Identifying Bird Strikes Using Genetic Analyses: Processing and Collecting ‘Snarge’

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Smithsonian Institution Feather ID Lab

- Provide identifications to FAA, Air Force, Navy
- “Snarge” = Snot & Garbage... bird ick
Identification Techniques

1. Whole feathers
2. Microscopy
3. DNA
When do we use DNA?

- No visible feather material
- Damaged or non-diagnostic feathers
- FY 2017 used DNA in 61% of final IDs
Why Use DNA?

• DNA increases species-level identifications
  – Introduction of DNA barcoding in 2001 increased species level IDs from 74% to 90% of all cases
  – Average 86% of cases sent to DNA return positive ID FY13-17
  – Currently conducting internal pilot study of sample quality to improve species level IDs past 90%
DNA Barcoding: the CO1 gene

• Using a single gene for species IDs
  – chicken genome* is ~ 1 billion bp
    (~21,000 genes)
  – CO1 barcoding uses 640 bases (A,C,T,G)
  – Rapidly evolving, allows good species resolution
  – Useful for birds, mammals, reptiles, amphibians, insects

*from https://www.ncbi.nlm.nih.gov/genome/?term=gallus%20gallus
Process in the lab

- Samples received
- Buffer stabilizes and stores DNA
Process in the lab

• Digestion & Extraction
  – break cell
  – phenol-chloroform extraction

• Purification
  – collect and centrifuge DNA, wash away unwanted proteins
Process in the lab

• Amplification and cleaning
  – PCR: target a specific section of DNA and increase the number of copies to tens of millions
  – Exosap, Sephadex: clean up tiny bits of non-target DNA
Process in the lab

• Add fluorescent tag to each base
• Sequence (read) DNA with ABI 3100 sequencer
Matching Sequences: Barcode of Life

• Compare sequences in publicly available database, *Barcode of Life Data Systems (BoLD)*

• 192,389 species of animal

• 5,598 species of bird (>50% of all bird species)

Barcode of Life Vouchers

- SI Bird Division contributed sequences for >1,800 species to BoLD
- Vouchers from Expeditions
- Vouchers from donations (ex. USDA)

MCAS Iwakuni, Iwakuni, Japan

Camp Lemmonier, Djibouti, Africa
Barcoding Limits

• A few groups can’t be accurately identified with any single gene

Herring Gull (1,150 g)  Ring-billed Gull (520 g)  Mew Gull (420 g)
Beyond Barcoding

• Diving Ducks can be identified with ND2

Canvasback (1,220 g)  Lesser Scaup (830 g)  Ring-necked duck (700 g)
Beyond Barcoding

• Subspecies level identifications with ND2

- Streaked Horned Lark
  (*Eremophila alpestris strigata*)
- Critical Habitat: Designated
- Listing Activity:
  
  Oct. 2001, added to candidate list

  Oct. 2013 listed as threatened under ESA
Beyond Barcoding

• Gender-typing – some groups have markedly different weights between gender
Future Extensions

• Aiding identification in mixed-species flocks

• Identifying stomach contents

• Identifying number of individuals

Image credit: USFWS
COLLECTING REMAINS FOR MOLECULAR IDENTIFICATIONS
Links to collecting birdstrike remains

**AIR FORCE**
Air Force Safety Center
https://www.safety.af.mil
BASH box > BASH information
Guidelines and General Information

**NAVY**
NAVY Safety Center
Aviation: https://www.public.navy.mil/NAVSAFECEN/Pages/aviation/index.aspx
Airfield Ops/BASH > Collecting Birdstrike Remains

**CIVIL**
https://wildlife.faa.gov
Types of birdstrike remains

Tissue

Blood

Feather
What to use to collect the remains

- Alcohol wipes (never BBQ wipes)
- Alcohol pads
- FTA cards
- Swabs
- Paper towel
- Coffee filter
- Anything clean, convenient and handy- plastic utensil, lost luggage tag, business card.

Whatever is being used to lift, wipe or scrape, the remains has to be totally dried before sending it SI Feather Lab.
TISSUE and BLOOD

Collect ALL remains available
Use clean collecting supplies
Keep remains as **dry as possible**

**DO NOT USE BLEACH, WATER OR OTHER CLEANSER**

- Scrap off dry tissue
- Wipe remains or blood off the surface with alcohol wipes
- Put on to FTA or leave it on collecting supply—**DRY BEFORE SEALING IN BAG**

Include all feathers
Whole or Partial Carcass - Feathers

• Pluck the best variety of feathers available
  – Breast, back, wing, tail
  – include any feathers with color or pattern
  – If only a small amount of material is present, send all feathers available

• Do not cut feathers.
  Plucked feathers usually have down and tissue attached for micro and DNA analysis

• Photos and field IDs are welcome, but do NOT replace sending feather material.

   AIR FORCE has implemented photo ids
FTA® DNA collecting cards

- Use sterile applicator to wipe off material
- Press applicator to card to transfer material
- Allow card and applicator to dry before sealing in bag
- Include applicator and any available feathers with the card

Cost: $6 to $8 each; the applicator 20 to 30 cents
Problematic for DNA identifications

- Moldy
- Nothing
- Irradiated
- Rotten

- No Ethanol
- Not Biological
- Shipping Address
- Decomposition
Shipping

* SHIPMENT FROM OUTSIDE THE U.S *
Any material from foreign countries must be treated prior to shipment and include the following documents:

1. Certificate of Origin
2. Certificate of Treatment
3. USDA APHIS permit for SI

Include **AFSAS, WESS, or FAA 5200-7 report**
Secure all material in re-sealable plastic bag

**REGULAR SHIPMENT**
(US Postal Service)

Smithsonian Institution
Feather Identification Lab
E-600, MRC 116
PO Box 37012
Washington, DC 20013-7012

**OVERNIGHT SHIPMENT**
(FEDEX, UPS, DHL- require street address)

Smithsonian Institution
Feather Identification Lab
E-600, MRC 116
10th & Constitution Ave., NW
Washington, DC 20560

If using **US postal service**, never to this address-
IRRADIATED
**Do’s**

- Collect best variety of material available
- Pluck feathers
- Use alcohol or FTA® cards to collect snarge
- Collect multiple impacts including birds found on the runway
- Send dried samples
- Use online reporting
- Include report number and contact information
- Practice good hygiene at all times
- Always follow the safety or BASH protocols for your organization

**Don’t’s**

- Don’t send single feathers if more material is available
- Don’t send carcasses or smelly samples unless frozen and shipped in a cooler overnight
- Don’t cut feathers or use tape
- Don’t use water, bleach or other cleansers to collect snarge
- Don’t send remains without incident report or contact information
Good collecting of birdstrike remains – better molecular identifications to species

Tyto alba

Cathartes aura

Aix sponsa

Barn owl

Turkey vulture

Wood duck
Current in house study to see what we can do to increase molecular identifications to species

Preliminary results
20% of the second sampling was successfully resolved to species
More difficult to resolve were samples that are moldy, rotten, irradiated, greasy or nothing is visible

No DNA from 1st sampling -> 2nd sampling

Insects identified from 1st sampling -> 2nd sampling if feather is found - micro

Regular DNA protocol -> Identifications
Thank you for sending in the remains.