Feather DNA Solves Sex Determination Mysteries and Identifies Cryptic Species of San Francisco Bay Area Birds

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Artwork by Edward Rooks

Background

- Often identify bird species by "field marks"; for some species field marks insufficient.
- Sexes of Yellow-rumped Warblers (YRWA) and cryptic species of Western Flycatchers (WEFL) are hard to distinguish visually, but…
  - Male birds have two Z sex chromosomes, females have Z and W (differ in size).
  - Bird species can be differentiated by mtDNA gene CO1.

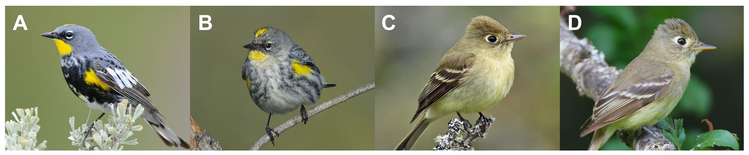


Figure 1. Pairs of morphologically similar birds. Male (A) and female (B) YRWA. Pacific-slope Flycatcher (C) and Cordilleran Flycatcher (D), two species of WEFL.

- The base (calamus) of feathers contain small amounts of DNA; invites novel research questions (Smith et al. 2003).

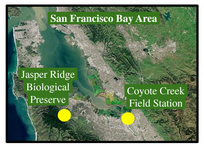
Research Questions

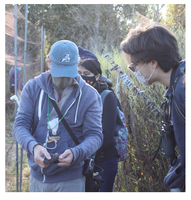
Can we determine the sex of Yellow-rumped Warblers migrating into the Bay Area with DNA from feathers?

Can we identify cryptic species of Western Flycatchers in the Bay Area with DNA from feathers?

Methods

1. Collect **feathers** at banding stations
   - YRWA: Collected from 2/28/21 to 4/10/21
   - WEFL: Collected 8/29/2021 and 9/1/2021





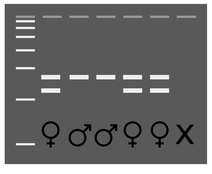
2. Extract DNA with QIAGEN DNeasy kit

Genomic DNA

3. PCR with sex chromosome primers for YRWA (Purwaningrum et al. 2019)

PCR Product

4. Agarose gel electrophoresis

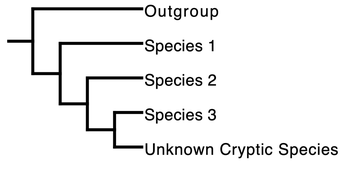


3. PCR with mtDNA CO1 primers for WEFL (Johnson & Cicero 2002)

PCR Machine

PCR Product

4. Sanger sequencing and BLAST®



Sex Determination

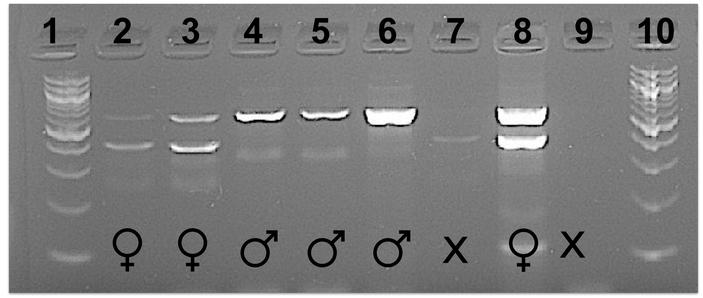


Figure 2. Representative agarose gel. Single prominent band indicates male (ZZ), two indicates female (ZW), none indicates PCR failure (e.g. band 7). 100bp ladders loaded in lanes 1 and 10, positive control (female Steller's Jay) in lane 8, and negative control (water) in lane 9. Only 7/14 YRWA samples shown.

- 13/14 YRW feather samples successfully distinguished by sex after PCR amplification and agarose gel analysis.
- 6 males identified, 7 females identified.
  - Confirmed morphology-based predictions by experts in field for 8 birds.
  - Among 5 birds with unknown sex, identified 1 male and 4 females.

Cryptic Species

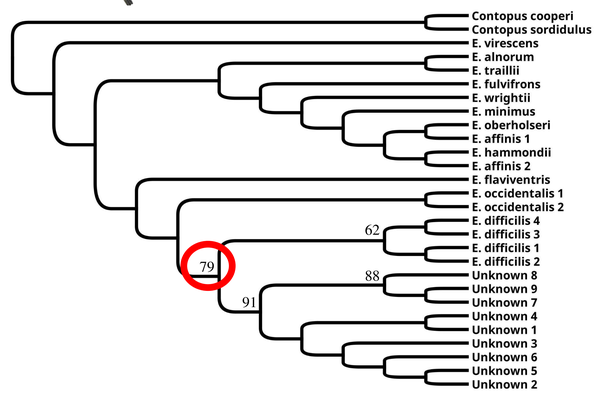


Figure 3. Phylogenetic tree identifying unknown samples as *Empidonax difficilis*. CO1 mtDNA sequences from nine unknown samples were aligned with a Genbank reference panel of *Empidonax* Flycatcher sequences that have been or could be found in the SF Bay Area. Bootstrap values are shown at relevant branches. *Contopus cooperi* is the outgroup. The red circle highlights the support for the unknown sequences being Pacific-slope Flycatchers (*E. difficilis*).

- 7/9 WEFL samples successfully identified to species (mean identity = 99.6%).
  - All 7 identified to Pacific-slope Flycatcher.
- 1 sample exhibited a unique, synonymous SNP (confirmed 3x = Unknowns 7,8,9).

Conclusions

Sex was successfully determined from ~85% of YRWA feather samples.

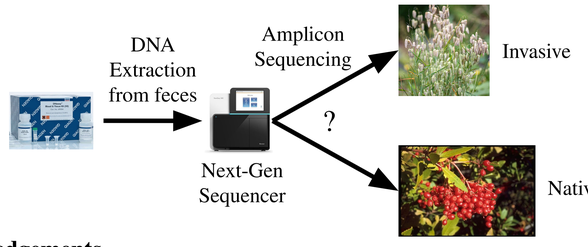
- Confirms usefulness of feather DNA for sex determination.
- Found an approximately equal sex ratio with no strong seasonal pattern.
- Indicates the presence of both sexes in the SF Bay Area from Feb 28 to Apr 10, 2021.

Species were successfully identified from ~75% WEFL feather samples.

- Confirms usefulness of feather DNA for identifying cryptic species.
- All identified were Pacific-slope Flycatcher, consistent with the species' accepted breeding distribution.
- One sample exhibited a polymorphism, suggesting hidden diversity within WEFL.

Future Directions

- Larger sample sizes collected over a longer time period & across multiple years promises to uncover:
  - Sex-specific ecology in YRWA (e.g. migration patterns, establishment of territories and nest building).
  - Hidden biodiversity among cryptic WEFL.
    - Detection of rare vagrant flycatchers (Goldberg & Mason 2017).
  - Seasonal and year-to-year variation among both YRWA and WEFL populations
  - Uncover morphological characteristics that may improve accuracy of field identifications.
- Molecular Scatology in a restored habitat:
  - Extract DNA from bird scat (feces).
  - Next-Gen Sequencing with universal plant ITS primers to determine if birds are consuming native or invasive plants in a habitat restoration.



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