

Identifying Bird Strikes Using Genetic Analyses: Processing and Collecting 'Snarge'

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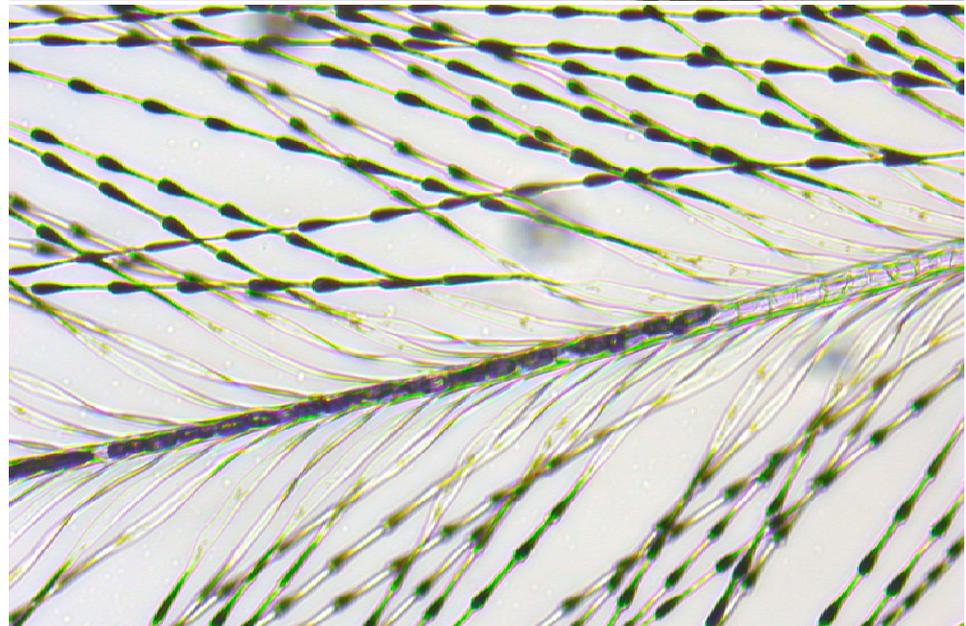
Smithsonian Institution Feather ID Lab

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



Identification Techniques

1. Whole feathers
2. Microscopy
3. DNA

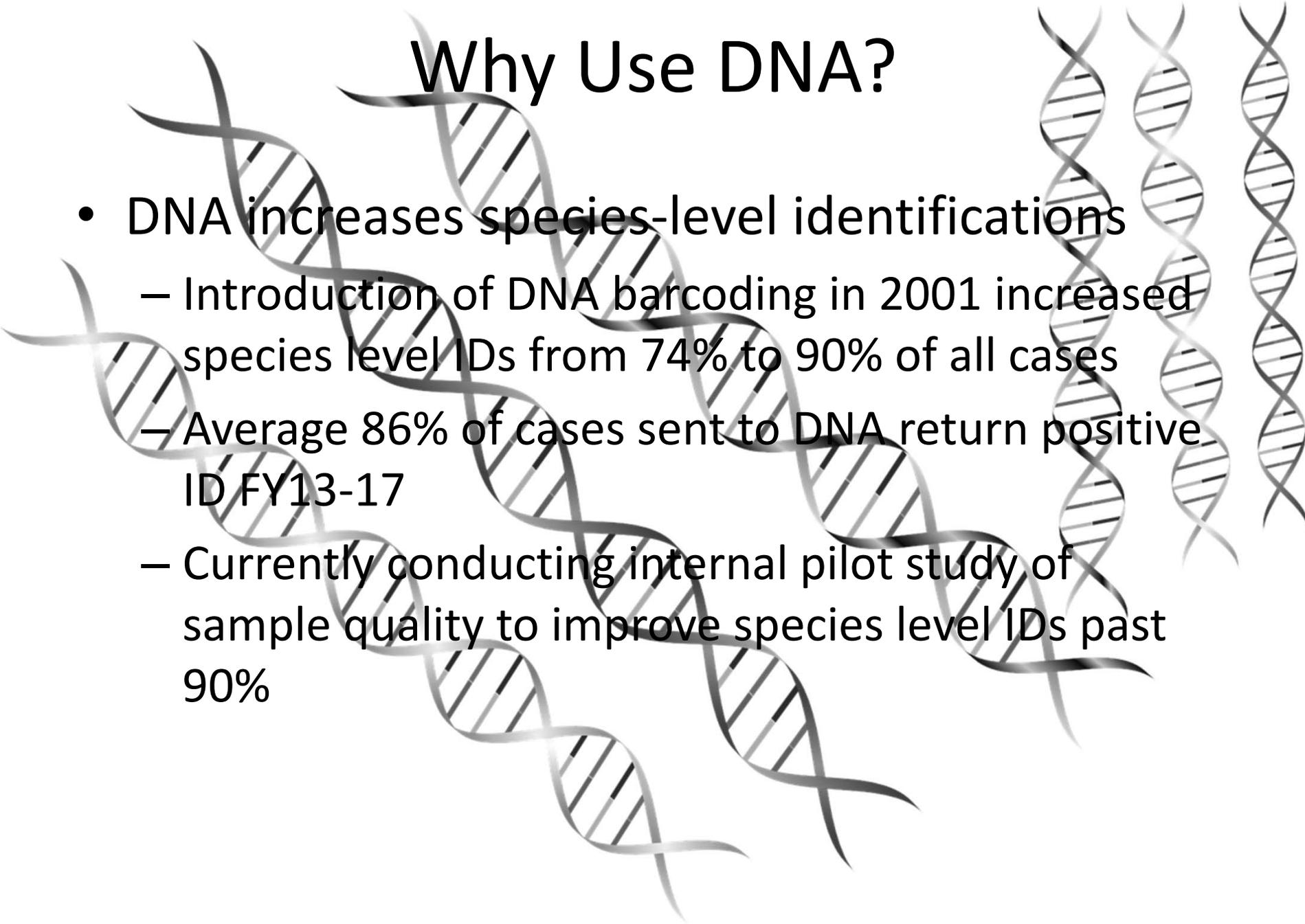


When do we use DNA?

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



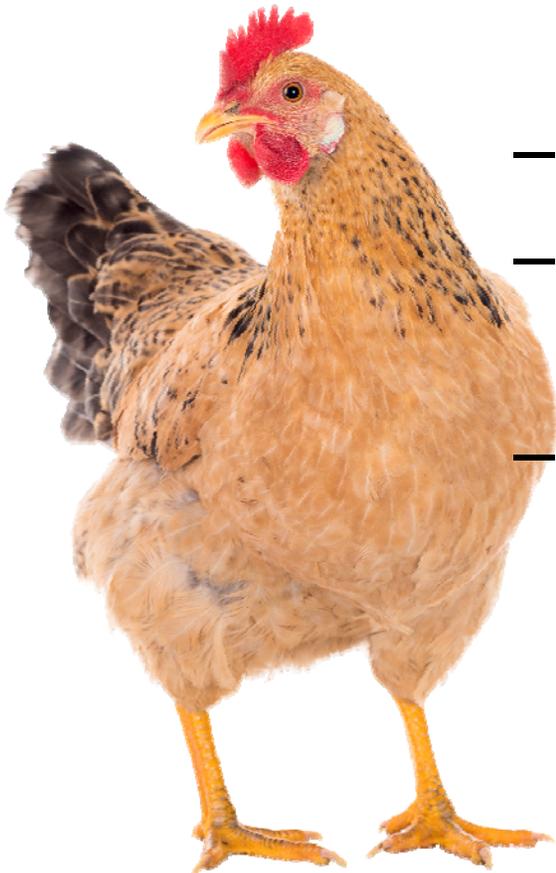
Why Use DNA?

The slide features several stylized DNA double helix structures. One large, semi-transparent helix is positioned on the left side, winding across the text. On the right side, there are three vertical, smaller DNA helices. The background is white, and the text is in a black, sans-serif font.

- 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>

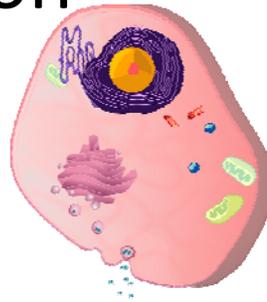
Process in the lab

- Samples received
- Buffer stabilizes and stores DNA



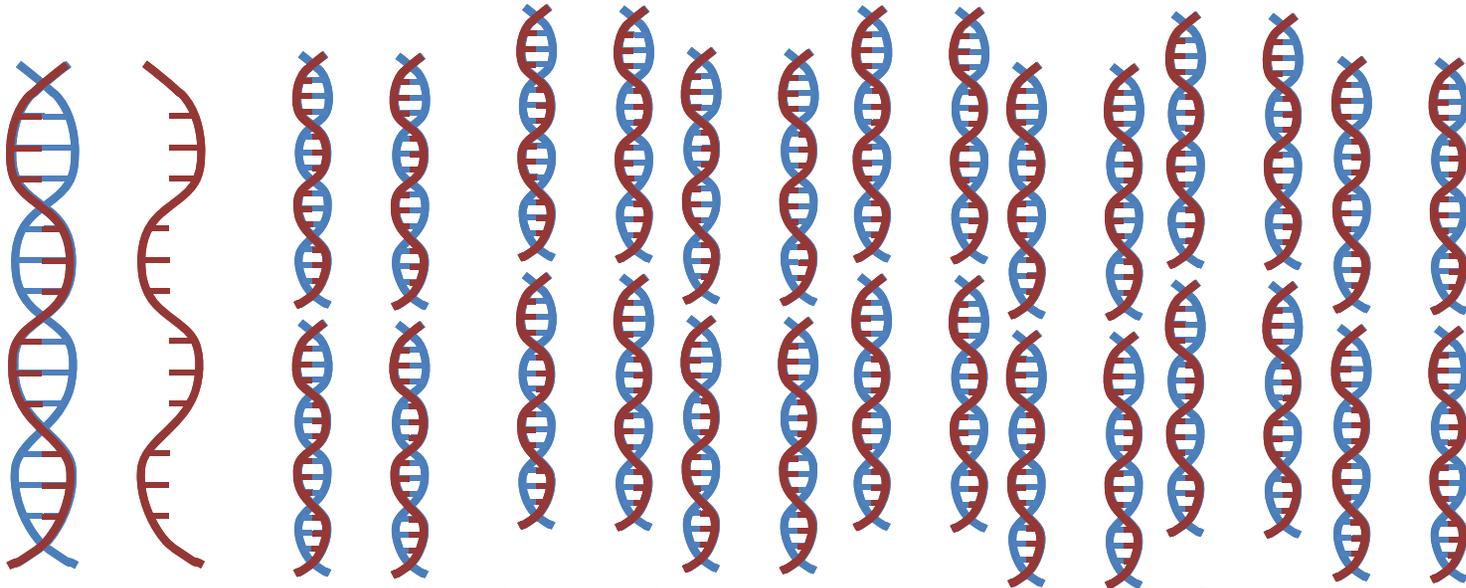
Process in the lab

- Digestion & Extraction
 - break cell
 - phenol-chloroform extraction
- Purification
 - collect and centrifuge DNA, wash away unwanted proteins



Process in the lab

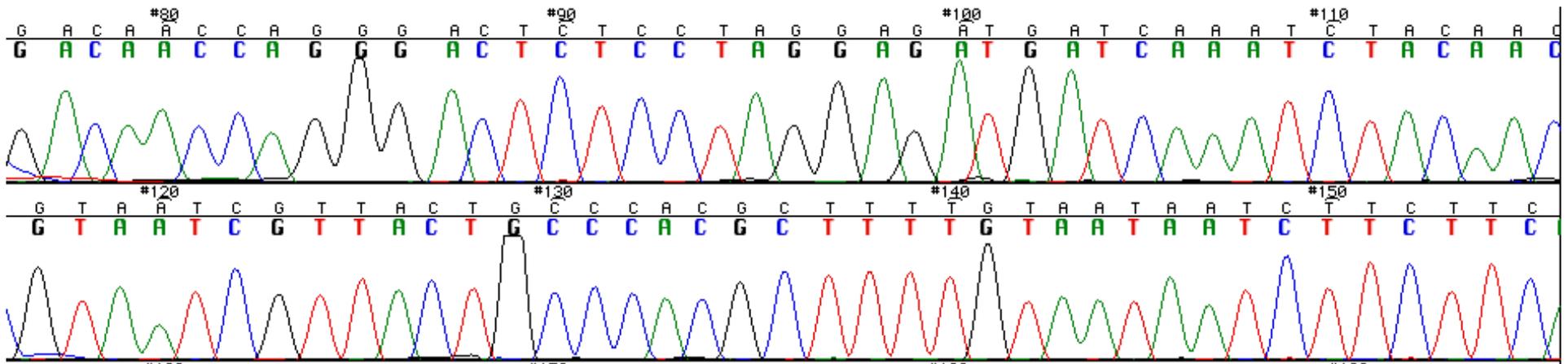
- Amplification and cleaning
 - PCR: target a specific section of DNA and increase the number of copies to tens of millions



- 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)

Enter sequences in fasta format:

```
TCAGTATACTTATCTTTGGGGCATGAGCTGGTATAGTAGGAACTGCC  
TCAGCCTACTTATCCGAGCAGAGCTTGGACAACCAGGGACTCTCCTAG  
GAGATGATCAAATCTACAACGTAATCGTACTGCCACGCTTTTGTAA  
AATCTTCTTCATAGTTATACCAATTATGATCGGAGGATTTGGAACTGA  
CTAGTCCCACCTTATAATTGGAGCACCTGACATAGCCTTCCCACGAATA  
ACAATATAAGCTTTTGACTTCTTCCCCATCATTCTCCTCCTACTAGCC  
TCCTCAACAGTTGAAGCAGGAGCAGGAACAGGCTGAACCGTATACCCT  
CCACTAGCCGGCAATCTAGCCACGCAGGAGCATCAGTTGACCTCGCT  
ATCTTCTCCCTCCACCTAGCAGGGGTCTCCTCCATCCTAGGTGCAATCA  
ACTTTATCACAACCTGCCATCAATATAAAACACCTGCCCTCTCACAATC  
CAAACACCCCTATTTCGTATGATCCGTCCTCATTACCGCCGTCCTACTAC  
TCCTCTCCCTCCCGTCTCGCTGCAGGCATCACTATACTCTTAACTGA  
CCGTAACCTAAACACACATTCTTCCAGCCAGCTGGAGGAGGCGGACCC  
CCATCTTATACCAACACCTATCTGATTCTTTGGTTCATTGGAAAGTTTAA  
AA
```

SUBMIT



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)



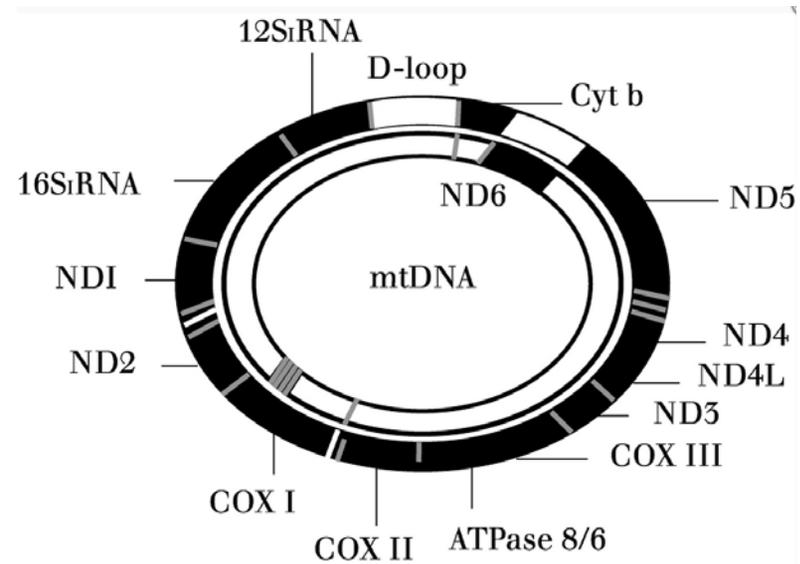
Ring-billed Gull (520 g)



Mew Gull (420 g)

Beyond Barcoding

- Diving Ducks can be identified with ND2



Canvasback (1,220 g)



Lesser Scaup (830 g)



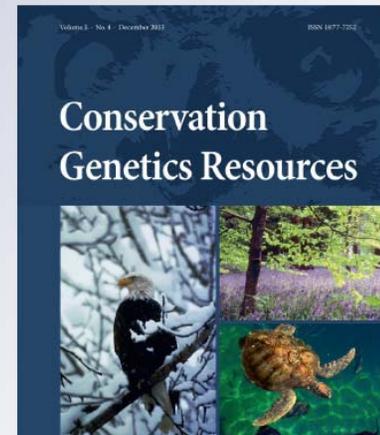
Ring-necked duck (700 g)

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

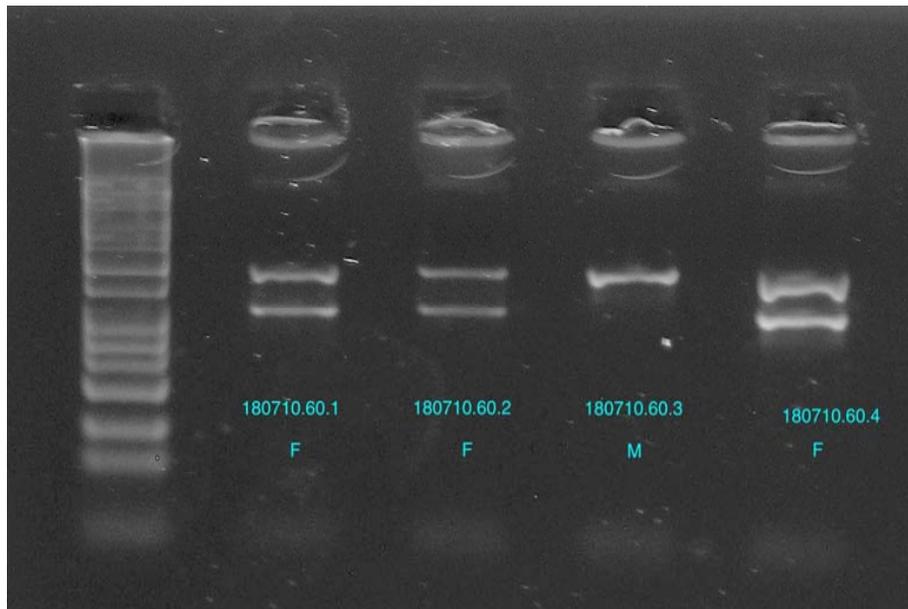
MtDNA ND2 sequence identifies Streaked Horned Lark (Eremophila alpestris strigata) from birdstrike to US Air Force F-15 at Portland International Airport, Oregon

Carla J. Dove, Nor Faridah Dahlan & Sergei V. Drovetski



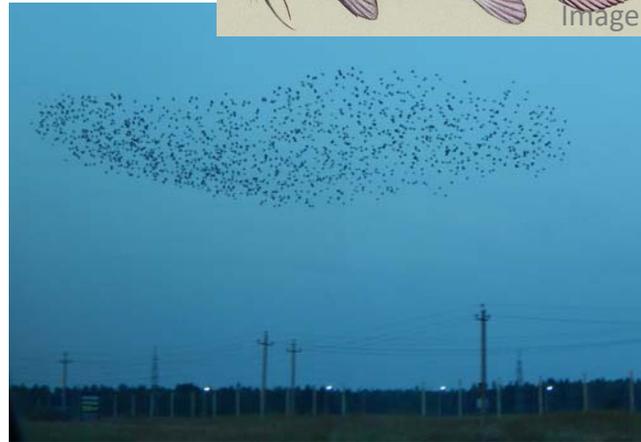
Beyond Barcoding

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



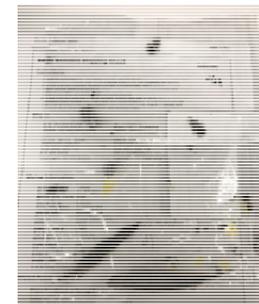
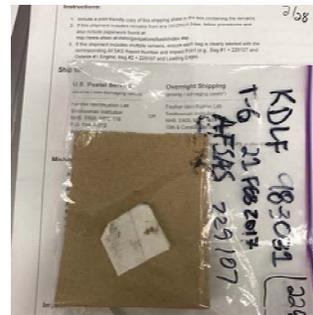
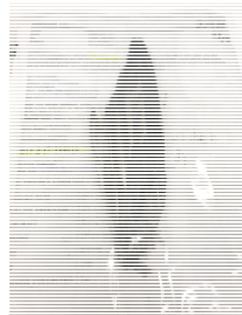
Future Extensions

- Aiding identification in mixed-species flocks
- Identifying stomach contents
- Identifying number of individuals





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



CIVIL

<https://wildlife.faa.gov>

NAVY

NAVY Safety Center

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

Types of birdstrike remains

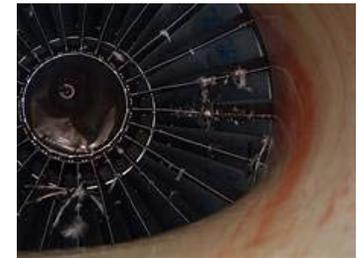
Tissue



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**

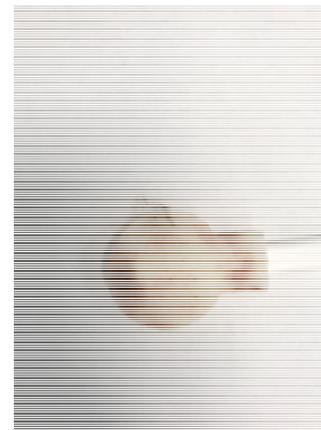
**DO NOT USE BLEACH, WATER OR
OTHER CLEANSER**

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



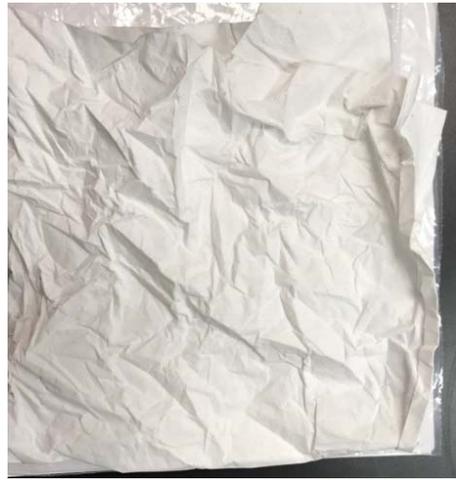
Cost: \$6 to \$8 each; the applicator 20 to 30 cents

Problematic for DNA identifications



Moldy

**No Ethanol
Not Dried**



Nothing

**Not Biological
Nothing**

7/12/2018
Destination
9/1-2
Review Strike Re

| | | |
|--|-------------------------------------|---|
| 1. Name of Operator/Carrier Unknown | 1. Name of Operator/Carrier KMKC | 1. Name of Operator/Carrier |
| 4. Aircraft Registration Unknown | 4. Aircraft Registration UNK | 4. Aircraft Registration |
| 6A. Flight Number Unknown | 6A. Flight Number UNK | 6A. Flight Number Suspect flight 2483 |
| 7. Airport Name/ID (KMR) F | 7. Airport Name/ID UMKC | 7. Airport Name/ID (KMR) ROGUE VALLEY INTL |
| 10. Height (AGL) 0 | 10. Height (AGL) 0 | 10. Height (AGL) |
| 12. Phase of Flight I. Unknown | 12. Phase of Flight I. Unknown | 12. Phase of Flight I. Unknown |

Irradiated

Shipping Address



Rotten

**Decomposition
No carcass**

Shipping

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



* 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



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**

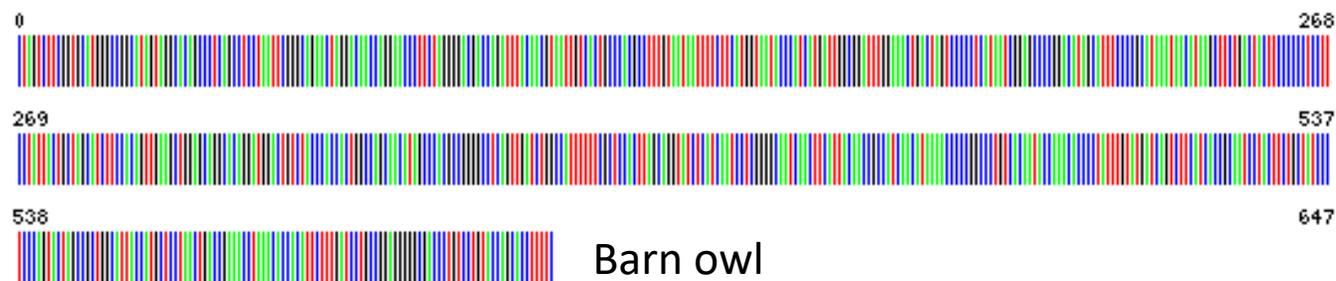
Don't's

- **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**

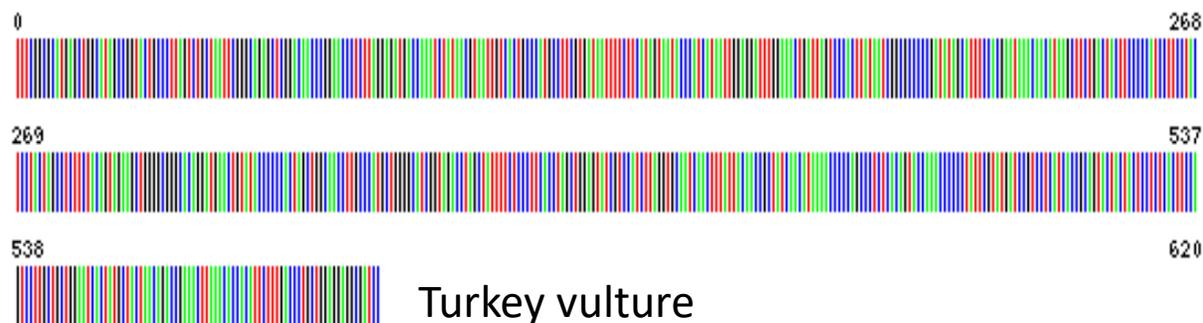
Good collecting of birdstrike remains – better molecular identifications to species



Tyto alba



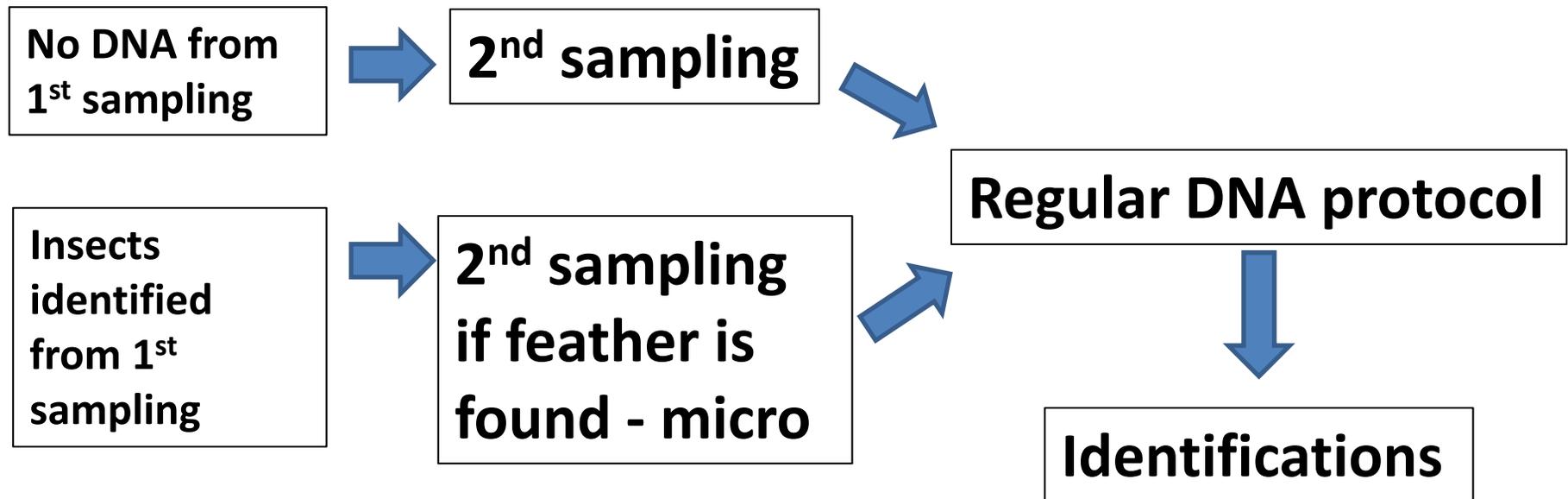
Cathartes aura



Aix sponsa



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

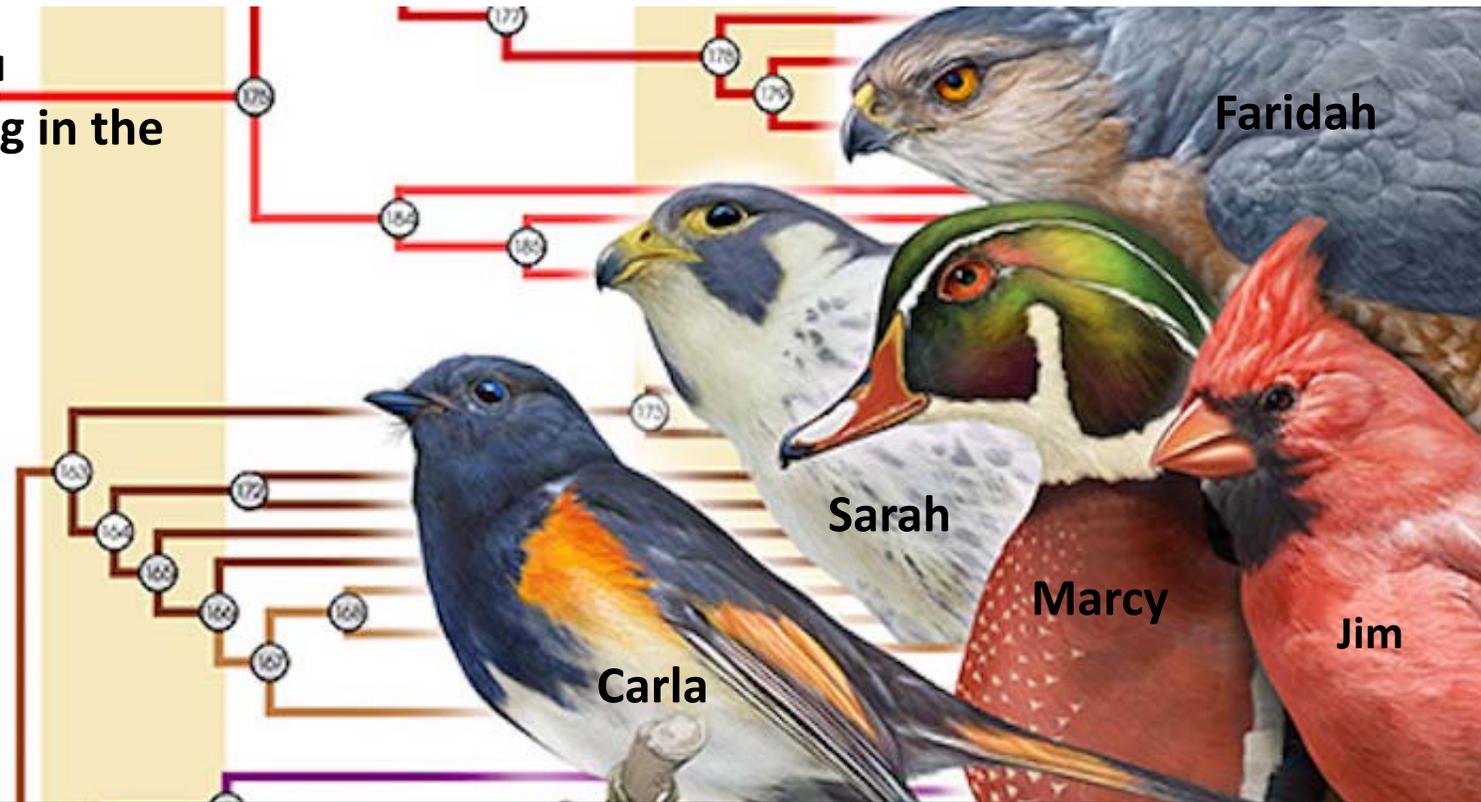


Preliminary results

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

Thank you
for sending in the
remains



Feather Identification Lab

Smithsonian
National Museum of Natural History