2015

Analysis of standard DNA procedures on feathers of late 19th to late 20th century Osprey (Pandion haliaetus)

Alia A. Hamdan
Virginia Commonwealth University, hamdanaa2@vcu.edu

Catherine B. Viverette
Virginia Commonwealth University, cbvivere@vcu.edu

Rodney J. Dyer
Virginia Commonwealth University, rjdyer@vcu.edu

Analysis of standard DNA procedures on feathers of late 19th to late 20th century Osprey (*Pandion haliaetus*)

Alia Hamdan[1, 2], Catherine Viverette[1], Dr. Rodney Dyer[2*]

Center of Environmental Studies[1], Department of Biology[2], Virginia Commonwealth University, Richmond, VA, USA

Introduction

Species with well-documented demographic histories and well known perturbations to gene flow provide good models for understanding how historic events impact contemporary population genetic structure[1,2]. Osprey (*Pandion haliaetus*), a marine bird-of-prey, experienced steep declines after widespread organochloride pesticide (e.g. DDT) use in the mid-twentieth century[3], however, population genetic consequences remain unknown. Use of historic specimens can aid population genetic studies[4,5], however, these samples can degrade over time impacting quantity and quality of extracted DNA[5]. We compared the concentrations of extracted DNA of Osprey feathers from museum and research collections to those of contemporary samples collected according to standard field collection protocols.

Objectives

The object of this study was to determine if feather samples from museum and research collections can be used for population genetic analysis of pre- and post-DDT effects in Osprey. A secondary objective is to promote the further development and use of museum and research collection samples in research; this avenue offers a noninvasive technique for studies of species of conservation concern through easily accessible materials.

Methods

82 Osprey feather samples from collection gathered for previous study:

- Date from late 19th to late 20th century
- 41 from Smithsonian Natural Museum of Natural History (A3-A72)
- 41 from VCU research collection (1999: A73-A118)

Standard genetics procedures:

- QIAGEN DNeasy extraction kit
- Polymerase Chain Reaction (PCR)
- Agarose gel electrophoresis, GeneRuler Ladder (100bp)
- Microsatellite primers (PHAL 12: forward primer: [HEX]TGCATCCTAATGAACCTTTGC; reverse primer: AGGCTGGTGGTTAAACATGG)[6]

Software: NanoDrop 8000 Spectrophotometer to measure DNA concentration, Invitrogen E-Editor Version 2.02 for gels, IBM SPSS Statistics 22 for statistics

Cathy Viverette

Rachel Barnes

Rachel Barnes

References:

1. Viverette *et al.* 2007. Finfish-waterbird trophic interactions in tidal freshwater tributaries of the Chesapeake Bay. Waterbirds 30: 50-62. **2.** Waldick *et al.* 2002. Evaluating the effects of historic bottleneck events: an assessment of microsatellite variability in the endangered, North Atlantic right whale. Molecular Ecology 11: 2241-2249. **3.** Watts and Paxton 2007. Ospreys of the Chesapeake Bay: Population recovery, ecological requirements, and current threats. Waterbirds 30: 39-49. **4.** Rawlence *et al.* 2009. DNA content and distribution in ancient feathers and potential to reconstruct the plumage of extinct avian taxa. Proceedings of the Royal Society B 276: 3395-3402. **5.** Horvath *et al.* 2005. An overlooked DNA source for non-invasive genetic analysis in birds. Journal of Avian Biology 36: 84-88. **6.** Dawson *et al.* 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility – as demonstrated for birds. Molecular Ecology Resources 10: 475-494. **7**. Hogan *et al.* 2008. Optimizing the use of shed feathers for genetic analysis. Molecular Ecology Resources 8: 561-567. **8.** Bayard de Volo *et al.* 2008. An improved extraction method to increase DNA yield from molted feathers. The Condor 110: 762-766.

Results

47 successful amplifications out of 82 samples: 8 of NMNH samples, 39 of VCU collection

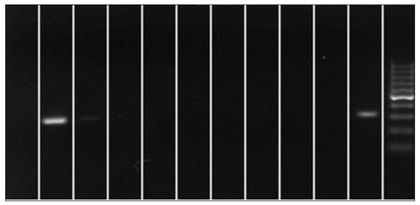
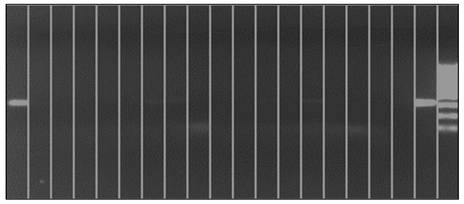


Figure 1. *Above*: Agarose gel electrophoresis of extracted and amplified samples A3-A18 with negative (-) control in well 10 and positive (+) control in well 11.

Figure 2. *Right Top*: Agarose gel electrophoresis of samples A20- A49, two - controls in wells 16 and 17, and + control in wells 18 and 19

Figure 3. *Right Bottom:* - control for A20-A49 in well 1



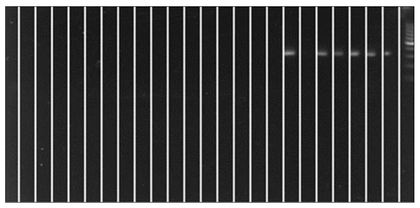


Figure 4. Samples A50-A78 (of A50-A97)

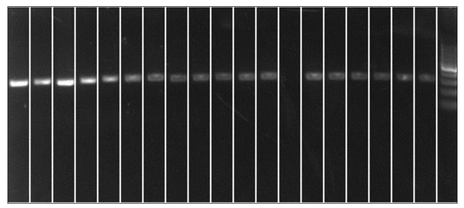


Figure 6. Samples A98-A118

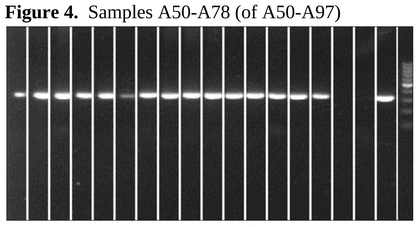


Figure 5. Samples A79-A97 (of A50-A97), – controls in wells 16 and 17, and + control in well 18

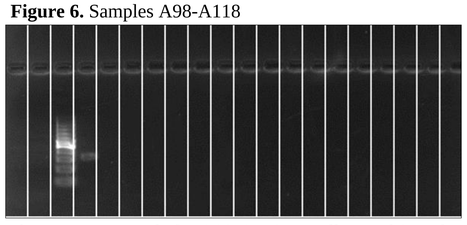


Figure 7. - controls for A98-A118 in wells 1 and 2, and + control in well 4

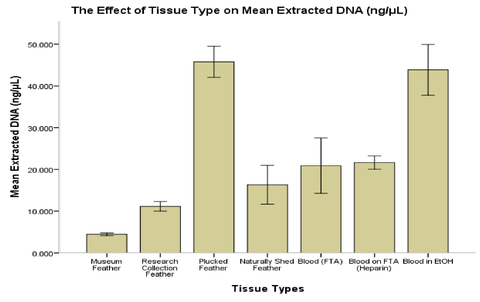


Figure 8. Mean (±SE) extracted DNA (ng/µL) among different tissue types: Museum feathers, VCU research collection feathers (1999), Plucked feathers, Naturally shed feathers, Blood (FTA), Blood (FTA) with heparin, and Blood in Ethanol (EtOH)

Table 1. Tukey HSD results from one-way ANOVA (α: 0.05, F= 12.15, p= 0.00) comparing mean extracted DNA (ng/µL) of various tissue types

Nanodrop Results

	Origin	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a,b]	Museum Feather	41	4.46178	
	Research Collection Feather	41	11.14663	
	Naturally Shed Feather	90	16.32186	16.32186
	Blood (FTA)	9	20.89667	20.89667
	Blood on FTA (Heparin)	41	21.63122	21.63122
	Blood in EtOH	28		43.84157
	Plucked Feather	176		45.76249
	Sig.		.620	.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 29.562.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Conclusion

The results show that historic feather samples can yield amplifiable DNA for population genetic studies. Historic feather samples collected from living organisms resulted in higher concentrations of genomic DNA than museum study skins. Storage conditions and degradation of specimens may affect the ability to extract amplifiable DNA. However, there is no significant difference in concentration of DNA obtained from historic feather specimens and shed feathers or some types of blood samples collected from live individuals which are commonly used in population genetic studies[4,5]. The highest concentration of DNA was obtained from blood and feather samples recently collected from live birds. However ability to collect blood samples or plucked feathers from wild individuals across a large area, especially for protected species sensitive to disturbance, can be limiting[7]. Including specimens from natural history museums and research collections can combat these limitations and may allow comparison of historic and contemporary population genetic structure over broader temporal and spatial scales. In addition, maintaining research collections and utilizing museum specimens can help build relationships between museum researchers, academics, and outside scientists.

Future Direction

Standard extraction techniques were used in this study with some modifications developed specifically for feathers[8]. Optimizing these procedures and adding additional steps in the extraction and PCR processes may yield better results for future studies[4] as well an increase in sampling size with a stringent focus on specimen condition[7]. The next step is determining the quality of the extracted DNA by genotyping multiple microsatellites of different sizes and sequencing a portion of the mitochondrial DNA. If the DNA is of sufficient quality, the samples will go on to be included in a current population genetic study of Osprey for pre- and post-DDT effects.

Juvenile Osprey on the Rappahannock River

Acknowledgements

I would like to thank my mentor Cathy Viverette, VCU Life Sciences Department, VCU-CES, and VCU-BIO, especially the Population Genetics Lab run by Dr. Rodney Dyer and the Verrelli Lab for use of their facilities. Also, we extend our gratitude to the VCU Rice Rivers Center, Florida Fish and Wildlife Conservation Commission, American Ornithologists Union, the International Osprey Foundation, the Virginia Society of Ornithology, and the VCU-William & Mary Center for Conservation Biology for their financial support. We also give special thanks to the Smithsonian Institute: Natural History Collections for providing the specimens for the study and Dr. Deborah Dawson from the University of Sheffield for the development and provision of the microsatellite primers. I would also like to thank Rachel Barnes for her photography.

VCU Life Sciences · VCU College of Humanities and Sciences · Smithsonian National Museum of Natural History · Rice Rivers Center VCU