. The bats exclusively sought out roosts on the north side of the fiord with the slope facing south, where solar irradiation yielded the warmest micro climate. From 2005 to 2014, bat boxes were erected at three sites in the study area. From 2012 to 2014 we only found maternity roosts in such structures, thus there was a clear shift in roost site selection by the soprano pipistrelle in the study area. Man-made structures, such as buildings and bat boxes, provided the warmest cavities, whereas natural cavities did not differ significantly from air temperatures. The bats did not find thermo neutral roosts most of the time, and this should affect the energy budgets, and thus the bats' behaviour. Direct observation of predation at roosts in build-up areas suggests that living close to humans could be costly to bats emerging in daylight conditions. No predation attempts were recorded at roosts in woodland habitats.

Key words: generalized additive model, roost temperature, Norway, bat boxes, predation

Tree cavities used as bat roosts in a European temperate lowland sub-Atlantic forest

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The availability of suitable roosts may be the major limiting factor for maternity colonies of bats in forests. Most studies on the use of tree cavities by bats have focused on analysing occupancy by a single species, and not by the entire bat community. To provide guidelines for forest management conducive to sustaining bats in a temperate lowland European forest, we studied the occupancy of tree cavities by bats of all species. In six different habitat types of 4 to 10 ha, all tree cavities were recorded and described according to 47 qualitative and quantitative descriptive variables. Logistic regression analyses were computed to predict the occupancy of cavities by bats, and to identify the most relevant variables for use as bat roosts. With or without potential competitors in the analyses, bats mainly used cavities in healthy main branches, with a large entrance located high above the ground. They did not use peeling bark or cavities on secondary branches, nor cavities covered by spider webs. Despite a large number of potential roosts in the area, bats tend to be selective and the types of roosts were less diverse than described in the literature. Not surprisingly guidelines for forest management aimed at bat conservation include keeping healthy old trees, which provide various types of cavities.

Key words: bats, community, tree roost, temperature, forest management

Large home range size in the ground foraging bat, *Mystacina tuberculata*, in cold temperate rainforest, New Zealand

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Large home range sizes are not unusual for small forest-dwelling bats living in cold temperate climates. However, lesser short-tailed bats (*Mystacina tuberculata*) feed on the ground for a proportion of their time, so their home range requirements may not conform to predictions based on other bat species. The home range size requirements of a population of *M. tuberculata* were investigated in cold temperate rainforest in the Eglinton Valley, Fiordland, New Zealand. Home range data was collected from a total of 21 out of 23 radio-tagged bats during late summer to early autumn of 1997 and 1998. Individual bats were followed for an average of 7.8 ± 3.2 (SD) days and collectively ranged over a total area of 14,710 ha. Thirteen colonial roosts were located within a central roosting area occupying 17 ha. A further 10 solitary roosts were located within individual foraging areas. Individual 100% minimum convex polygons varied considerably in size from 127.3 to 6,223.4 ha (median = 478.5 ha) with a range length of 2.2–23.0 km (median = 5.0 km). Cluster analyses revealed individual home ranges contained multi-nuclear patches of activity. Despite their large home ranges, bats concentrated activity (85%) in small core areas of 0.6–270.3 ha (median = 26.5 ha), with 85% of core areas less than 62 ha in size. Moderate levels of home range overlap amongst individuals (median = 26.8%) decreased to low levels for individual core areas (median = 3.7%). Our results show that *M. tuberculata* have relatively large home ranges, similar to many other small temperate rainforest bats, and implies that conservation areas designed for *M. tuberculata* should be large. Our predictions should be tested on populations of this species in areas with more abundant resources and milder climates.

Key words: lesser short-tailed bat, vespertilioniformid, Yangochiroptera, Noctilionoidea, Mystacinidae, radio-tracking, movements, beech forest, Nothofagaceae

Sex and season differences in the echolocation pulses of big brown bats (*Eptesicus fuscus*) and their relation to mating activity

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Although several studies have reported inter- and intra-individual variation in the echolocation pulses of big brown bats, relatively few have explored its function in the context of courtship and mating. In this study, we tested for sex and seasonal differences in the echolocation pulses of male and female big brown bats, and related these differences to mating activity. We recorded the echolocation pulses of adult female and male big brown bats in a controlled laboratory environment during the mating and three non-mating seasons. Factor analysis was used to reduce the number of call variables into two principle components (PC1 and PC2). Call components related to PC2 were significantly sexually dimorphic in the mating season. However, no call components were significantly sexually dimorphic in the non-mating season. In addition, we found a significant correlation between PC1 and the mating score of male bats. There was no significant correlation between principle components and the mating score of female bats. Our results provide additional support for the current literature that suggests a communicative function for bat echolocation pulses. Furthermore, it suggests that differences in the echolocation pulses of male and female bats may be important in the mating activity of this group.

Key words: bats, communication, echolocation pulses, sexual dimorphism, mating

Abundance patterns of ectoparasites infesting different populations of *Miniopterus* species in their contact zone in Asia Minor

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Although closely related host species are similarly susceptible to infestations of parasites, even small differences in their morphology, feeding behaviour or population history may affect parasitic infestation. In the present study we analyse the abundance of two species of nycteribiid flies (Diptera, Nycteribiidae) and one wing mite (Mesostigmata: Spinturnicidae) infesting populations of *Miniopterus schreibersii* and *M. pallidus*, which comprise a cryptic species complex of *Miniopterus* species in Asia Minor. We focus on the putative contact zone between these two taxa in Central Anatolia. We conducted our study in seven caves with large ($\geq 1,000$ individuals) maternity aggregations: three housing Anatolian *M. schreibersii*, one housing Levantine *M. schreibersii*, and three housing Anatolian *M. pallidus*. Sex-biased parasitism was found only twice: female-biased in *Spinturnix psi* on *M. pallidus*, and male-biased in *Nycteribia schmidlii* on *M. schreibersii*. Differences in the flies' abundance between Anatolian *M. schreibersii* and *M. pallidus* were found only in *N. schmidlii* (for female host), but not for *Penicilidia dufourii*. There was a significant difference in the wing mite abundance, both between hosts and sexes. Unexpectedly, we observed a large difference in the load and type of parasites between *M. schreibersii* from Levant (separated from other *M. schreibersii* colonies by a few colonies of *M. pallidus*) and the rest of *M. schreibersii*. In Levant, the wing mites did not infest bats. Instead, they carried almost threefold larger load of the flies than in other bent-wing bat colonies. It is possible that the decline of wing mites is associated with increasing quantities of flies. One hypothesis regarding the absence of *S. psi* in the Levant colony, is that it is correlated with a significant decrease in the size of the bat's population in the past (as indicated by both mtDNA and microsatellite studies) and their disconnection from the continuous range of other *M. schreibersii*. We did not find any correlation between parasite load and health status of the host.

Key words: cryptic species, ectoparasite infection, *Miniopterus* phylogeny, Nycteribiidae, Spinturnicidae, Asia Minor

Understanding bat-habitat associations and the effects of monitoring on long-term roost success using a volunteer dataset

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In gregarious species, the choice of colony location is especially crucial as the costs associated with breeding near conspecifics are important and the quality of a breeding patch is known to affect individual fitness. Consequently one could expect robust decision-making rules regarding colony location. The conceptual framework of animal aggregation with regards to habitat selection emphasizes that the presence and success of conspecifics are cues to habitat selection. Based on this, we explored how the distribution of breeding colonies could inform us about how habitat selection operates in bats. The data set we used was provided by a volunteer network whose first aim is to advise citizens facing bats in distress or bats in their homes. The dataset contained information on the locations of 105 serotine (*Eptesicus serotinus*) breeding colonies in a French region primarily dominated by an agricultural landscape. The methodology used for calculating habitat availability was based on the comparison of habitats surrounding serotine colonies to habitats surrounding random points. We found that serotine bats positively select pastoral and aquatic habitats regardless of the comparison used. The strong correlation between our results and those obtained with radio-tracking or acoustic methods underlines the robustness of this spatial distribution approach. The analysis of the history of the serotine colonies over a period of nearly 20 years showed that when attics are restored by the owners without the help of the bat rescue network, the probability of a departure of colony is greater. In addition, monitoring reduces the occurrence of an unsympathetic building restoration.

Key words: habitat selection, distribution, local wildlife, protection, *Eptesicus serotinus*

A recent bat survey reveals Bukit Barisan Selatan Landscape as a chiropteran diversity hotspot in Sumatra

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Bukit Barisan Selatan National Park is one of the last refuges protecting intact forest and a representative mammalian fauna in Sumatra. However, knowledge of bat diversity in the area is limited. From 2010 to 2012, 47 bat species were recorded through a series of surveys in 12 localities within and around the national park. An additional six species from the area were identified from the mammal collection of the Museum Zoologicum Bogoriense, Indonesia. At least seven of the species reported in this study are new records for Sumatra, including *Kerivoula krauensis*, *K. lenis*, *K. minuta*, *Murina rozendaali*, *Myotis horsfieldii*, *Myotis* cf. *borneoensis*, and *Rhinolophus borneensis/celebensis*. Moreover, a finding of two distinct morphs of *Chironax melanocephalus* coexisting in the study area indicates another possible undescribed species. With 60 species, we consider Bukit Barisan Selatan Landscape to be a Southeast Asian bat diversity hotspot and of critical importance in maintaining bat diversity in Sumatra.

Key words: Chiroptera, new record, lowland rainforest, Indonesia, identification keys, coffee agriculture

A molecular approach to the study of avian DNA in bat faeces

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The molecular identification of prey in faeces is an efficient non-invasive technique to study diet which requires both a satisfactory method of DNA extraction and the design of specific primers to selectively amplify prey's DNA. In this study we evaluated and compared the efficiency of two total DNA extraction methods and five primer pairs for the molecular identification of birds from scats, in particular from the giant noctule bat (*Nyctalus lasiopterus*). A modified DNA stool Mini Kit of Qiagen was tested against a modified silica method with a guanidinium thiocyanate (GuSCN) applied after freezing and pulverizing the samples. We also checked two published vertebrate- and bird-generalist primer pairs and three bird-specific primer pairs designed by us (two pairs targeting the cytochrome *b* and one the cytochrome oxidase subunit I genes) that amplified shorter DNA fragments. The results show that pulverizing the scat remains before extraction was a very important step, presumably facilitating access to the well preserved DNA located inside the rachis of the feathers. The combination of our bird-specific designed primers showed a higher amplification rate than the generalist primers and allowed successful bird identification from the feathers excreted by the giant noctule bat in all the scat samples analyzed, independent of the preservation method used (dried and frozen). These methodological improvements will allow not only the study of the avian diet composition of the enigmatic giant noctule, but the extension of this methodology to other bird predators such as raptors.

Key words: giant noctule bat, faeces, DNA extraction, conservation, primer design, bird DNA, amplification success