

**FURTHER DEVELOPMENTS OF IDENTIFICATION OF BIRDSTRIKE REMAINS
BY DNA ANALYSIS**

Phil Mountain & Chris Conyers

Central Science Laboratory, Sand Hutton, York. UK. YO41 1LZ
Tel: +44 19040462180, Fax: +44 1904462111
Email: p.mountain@csl.gov.uk

Abstract

DNA analysis can allow identification of birdstrike remains (that do not provide sufficient visual information for microscopic identification) to species specific level. However, a high quality genomic deoxyribonucleic acid (DNA) with uninterrupted gene sequences is required.

A 60% success rate has been achieved from the 85 DNA analyses of birdstrike remains carried out by CSL to date. The negative results were attributed to several factors: human contamination, contamination from other organisms and the inhibition of DNA by the presence of carbonised residue, that is found within engines. Measures to reduce the risk of contamination are discussed. In addition, feathers with no remains of cellular material were also seen to fail. Excessive degradation of samples, such as exposure to high temperatures, will also degrade DNA and result in a negative result. Failures also occur due to current database (Genbank) limitations. If a close match is unattainable, due to the bird sequence not being on the database, then a failure will ensue.

The library of sequences of commonly struck species is being constantly augmented by CSL. Of the 152 species identified by CSL as being involved in birdstrikes, or being representative of species frequently struck in Europe, 52 need DNA sequencing in order to provide a more comprehensive database from which birdstrike DNA sequences can be compared and therefore successfully identified. CSL has successfully obtained sequences for 9 of the species and are awaiting sequences from the 38 samples that we have obtained to date.

Samples of feathers lacking DNA may be identified in the future using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF). The technique will require considerable validation using reference material, but may be a valuable way forward in the future where feather material is DNA-deficient. This process would allow feather samples that lack cellular material (commonly submitted birdstrike remains) to be successfully identified to species level.

Key words: DNA, birdstrike remains, Genbank, MALDI-TOF, identification

Primary DNA applications for the aviation industry.

- Birdstrike incidents, where insufficient remains exist for positive identification by conventional methods.
- Identification of Birdstrike remains from engine ingestion incidents.

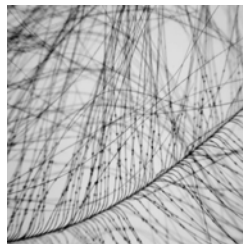
Sample quality

- Samples can contain large amounts of contour, flight and downy feathering as well as flesh.
- This sample was identified to family level using microscopic feather identification.



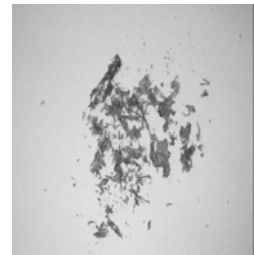
Good quality sample

- Downy feathering identified the remains as Pigeon *Columba sp.*
- The location of the incident allowed a weight range to be reliably assigned, as such DNA analysis was not required.



Typical quality sample

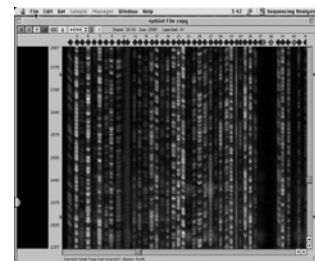
- This dried fleshy sample was received from an incident at an unknown location.
- DNA analysis gave a positive result as a large Gull *Larid sp.* enabling a weight range to be given.



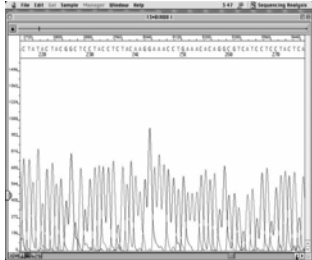
The DNA process

- The DNA process uses an enzyme that catalyses the formation and repair of DNA.
- High quality DNA is required to produce this product (PCR).
- The PCR product is used to identify genetic markers that can identify individual species.

PCR product sequencing image

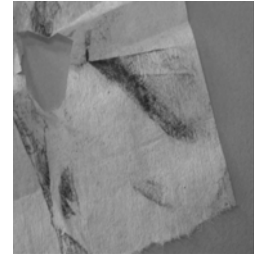


Bird sequencing analysis data



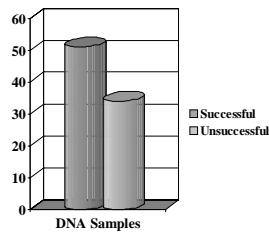
Poor quality sample

- This swab was taken from an incident at an unknown location.
- The swab appeared to contain an oily / sooty residue; DNA analysis proved negative.



DNA Success rates

- 85 Analyses
- 60% Success
- 40% Unsuccessful



Primary reasons for failure

- Failures due to lack of PCR product, this can result from the following:
- Denaturing due to exposure to high temperatures.
- Feather structures lack DNA, as they are made of keratin (protein), therefore, traces of blood supply need to be present in the sample.
- Swabs mainly containing soot / oil residue will inhibit the DNA extraction / PCR reaction process.

Database Limitations

- CSL identified 152 species that were representative of species frequently struck in Europe.
- Of these, 52 did not have sequences held on Genbank.
- We are in the process of sequencing 47 of the 'missing' species.

Secondary reasons for failure

- 10% Biological contamination
- (6% human contamination).
- (4% contamination from other sources i.e. insects or mammals).

Potential New Developments

- MALDI-TOF - a form of Mass Spectrometry - may be able to identify feather samples that lack any DNA material.
- However, the technique will require considerable validation, which may inhibit its use in the identification of birdstrike remains.

In Summary

- Birdstrike remains may not contain DNA material or the DNA may be denatured or contaminated, preventing positive identification.
- Adding species to Genbank will provide a more comprehensive database on which to find matches, CSL is currently working towards this.
- It has been shown that DNA analysis is a valuable tool in identifying Birdstrike remains that lack any visual clues as to their identity.