

DNA Metabarcoding for diet analysis of Bechstein's bats in Bernwood Forest

This project was carried out in collaboration with Chris Damant at Bernwood ECS Ltd, who has worked for several years on the population of Bechstein's Bats in Bernwood Forest, Buckinghamshire.



The Bechstein's Bats of Bernwood

Bechstein's bats were only recently (2010) discovered in the Bernwood Forest area as part of the Bat Conservation Trust's national Bechstein's Bat Survey. Early results from research being conducted at the University of Exeter suggests that this population is the most isolated in the UK and has relatively low genetic diversity (P Wright, *pers. comm.* to C. Damant). The population is therefore of conservation significance, and is currently vulnerable to disturbance and habitat loss associated with the construction of HS2.

Habitat use

Bechstein's bat is predominantly associated with ancient broadleaf woodlands, but recent radio tracking studies of the Bernwood population(s) have found that they also make use of adjacent areas of ancient semi-natural and secondary woodland, pasture, hedgerows, wetlands, and river corridors. The importance of such habitats as foraging grounds is unknown, and is an important consideration in terms of ensuring effective conservation of this unique population.

In this pilot project, we show how DNA metabarcoding can be used to reveal the relative contribution of woodland and non-woodland prey species to the diet of the Bechstein's bats in Bernwood Forest. The aim is to increase the understanding of the foraging behaviour and habitat use of this important population.

Bechstein's bats are thought to be predominantly a gleaning species of ancient semi-natural woodlands, with non-airborne beetles, centipedes, earwigs and spiders making up a high percentage of the prey food, along with moths and flies.

Traditional approaches to bat diet analysis involve detailed microscopic analysis of the fragmentary remains of the prey, but this is incredibly difficult and time consuming and can only feasibly be done for hard-bodied species. DNA analysis of faecal matter provides a higher resolution identification of prey species regardless of life-stage, size or body type.

Methods

Eight faecal pellets were collected from individual Bechstein's bats from three separate maternal colonies in the Bernwood Forest area on 16th - 18th August 2016. Faecal samples were collected either directly from the captured bats or from cotton bags and subsequently placed in eppendorf tubes with a storage solution. Samples were stored in a fridge before despatch to NatureMetrics for processing.

In the lab, we extracted DNA from each individual faecal pellet and amplified part of the CO1 gene using primers designed to target animal DNA. The amplified DNA was then sequenced on an Illumina MiSeq sequencer, returning hundreds of thousands of short sequence reads across the eight samples. These

were quality filtered and clustered into species-level taxonomic units using a custom bioinformatics pipeline.

The resulting species list was entered into Natural England's Pantheon database (<http://www.brc.ac.uk/pantheon/>) to derive habitat associations, or Broad Assemblage Types (BAT), for each species. This is the first time that DNA-based community analysis has been combined with the Pantheon database to infer habitat characteristics.

Results

The table below shows the results of the analysis.

| | | Location 1 | | | Location 2 | | | Location 3 | |
|---|--|------------|---|---|------------|---|---|------------|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|  | Bechstein's bat (<i>Myotis bechsteini</i>) | ○ | ○ | ○ | ○ | ○ | ○ | ○ | ○ |
|  | Common Oak midget moth (<i>Phyllonorycter quercifoliella</i>) | | | | | | | | ○ |
|  | Dun-bar moth (<i>Cosmia trapezina</i>) | | | ○ | | | | | |
|  | Engrailed moth (<i>Ectropis crepuscularia</i>) | | ○ | | | | | ○ | |
|  | Green silver-lines moth (<i>Pseudoips prasinana</i>) | | ○ | | | | | | |
|  | Pale oak beauty moth (<i>Hypomecis punctinalis</i>) | | | | | | | | ○ |
|  | Straw underwing moth (<i>Thalpophila matura</i>) | ○ | | | | | | | |
|  | Crane fly (<i>Molophilus occultus</i>) | | | | | | | | ○ |
|  | Crane fly (<i>Nephrotoma</i> sp.) | ○ | ○ | | | | | | |
|  | Harvestman (<i>Leiobunum rotundum</i>) | | | | ○ | | | | |
|  | Acari mite sp. | | | | | | | | ○ |
|  | Fly (<i>Pegoplatia infirma</i>) | | | | | | | | ○ |
|  | Ant (<i>Myrmica ruginodis</i>) | ○ | | | ○ | | ○ | | ○ |
|  | Oak bush cricket (<i>Meconema thalassinum</i>) | | | | ○ | ○ | | | |
|  | Speckled bush-cricket (<i>Leptophyes punctatissima</i>) | | ○ | | | | | | |
|  | Common earwig (<i>Forficula auricularia</i>) | | ○ | ○ | ○ | ○ | ○ | | ○ |
|  | Darkling beetle (<i>Prionychus ater</i>) | | | | | | ○ | | |
|  | Click beetle (<i>Stenagostus rhombeus</i>) | | | | | | | ○ | |
|  | Proturan (<i>Fujientomon dicestum</i>) | ○ | | ○ | | | ○ | ○ | ○ |
|  | Earthworm (<i>Allolobophoridaella eiseni</i>) | ○ | | | | | | | |

 Tree associated

 Open-habitat associated

 Tree and Open-habitat associated

 Tree and Wetland associated

The table shows which species are present in each of the eight samples (a white circle indicates a detection), and the habitat with which each species is typically associated.

In every case, the dominant source of DNA is Bechstein's bat, providing a very high-confidence confirmation of bat species identification. The remaining DNA sources comprise the prey species. For instance, by reading down the right-most column, we can see that bat 8 appears to have fed on two moth species, a crane fly, a mite, a fly, an ant, an earwig, and a proturan.

The prey species detected are predominantly associated with broadleaf woodland and semi-open habitats, with the exception of a single species (the crane fly *Molophilus occultus*) that is associated with wetlands.

In general, earwigs and ants appear to be popular prey items, which is consistent with what's known about Bechstein's tendency to forage by gleaning prey rather than intercepting it in flight. The presence of moths, beetles, arachnids, crickets and flies is also expected.

The occurrence of the earthworm *Allolobophora eiseni* (closely associated with woodland sites and sites with more acid soils) is noteworthy and could represent evidence for added prey diversity gleaned from either the woodland floor, off the bark of trees and/or from within rot holes and potential roosts.

The advantage of using molecular analyses over microscope-based analyses lies in **higher detection** probabilities for prey items that would otherwise be incredibly difficult to identify, for example soft bodied species such as the earthworm encountered here. Moreover, these analyses provide **reliable species-level identifications for the majority of species**, which would be virtually impossible using conventional morphological approaches.

The sample size in this pilot is (of course) too limited for us to draw significant conclusions regarding the diet of Bechstein's bats in Bernwood forest; however, it does demonstrate the potential of the metabarcoding approach for gathering this type of data, and with greater sampling effort going forward we hope to gain important insights into seasonal diet, foraging behaviour and habitat use of the Bechstein's of Bernwood.