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SHORT NOTES

Avian host DNA isolated from the feces of white-winged vampire bats (*Diaemus youngi*)

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INTRODUCTION

Studies on the feeding ecology of vampire bats (Phyllostomidae: Desmodontinae) have been limited by the diagnostic techniques available for identifying host taxa. A precipitin-based test has been the standard method for determining their domestic host preferences (e.g., Cardoso, 1995). This postmortem immunological assay provides a presence or absence indication of a particular host's blood in a vampire bat's gut contents (Greenhall, 1970). However, the precipitin test has significant limitations. The bat must be sacrificed for its stomach sample, a particular difficulty for the rare species *Diaemus youngi* and *Diphylla ecaudata*. The precipitin test has low utility for identifying wild hosts (Greenhall, 1970), as it requires the harvesting of antibodies, usually in rabbits, using serum collected from all potential host organisms. For this reason, antibody techniques are generally considered too costly (time-wise and financially) for investigating a diverse potential prey range (Symondson, 2002). Indeed, harvesting antibodies of all the potential sympatric avian host species of a vampire bat would be unfeasible. Third, cross-reactivity among related host taxa is expected, and the precipitin technique is therefore unlikely to be diagnostic at the level of families or genera.

An alternative and potentially powerful approach is to use the host DNA as a marker for host identification. Although previous studies demonstrate that prey DNA can be isolated and amplified via PCR from feces (Hoss *et al*., 1992; Sutherland, 2000; Jarman *et al*., 2002, 2004; Jarman and Wilson, 2004; Deagle *et al*., 2005) and even fossilized feces (e.g., Kuch *et al*., 2002), vampire bat fecal samples are a uniquely implausible PCR template due to the lack of robust tissue and very high concentration of PCR-inhibiting heme compounds. The resistance of prey tissue to digestion is a factor determining the extent to which the targeted fecal DNA is degraded (Jarman *et al*., 2004). Past successful PCR-based

analyses of vertebrate fecal samples have involved food items with fairly robust tissues such as plants (Hoss *et al*., 1992), krill (Jarman *et al*., 2002; Jarman and Wilson, 2004), insects (Sutherland, 2000; G. McCracken, personal comm.), fish and squid (Deagle *et al*., 2005). In contrast, blood contains no hard tissues to protect DNA from digestive processes. The ease of amplifying prey DNA decreases with the degree of digestion (Johanowicz and Hoy, 1996; Asahida *et al*., 1997), and previous successful PCR-based analyses of blood meal (e.g., Prior and Torr, 2002; De Benedicts *et al*., 2003) involved samples obtained from insect abdomens in which the blood meal was not completely digested. No previous studies have isolated and amplified DNA from completely digested blood meals. In addition, vampire bat feces are largely composed of heme compounds resulting from the digestion of hemoglobin within avian and mammalian blood; these compounds are especially strong PCR inhibitors (e.g., Akane *et al*., 1994; Morata *et al*., 1998).

Materials and Methods

Avoiding cross-sample contamination is a critical consideration in studies such as this one, which targets degraded DNA templates and that involves a double PCR amplification. All molecular assays were conducted in a laboratory where pre-PCR and post-PCR reactions were spatially separated and assembled using dedicated equipment. Extraction and PCR reactions were assembled using aerosol-resistant pipette barrier tips. Our extensive inclusion of negative control reactions is described below.

Fecal samples were collected from ten captive *D. youngi* held at the New Mexico Bat Research Institute in Tijeras, NM from 1–16 January 2003. The bats fed on live chickens. Fecal collection shelves were installed beneath the bat roost in each aviary, and 0.1–0.8 g of each fecal pellet was preserved in 1 ml of a buffer described and shown to be effective for preserving fecal DNA by Frantzen *et al*. (1998). Fecal samples from wild *D. youngi* were obtained from two individuals captured at a roost site near Fyzabad, Trinidad on 8 August 2003, and from two individuals captured at a poultry farm in Salazar, Trinidad (10°09'N, 61°39'W) on 22 July 2004. Wild bats were held in new cloth bags or clean cages until they defecated, and fecal pellets were then preserved in the same DNA preservation buffer.

We used the Qiagen® Mini-Stool Kit following the manufacturer's recommended methods for extracting DNA from human stools, except the InhibitEX™ tablets were not incorporated, making the protocol possible with the contents of the Qiagen® DNeasy Tissue Kit along with Buffer ASL (Stool Lysis Buffer, Qiagen Cat. No. 19082). Negative controls consisting of buffers processed without fecal material were included when performing DNA extractions at a ratio of 1 negative control: 1 sample. These negative controls were physically interspersed between samples. A PCR product from a subsequent polymerase chain reaction (PCR) for any of these negative controls, indicating possible DNA contamination, resulted in our discarding of the entire batch of extractions.

We investigated the nuclear gene RAG-1 (recombination activating gene 1) as a marker to identify the avian DNA in fecal samples. This gene is highly conserved across Aves (Groth and Barrowclough, 1999) but varies enough at the nucleotide level to allow identification of host taxa. Avian-specific primers were designed using Primerselect™ and the large number of avian RAG-1 sequences available in GenBank. We used the primers f2480 (CGTGACAGAGTGAAGGGTGTT) and r2635 (CTTCCTGAGGTGTTTGTCAAGAGTYA), which target a 200 base pair DNA fragment including primers. Optimized conditions for these primers for 10 µl PCR reactions consist of 20 mM Tris-HCl (pH 8.0), 50 mM KCl, 3.0 mM $MgCl_2$, 200 mM dNTPs, 2 pmoles of each primer, 0.2 units of Taq DNA polymerase (Invitrogen™), 1 µg of bovine serum albumin and 1 µl of the DNA extract. Thermal cycling conditions were: 95°C for 4.5 min; 35 cycles of 95°C for 45 s, 57°C for 45 s, and 72°C for 80 s; and then 72°C for 5.5 min.

PCR amplification of markers required two successive amplifications to produce enough product to visualize on an agarose gel, with the second amplification seeded with 1 µl of product from the first amplification. PCR reactions that yielded single visible bands of the expected size were sequenced using BigDye™ Terminator cycle sequencing chemistry, v3.1 (Applied Biosystems) and an Applied Biosystems 3100 Genetic Analyzer using the amplification primers and following the manufacturers recommended protocols. The resulting chromatograms were imported into the program

Sequencher™ (Gene Codes Corporation, Michigan, USA) and compared to a panel of avian and mammalian reference sequences, and to the complete GenBank database using the BLAST search function.

Results

Using a DNA-based method, we were able to amplify and sequence chicken (*Gallus gallus*) DNA from captive and wild vampire bat fecal samples. The DNA purification methods employed in this study (Qiagen) combined with targeted PCR reactions amplifying 200 bp fragments are able to provide clean sequences of avian DNA from vampire bats that have parasitized birds. DNA extracts contained amplifiable avian DNA from bat feces, albeit in very low yields; PCR reactions required a reamplification of the PCR product to yield enough product to cycle sequence. Only two out of a total of seven tested fecal samples produced visible PCR product bands using the method described above. We amplified the RAG-1 locus from one sample of feces obtained from the captive vampire bats, and the resulting sequence was identical to corresponding *Gallus gallus* sequences in GenBank. As expected, given that the wild vampire bats were captured at a poultry farm, the resulting RAG-1 sequence derived from the wild samples similarly matched the corresponding region of the *Gallus gallus* RAG-1 sequence in GenBank.

Discussion

Host DNA in vampire bat feces is a highly degraded and difficult PCR template. Working with this material therefore requires unusually close attention to the possibility of sample contamination and use of some techniques analogous to those employed in studies of ancient DNA (Willerslev and Cooper, 2005), where contamination is a similarly high-risk consideration.

Our demonstration that avian host DNA can be isolated, amplified, and identified from vampire bat blood meals opens the door to further application of DNA-based analytical techniques to unexplored areas of vampire bat feeding ecology. Noninvasive sampling techniques such as those validated here are particularly appropriate for rare species such as *Diaemus* and *Diphylla*, which specialize on avian blood. We targeted the RAG-1 gene, a nuclear locus being sequenced in comprehensive phylogenetic surveys that will ultimately include all genera of birds (Cracraft *et al*., 2004). The forthcoming availability of these reference sequences in public databases means that avian DNA sequences obtained from vampire bat feces can be compared immediately against a robust set of reference sequences, without the need to obtain and sequence new reference samples de novo from all potential host taxa. Therefore, a DNA-based method of investigating host preference can effectively address the uncharacterized wildlife hosts of vampire bats. With the exception of a squirrel host identified from a *Desmodus* in Mexico (Greenhall, 1972), virtually no data are available on the patterns of wildlife parasitism by any population of vampire bats. Characterization of the wild host species exploited by vampire bats would be a vital contribution to their management (Greenhall, 1972, 1988).

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Literature Cited

Akane, A., K. Matsubara, H. Nakamura, S. Takahashi, and K. Kimura. 1994. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. Journal of Forensic Science, 39: 362–372.

Asahida, T., Y. Yamashita, and T. Kobayashi. 1997. Identification of consumed stone flounder, *Kareius bicoloratus* (Basilewsky), from the stomach contents of sand shrimp, *Crangon affinis* (De Haan) using mitochondrial DNA analysis. Journal of Experimental Marine Biology and Ecology, 217: 153–163.

Cardoso, M. 1995. Prey-type of the vampire bat *Desmodus rotundus* from mid-western Brazil, revealed by tests of precipitin on stomach blood meal. Chiroptera Neotropical, 1: 31–32.

Cracraft, J., F. K. Barker, M. J. Braun, J. Harshman, G. Dyke, J. Feinstein, S. Stanley, A. Cibois, P. Schikler, P. Beresford, J. García-Moreno, M. D. Sorenson, T. Yuri, and D. P. Mindell. 2004. Phylogenetic relationships among modern birds (Neornithes): toward an avian tree of life. Pp. 468–489, *in* Assembling the tree of life (J. Cracraft and M. J. Donoghue, eds.). Oxford University Press, New York, 592 pp.

Deagle, B. E., D. J. Tollit, S. N. Jarman, M. A. Hindell, A. W. Trites, and N. J. Gales. 2005. Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Stellar sea lions. Molecular Ecology, 14: 1831–1842.

De Benedictis, J., E. Chow-Shaffer, A. Costero, G. G. Clark, J. D. Edman, and T. W. Scott. 2003. Identification of the people from whom engorged *Aedes aegypti* took blood meals in Florida, Puerto Rico, using polymerase chain reaction-based DNA profiling. American Journal of Tropical Medicine and Hygiene, 68: 437–446.

Frantzen, M. A. J., J. B. Silk, J. W. H. Ferguson, R. K. Wayne, and M. H. Kohn. 1998. Empirical evaluation of preservation methods for faecal DNA. Molecular Ecology, 7: 1423–1428.

Greenhall, A. M. 1970. The use of a precipitin test to determine host preferences of the vampire bats, *Desmodus rotundus* and *Diaemus youngi*. Bijdragen tot de Dierkunde, 40: 36–39.

Greenhall, A. M. 1972. The problem of bat rabies, migratory bats, livestock and wildlife. Pp. 287–293, *in* Transactions of the 37th North American wildlife and natural resources conference (J. B. Trefethen, ed.). Wildlife Management Institute, Washington D.C., 401 pp.

Greenhall, A. M. 1988. Feeding behavior. Pp. 207–214, *in* Natural history of vampire bats (A. M. Greenhall and U. Schmidt, eds.). CRC Press Inc., Boca Raton, Florida, 246 pp.

Groth, J. G., and G. F. Barrowclough. 1999. Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. Molecular Phylogenetics and Evolution, 12: 115–123.

Hoss, M., M. Kohn, S. Pääbo, F. Knauer, and W. Schroeder. 1992. Excrement analysis by PCR. Nature, 359: 199.

Jarman, S. N., and S. G. Wilson. 2004. DNA-based species identification of krill consumed by whale sharks. Journal of Fish Biology, 65: 586–591.

Jarman, S. N., N. J. Gales, M. Tierney, P. C. Gill, and N. G. Elliot. 2002. A DNA-based method for identification of krill species and its application to analyzing the diet of marine vertebrate predators. Molecular Ecology, 11: 2679–2690.

Jarman, S. N., B. E. Deagle, and N. J. Gales. 2004. Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. Molecular Ecology, 13: 1313–1322.

Johanowicz, D. L., and M. A. Hoy. 1996. *Wolbachia* in a predator-prey system: 16S ribosomal DNA analysis of two phytoseiids (Acari: Phytoseiidae) and their prey (Acari: Tetranychidae). Annals of the Entomological Society of America, 89: 435–441.

Kuch, M., N. Rohland, J. L. Betancourt, C. L. Latorre, S. Steppans, and H. N. Poinar. 2002. Molecular analysis of a 11,700-year-old rodent midden from the Atacama Desert, Chile. Molecular Ecology, 11: 913–924.

Morata, P., M. I. Queipo-Ortuno, and J. D. Colmenero. 1998. Strategy for optimizing DNA amplification in a peripheral blood PCR assay used for diagnosis of human brucellosis. Journal of Clinical Microbiology, 36: 2443–2446.

Prior, A., and S. J. Torr. 2002. Host selection by *Anopheles arabiensis* and *A. quadriannulatus* feeding on cattle in Zimbabwe. Medical and Veterinary Entomology, 16: 207–213.

Sutherland, R. M. 2000. Molecular analysis of avian diets. Ph.D. Thesis, University of Oxford, Oxford, UK, 163 pp.

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Molecular Ecology, 11: 627–641.

Willerslev, E., and A. Cooper. 2005. Ancient DNA. Proceedings of the Royal Society of London, 272B: 3–16.

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