Insects in the diet of the hazel dormouse (*Muscardinus avellanarius*): a pilot study using DNA barcoding

Photograph by Paul Chanin

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ABSTRACT

The common dormouse is primarily herbivorous, specialising in high quality food. Insects have also been recorded in the diet, identified from their fragmented remains in faeces. We investigated the use of 'DNA barcoding' using mitochondrial DNA as an alternative. Samples were collected from seven sites in England. Half of the 26 samples contained insect DNA, from four species of Lepidoptera and one Dipteran. We conclude that this is a practical approach to investigating dormouse predation on insects and discuss its limitations.

Introduction

The hazel or common dormouse, (*Muscardinus avellanarius* L.) is an arboreal rodent found across much of central Europe from UK to the Urals and from the Baltic to the Mediterranean. In Britain it is largely confined to central and southern counties of England and Wales where, as elsewhere in Europe, it has declined in numbers and distribution in the 20th century (Bright *et al*. 2006).

Like most rodents the common dormouse is primarily herbivorous, specialising in high quality food in the form of flowers, fruits and seeds (Bright & Morris, 2008). Lacking a caecum, it is not equipped to digest cellulose and it also avoids seeds that are chemically defended (Bright & Morris, 1993). Juškaitis (2008) pointed out that published information on the diet of common dormice is contradictory, with some authors asserting that dormice are entirely vegetarian, others that they are omnivorous. However, predation on insects has been found by Richards *et al*. (1984) and Morris (personal communication) who analysed dormouse faeces, and by Holisova (1968) who looked at stomach contents. Richards *et al*. (*op. cit*.) recorded the remains of Aphidae, adult and larval Lepidoptera and other, unidentified adult insects. Aphidae were recorded in June, larval Lepidoptera in June and July and adult insects throughout the year. Overall insects were observed most frequently in June (more than 50% of items).

The use of 'DNA barcoding' using mitochondrial DNA to study the diet of insectivorous mammals (bats) was pioneered by Zeale *et al*. (2011). Here we report a pilot study to determine the extent to which insect DNA can be recovered from dormouse faeces and we discuss the extent to which this method can be used in studies of dormouse diet.

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Key words: dormouse, *Muscardinus avellanarius*, insects, diet, DNA barcoding

Chanin, P., O'Reilly, C., Turner, P., Kerslake, L.Birks, J. & Woods, M. (2015) Insects in the diet of the hazel dormouse (Muscardinus avellanarius): a pilot study using DNA barcoding. Mammal Communications **1**: 1- 7

METHODS AND MATERIALS

Dormouse faeces were collected from a sample of animals which were handled during routine monitoring for the National Dormouse Monitoring Programme (NDMP; Bright *et al*., 2006). Samples were obtained from seven sites in southern England (**Table 1**). Since samples were collected directly from live animals during handling, there was no risk of misidentifying the origin and the date was precisely known. Samples were placed either in 2ml microfuge tubes or ziplock bags and sent to Waterford Institute of Technology for genetic analysis.

DNA was isolated from individual pellets using the method described in O'Reilly *et al*. (2008) with minor modifications. The pellets were sampled in 500 µl S.T.A.R. Buffer (Stool Transport and Recovery buffer, Roche) and DNA was isolated from 150 µl of sample using the ZR Genomic DNA isolation kit (Zymo Inc.) according to the manufacturer's instructions. Insect DNA was PCR amplified from the samples using primers ZBJ-ArtF1c and ZBJ-ArtR2c as described in Zeale *et al*. (2011). PCR products were analysed on 1.5% agarose gels and purified using the DNA Clean & Concentrator™-5 kit (Zymo Inc). Purified PCR products were cloned into the pDrive cloning vector (Qiagen) and transformed into high efficiency.

NovaBlue GigaSingles™ Competent Cells (Novagen). Plasmid DNA was isolated from individual clones using the GenElute™ Plasmid Miniprep Kit (Sigma-Aldrich) and the inserts were sequenced in both directions using the T7 promoter and M13 reverse primers using the ABI BigDye 3.1 system on an ABI310 sequencer (Applied Biosystems).

DNA sequences were analysed using SeqMan and Megalign software (DNASTAR Inc) to compare sequences, primer and vector sequences were removed and a consensus sequence was constructed for each insert. Between 2 and 5 clone inserts were sequenced from each PCR product. The sequences were then compared to sequences on the Barcode of Life Database (BOLD), Ratnasingham & Hebert (2007)) and the GenBank (NCBI) databases to identify the closest match sequence. As recommended in Zeale *et al*. (2011) sequences were identified to family, genus or species depending on whether the similarities exceeded 91.0%, 94.9% and 99.3% respectively.

Table 1. Sources of samples and number collected each month. Ordered from west to east.

Site Location	Grid ref.	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Cornwall	SX1070	1	2	1			1			
Devon	SS4720			2						
Devon	SS4815			1			1			
Devon	SX8266		1		1					
Somerset	ST3624						1	1		
Bristol	ST5573				2		6			
Buckinghamshire	SP8345			1	1	1			1	1

RESULTS

Twenty six samples were collected from seven sites and insect mtDNA was recovered from thirteen of these (50%). Dates and places of collection are shown in **Table 1**. Faecal samples containing insect remains had from one to three species present, totalling 17, mean: 1.31. Four species of Lepidoptera were identified and one sample included DNA from a species of Cecidomyiid fly (**Table 2**).

There were nine samples containing *Operophtera brumata* (L.) (winter moth). *Eupsilia transversa* (Hufn.) (satellite moth) was the second most frequent species (five instances) and one example each of *Blastobasis adustella* (Walsingham), and *Panolis flammea* ([D.&S.]) (pine beauty moth) was recorded. All of 13 samples collected between April and July inclusive contained insect DNA but none was recovered from 13 samples collected between August and October. These negative results were confirmed by a second attempt at extraction.

Table 2. Insect species eaten, frequency in the diet (N) and phenology. Shading indicates that the life stage is available to dormice

Species	N	Larval food	Larvae active	Adults flying
Operophtera brumata	9	Trees and shrubs	April–July	October–January
Eupsilia transversa	5	Deciduous trees	April–June	October–April
Blastobasis adustella	1	Various plants including decaying matter	March–September	July–October
Panolis flammea	1	Pinus spp.	June–July	March–April
Cecidomyiidae	1	Depends on sp.	NA	NA

Ambiguous Identifications:

One record of *Eupsilia* was only identified to genus. However, since only one species (*E. transversa*) is recorded from the UK, it was assigned to that.

One sample showed 100% identity to *Hemioctenophora euplexiodes*, Noctuidae and showed 99.3% identity with *Spodoptera umbraculata* (Walker). Only two barcode sequences were available for *H. euplexiodes* on the BOLD database and both of these were from specimens from Japan. *S. umbraculata* is an Australian species although a congener (*S.exigua* Hubner) is known from the UK. It is possible that this sample is from a species which has not yet been sequenced.

Cecidomyiidae spp., Nematocera, Diptera were identified with 96.79% similarity which Zeale *et al*. (2011) regarded as adequate for identification to family. This family includes a large number of gall forming species as well as predatory (or parasitoid species) and species that feed on fungi and decaying wood (Dipterists' Forum website).

DISCUSSION

Insect phenology and predation

Winter and satellite moths which form 74% of the items identified are only available to dormice as larvae and pupae with the adults being active while dormice are in hibernation. Dormouse faeces containing the remains of these species were only recorded in July or earlier, suggesting it is the larval stages that were taken. Other Lepidoptera species could have been taken as adults. On these grounds it is plausible to suggest that larval Lepidoptera are a significant source of food for dormice during the first half of the summer, at a time when high quality plant products consist mainly of nectar and pollen with few fruits or seeds available.

Cecidomyiids are characteristically small (0.5-3mm, rarely up to 8mm) and it is probable that dormice consume them when feeding on the galls that many species create.

Comparison with previous studies

Richards *et al*. (1984) examined dormouse faeces under the microscope and were able to identify plant remains in some detail, to species for leaves and pollen in many cases. Insects were identified as larval Lepidoptera, aphids or other adult insects. The last category consisted most frequently of wing scales from adult Lepidoptera but other insect remains were not identified further. Adult insects were recorded in every month from May to October but lepidopteran larvae were only found in June and July. A preliminary study by Pat Morris (unpublished) revealed fragments of insect mouthparts, eyes and legs. In seven dated samples he recorded insects four times in August and once each in May, June and July.

There are no other systematic studies of the diet of dormice using faeces. In reviewing the literature on feeding by dormice, Juškaitis (2008) highlights the fact that there are divergent views on insectivory in dormice. Some captive dormice have fed on insects, others have ignored them. He also points out that a study of the stomach contents of dormice from Slovakia included the remains of insects, chiefly larvae, (Holisova, 1968). Another from Russia revealed the remains of chitin from coleopteran exoskeletons which the author believed had been ingested accidentally. Other evidence of dormice feeding on insects comes from field observations by Bright & Morris (1996).

CONCLUSIONS

We have demonstrated that the bar-coding of insect mtDNA has a practical application in the study of the diet of dormice, and that in the areas from which we collected samples the dominant prey items were Lepidoptera, mainly at the larval stage. Our samples are too small to discern patterns of predation other than a change from early in the year when Lepidoptera were found in all samples to later when none were found.

A limitation in the method is that the bar-coding is not sufficiently precise to prevent species which do not occur in the area from being 'identified' but such errors do not invalidate the procedure. This reflects the incomplete nature of the DNA databases which is rapidly changing as more and more sequences become available.

Collecting sufficient samples from dormice is difficult because our method - obtaining them from animals that were being handled as part of a national monitoring programme - produced a low yield. However the fact that over 200 sites are being monitored in this way means that a large scale study over a wide area would be practical. We limited ourselves to a small preliminary sample but the network of surveyors in the NDMP have been very willing to supply material for other studies (e.g. Md Naim, 2010).

For local studies, it may be practical to use the approach adopted by Richards *et al* (1984) who placed trays baited with carrots in dormouse habitat and collected faeces from these at weekly intervals obtaining 20-30 samples per month for most of the year. Care should be taken to ensure that the faeces of other rodents such as woodmice and bank voles are not confused with those of dormice but this can be controlled by using molecular methods.

The standard sequencing technology used in this study was labour intensive and analysis costs £10 (€12; US$15) per sample. However, the next generation sequencing techniques now available would provide a cost effective method for a large scale study (Pompanon *et al*., 2012).

A further problem is that there is no simple molecular method for determining the number of prey items present in a dormouse faecal pellet. However, by combining it with conventional faecal analysis as practiced by Richards *et al*. (1984), it may be possible to count the number of larvae present using hard parts. Richards *et al* (1984) could identify spiracles and crochets (gripping hooks on the prolegs) from larval Lepidoptera and it may be possible to devise appropriate multipliers based on their density in scats, possibly informed by feeding experiments.

ACKNOWLEDGEMENTS

We are very grateful to the Whitley Wildlife Conservation Trust for funding the cost of analytical materials. Hannah Broughton, Leonardo Gubert and several contributors to the National Dormouse Monitoring Programme very kindly helped in collecting samples for us and Pat Morris made available the results of unpublished studies of dormouse diet. We are particularly grateful to the late David Stradling, chair of the Whitley Wildlife Conservation Trust, who ensured that the manuscript followed entomological conventions.

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