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METHODS AND RESOURCES

Technical note: successful DNA amplification of DNA from non-destructive buccal swabbing in Vespertilionid and Rhinolophid bats

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Abstract

Acquiring DNA from wild bats (Mammalia: Chiroptera) is typically undertaken utilizing highly invasive (but non-lethal) sampling techniques comprising wing biopsies and occasional blood samples. While non-invasive sampling is possible through the extraction of DNA from faecal samples, it is not always possible to acquire samples from individual bats whilst conducting fieldwork, and as such, this method is primarily applicable to roost occupancy identification. Similarly, wing swabbing is liable to cross-contamination from roost mates. Here we present the first use of oral (buccal) swabbing for successful, species-resolution DNA sequencing of Vespertilionidae and Rhinolophidae in 10 bat species (nine Vespertilionidae and one Rhinolophidae) from the UK.

Keywords Conservation · Population genetics · Sampling techniques · Buccal swabbing

Text

The use of genetic sampling for conservation ecology is becoming prevalent and may be particularly important for vulnerable taxa such as bats (Mammalia: Chiroptera). When studying Chiroptera, the most common technique for obtaining DNA samples is wing biopsies (Manjerovic et al. 2015; Player et al. 2017) and occasionally blood sampling (Walker et al. 2016; Russo et al. 2017). Wing biopsies are problematic, as they require extensive training and, in some areas, permits which are difficult or prohibitively expensive to attain. Additionally, biopsies have been shown to have slow healing rates prior to and during hibernation (Player et al. 2017) and are thus less viable in temperate areas. Consequently, the emphasis on demonstrating the efficacy of non-invasive techniques for obtaining DNA samples is increasingly important (Boston et al. 2012).

Collection of faecal samples (Puechmaille et al. 2007; Walker et al. 2016) and wing swabs (Walker et al. 2016; Player et al. 2017) have been used as non-invasive methods in bats, but each has drawbacks. Most bat species form colonies and whilst collection of faeces from roosts can identify species (or multiple species), in order to collect an individual bat's faecal sample, surveyors must hold each bat captive until it produces a faecal pellet, and there is potential for cross-contamination of samples from holding bags. Wing swabbing has potential for cross-contamination from roost mates (which may not be the same species) (Player et al. 2017).

Oral (buccal) swabbing has been used as a less invasive DNA sampling procedure in many vertebrate groups, including birds (Handel et al. 2006; Vilstrup et al. 2018), reptiles (Miller 2006; Beebee 2008) amphibians (Pidancier et al. 2003; Maddock et al. 2014) and small mammals (Naim et al. 2012) as well as in metabarcoding studies of diet (Nota et al. 2019). As far as we are aware, few studies have used buccal swabbing in bats, comprising Ramirez (2011) who successfully used buccal swabs to extract DNA

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from the *Leptonycteris yerbabuenae* (Phyllostomidae), and Corthals et al. (2015) who used buccal brushes to collect epithelial cells from members of the families Mormoopidae and Phyllostomidae, successfully amplifying DNA. However, the technique of Corthals et al. (2015) was invasive as they used brushes rather than swabs for long sampling periods (60 s), frequently causing bleeding in the mouths of bats. The only published work on the use of buccal swabs to extract DNA from the Vespertilionidae is that of a single oral swab from *Myotis californicus* (Walker et al. 2016) which was successfully amplified to Genus level only. Here we report a species-level, non-invasive buccal swabbing sampling method for bat DNA for Vespertilionidae and Rhinolophidae. Samples were extracted from swabs of 24 individual bats comprising ten species in five genera (*Myotis daubentonii, Myotis mystacinus, Myotis nattereri, Nyctalus leisleri, Nyctalus noctula, Pipistrellus nathusii, Pipistrellus pipistrellus, Pipistrellus pygmaeus, Plecotus auritus* and *Rhinolophus hipposideros*).

Buccal swabs were taken from bats during ongoing monitoring (bat box and trapping) programmes in the West Midlands, UK. All surveys adhered to standard UK (Collins 2016) or European (Battersby 2010) guidelines, using standard methodology (Kunz and Kurta 1988; Barlow 1999). Bats were identified as per Dietz and Kiefer (2014). Animals were handled whilst wearing clean, disposable latex gloves which were changed between bats. A MSDS Dryswab™ Mini Σ-Swab® tip sterile, polyurethane swab (MW943; Medical Wire & Equipment Co., Corsham, UK) was used for each bat. Each swab has a flexible, plastic 150 mm shaft with a cellular foam mini-bud designed for paediatric nasopharyngeal swabbing. Samples were obtained by encouraging the bat to gape, inserting the swab into the bat's mouth and rotating for 20 s, concentrating on the cheeks and the tongue. Unlike the aforementioned use of brushes in other studies, we found that no bleeding was caused with this swab type and duration. Whilst quantifying stress in bats is difficult (unless sampling cortisol, which itself increases stress), our swabbing was overseen by handlers with over 20 years of field experience, who assessed that the levels of stress caused to the bats by the use of the foam swabs was comparable to standard survey techniques such as measurement of dentition. Each swab tip was immediately placed in a 2 ml screw-top vial filled with 100% molecular grade ethanol and transferred to a -20 °C freezer within six hours. Individuals were marked with non-toxic chalk paint to avoid duplicate sampling if re-captured.

DNA was extracted using Qiagen DNeasy® Blood and Tissue Kits, following manufacturer's instructions, except lysing was at 56°C for 30 minutes, vortexing at 15 and 30 minutes and utilising only 100µl of buffer AE to suspend final DNA extracts. We quantified 2µl of extracted DNA elutions using a Thermo Scientific™ NanoDrop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA). Individuals of each species were amplified using PCR for the non-coding 16s rRNA (*16s*) mitochondrial marker using the primers 16sAL (5'-CGCC TGTTT ATCACG-3') and 16sBH (5'-CCGGTCTGAACT CAGATC ACG-3' (Palumbi et al., 1991) as they are universal for most tetrapods, and thus would identify cross-contamination from other organisms (e.g., humans). Reaction volumes for PCR at 25 µl were: 12.5 µl of MyTaq™ Red Mix, 6.5 µl of ddH_2O, 1 µl each of the forward and reverse primers (10µM) and 4 µl of template DNA. Cycling was undertaken using a Techne Prime Thermal Cycler; conditions were: denature at 94 °C for 60s; followed by 35 cycles of 94 °C for 30s, 50 °C for 30s, and 72 °C for 30s; and final extension of 72 °C for 5 min. PCRs were assessed for successful amplification with a 1% agarose gel and were then prepared for sequencing using the BigDye™ Terminator v3.1 cycle sequencing kit. Sequencing took place on the University of Wolverhampton's in-house ABI Applied Biosystems 3500XL DNA analyser. Sequences were analysed and checked using Geneious Prime v 2022.1.1 (Biomatters Ltd., 2022) and species identity checked using NCBI nucleotide BLAST searches (National Centre for Biotechnology Information (NCBI), 1988).

DNA yields shown in Nanodrop analyses were low for all samples and were undetectable in many (Table 1), however Nanodrop values are unreliable for quantification of very low DNA yields. Despite low yields, successful amplifications were achieved for all individuals of all species in the study and BLAST searches supported species ID.

We have demonstrated that it is possible to obtain DNA from ten Vespertilionidae and one Rhinolophidae species using a buccal swabbing method that provides enough DNA concentration to generate Sanger sequence data. This method requires field training and specific swab types but can readily be learned and applied by anyone with competency in handling wild bats. It precludes the need for other, more invasive methods. Further work is required to determine whether oral swabbing can be utilised for other genetic methods (e.g., microsatellite analyses).

Table 1 Quantities of DNA extracted and PCR success. Nucleic Acid Concentration, A260/A280 resultant from Nanodrop analysis; Qubit Fluorometric Quantification results; Query length and maximum percentage Blast Match

				Nanodrop		Qubit		BLAST
Latitude	Longitude	Family	Species	Conc.	260/280	ng/ųl	ng/ml	Ident.
52.520493	-1.9665904	*Vespertilionidae*	*Myotis daubentonii*	-	2.05	-	-	95.83%
52.520493	-1.9665904	*Vespertilionidae*	*Myotis daubentonii*	-	3.27	-	-	97.93%
52.582097	-1.8617896	*Vespertilionidae*	*Myotis mystacinus*	-	2.49	0.06500	0.65	100.00%
52.561178	-1.9372785	*Vespertilionidae*	*Myotis mystacinus*	1	1	0.18700	1.87	95.15%
52.561178	-1.9372785	*Vespertilionidae*	*Myotis mystacinus*	1.7	0.93	0.10900	1.09	100.00%
52.568248	-1.8438631	*Vespertilionidae*	*Myotis mystacinus*	3.5	1.04	0.44500	4.45	99.06%
52.568474	-1.844305	*Vespertilionidae*	*Myotis mystacinus*	-	1.56	0.47200	4.72	94.56%
52.548494	-1.9224905	*Vespertilionidae*	*Myotis nattereri*	-	2.88	0.07800	0.78	99.35%
52.548683	-1.9224164	*Vespertilionidae*	*Myotis nattereri*	-	3.07	-	-	96.73%
52.568474	-1.844305	*Vespertilionidae*	*Nyctalus leisleri*	-	1.7	-	-	94.81%
52.507699	-1.7848493	*Vespertilionidae*	*Nyctalus leisleri*	5.1	1.68	6.78000	67.8	96.34%
52.507699	-1.7848493	*Vespertilionidae*	*Nyctalus leisleri*	2.7	1.74	1.33000	13.3	99.16%
52.561288	-1.9396238	*Vespertilionidae*	*Nyctalus noctula*	-	1.57	0.10200	1.02	96.64%
52.561315	-1.9399483	*Vespertilionidae*	*Nyctalus noctula*	-	2	-	-	96.10%
52.520925	-1.9675186	*Vespertilionidae*	*Pipistrellus nathusii*	-	1.94	0.06800	0.68	100.00%
52.523269	-2.0399716	*Vespertilionidae*	*Pipistrellus pipistrellus*	-	-	-	-	100.00%
52.524284	-2.0411664	*Vespertilionidae*	*Pipistrellus pipistrellus*	-	1.73	-	-	95.98%
52.653792	-1.9393787	*Vespertilionidae*	*Pipistrellus pygmaeus*	-	2.16	0.05400	0.54	94.59%
52.653792	-1.9393787	*Vespertilionidae*	*Pipistrellus pygmaeus*	-	1.85	-	-	99.38%
52.653792	-1.9393787	*Vespertilionidae*	*Pipistrellus pygmaeus*	-	1.87	-	-	100.00%
52.561169	-1.9372638	*Vespertilionidae*	*Plecotus auritus*	-	1.7	-	-	94.31%
52.562277	-1.9399912	*Vespertilionidae*	*Plecotus auritus*	-	3.67	-	-	99.28%
52.515240	-2.0774818	*Rhinolophidae*	*Rhinolophus hipposideros*	-	1.55	0.24100	2.41	96.54%
52.515204	-2.0775849	*Rhinolophidae*	*Rhinolophus hipposideros*	1.6	1.67	0.43600	4.36	98.51%

- denotes no discernible yield

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Author contributions Study conception and design was by MH, SM and CY. Material preparation, data collection and analyses were performed by MH, SB, RM and SM. The first draft of the manuscript was written by MH and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets (gene sequences) generated during this study will be made available in the Genbank repository. After acceptance, Genbank numbers will be included in Table 1.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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