



Shvemetzphotography
J6Wenile eagle

Preliminary population genetic analysis of Bald Eagles using microsatellite markers developed for White-tailed Sea Eagles

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Abstract

The Bald Eagle (*Haliaeetus leucocephalus*) is considered a federally threatened species in the United States. Population studies with these birds are difficult, however, because adults are not easily banded and sexes are monomorphic. In this study, we used microsatellite primers developed for White-tailed Sea Eagles and applied them to Bald Eagles in order to find polymorphic markers, which can aid in inter- and intra-population studies. Birds were also sexed by using PCR (polymerase chain reaction) to amplify a segment of the W and Z chromosomes. Bald Eagle DNA was extracted from blood dried on gauze or preserved in phosphate buffered saline (PBS). It was then amplified using PCR and the products were visualized on polyacrylamide gels. All ten loci produced amplified product. Four of these were polymorphic and therefore useful for population studies. Fluorescent primers were developed for the polymorphic loci for automated genotyping on a Beckman CEQ8000. Although automated genotyping has many advantages when compared to manual gels, several issues, including interpretation of peaks and primer failure were problematic in this study. Ninety-nine juvenile Bald Eagle samples, collected from the northeastern United States (Maine, New York, New Hampshire), were genotyped at the polymorphic loci and sexed. Loci contained 2 to 5 alleles, which were evenly distributed across the sample set; allele frequencies will be reported. Juveniles sampled for this study were 51.6% male and 48.4% female. These preliminary results show these markers will be very valuable in future population studies concerning site fidelity, territoriality, and gene flow.

Introduction

In 1976 the US Fish and Wildlife Service listed the Bald Eagle as a national endangered species. The decline in Bald Eagle populations was primarily due to DDT, other anthropogenic contaminants, and habitat destruction. These and other factors combined led to near extirpation of Bald Eagles from much of their range in the United States. In recent years, however, Bald Eagle populations have rebounded, which may even result in their delisting (www.fws.gov/endangered/). Population genetic studies will help elucidate the long term effects of these population fluctuations as well as better understand the demographics and behaviors of this species.

Polymorphic microsatellite markers are important tools for population genetic studies, paternity assessment and unambiguous identification of individuals for forensic and ecological applications (Martinez-Cruz, et al., 2002). Demographic studies also are enhanced when the sex of each individual can be determined. Since Bald Eagles are sexually monomorphic, a method of sexing that uses DNA collected for the population genetics study would be the most useful.

We had 2 major goals for this study:

1. Use microsatellite primers developed for other eagle species to amplify polymorphic microsatellite loci in Bald Eagles. In this case, there were no primers available to amplify Bald Eagle DNA, so we used cross-species amplification, which makes use of primers developed for other eagle species (Busch et al., 2005). The primers that we used were from White-tailed Sea Eagles (*Haliaeetus albicilla*; Hailler et al., 2005). This technique has been used in many studies, including the use of Gyrfalcon microsatellites in Golden Eagles and other raptors (Nesje and Roed, 2000).

2. Use PCR to differentially amplify a segment of the W and Z chromosomes in order to determine Bald Eagle sex from DNA. Our lab has successfully used primers developed by Itoh et al. (2001) to molecularly sex a number of different bird species. In this study we used two different primer sets to determine the best primer combination for Bald Eagle sex determination.

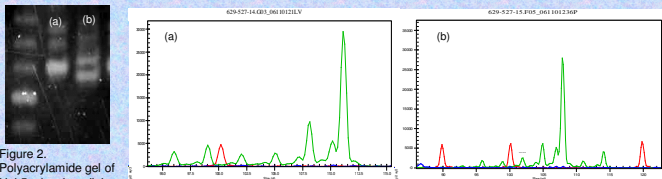


Figure 2. Polyacrylamide gel of Hal 5, showing alleles at (a) 110 bp, (b) 107 and 113 bp.

Figure 3. Electropherograms of the same two individuals in Figure 2, respectively, which can be interpreted as (a) 110 bp or 107 and 110 bp and (b) 107 or 107 and 113 bp.

Methods

- DNA extracted from blood on gauze, filter paper, and in PBS using Qiagen DNeasy tissue kit
- DNA amplified using PCR. Products observed using gel or capillary electrophoresis.
 - Microsatellites-
 - 25 µl PCR reactions: 0.4uM each primer, 0.2 mM each dNTP, 1.5 mM MgCl₂, 0.5 U Taq DNA polymerase (NEB), and 20 ng template DNA in buffer containing 20 mM Tris-HCl and 50 mM KCl (pH 8.0)
 - Hal 5 - Platinum Taq (Invitrogen) and 2.0 mM MgCl₂; Hal 9 - FastStart Taq (Roche) and 2.0 mM MgCl₂
 - Thermal Profile: 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, annealing temperature for 30 seconds (Table 1), 72°C for 30 seconds; annealing temperature for 1 minute, 72°C for 10 minutes
 - Observed on 7.7% polyacrylamide gel, stained with Sybr Gold (Invitrogen)
 - Sexing-
 - 25 µl PCR reactions: 0.4uM each primer (AWSO-5, NRD-4, SINT-F, and SINT-R, Itoh et al. 2001), 0.2 mM each dNTP, 1.5 mM MgCl₂, 0.5 U Platinum Taq DNA polymerase, and 100 ng template DNA in buffer containing 20 mM Tris-HCl and 50 mM KCl (pH 8.0)
 - Thermal Profile: 94°C for 3 minutes; 31 cycles of 94°C for 30 seconds, 62°C for 20 seconds, 72°C for 45 seconds; 72°C for 5 minutes
 - Observed on 2.0% agarose gel, stained with ethidium bromide
 - Beckman CEQ 8000 Genetic Analysis System
 - 15 µl PCR reactions: 0.02 uM forward labeled primer (Prologo), 0.07 uM reverse unlabeled primer, 0.2 mM each dNTP, 2.5 mM MgCl₂, 0.5 U Taq DNA polymerase, and 15 ng template DNA in buffer containing 20 mM Tris-HCl and 50 mM KCl (pH 8.0)
 - Coloadng was possible with Hal 3 and Hal 5, using half volume of each

Table 1. Summary of the loci used in cross-amplification to Bald Eagles

Loci	Primer Sequence	Repeat Sequence	No. Alleles	Size Range	Annealing Temp.
Hal 3	F: GGGCATCCCTCAATCTGTTAC R: ATGTTCCCGACTAGGCCCTTTC	(CAA) ₆	2	138-142 bp	57 °C
Hal 5	F: GGGCAAAACCCCTGTGAATACC R: GTGGTCCGTGGGACACG	(AGG) ₁₀	3	107-113 bp	60 °C
Hal 9	F: TGAGCTTTGTAGTAGCAGGTGGT R: TGCAAAAATAGAGCCAAATAGCC	(AC) ₁₇	5	136-160 bp	64 °C
IEAAAG05*	F: GTCTGAATCCAGGACAGGCGAC R: GGGCAAAAGCTCATGTTTTC	(AAAG) ₇	2	122-126 bp	65 °C
Hal 1	F: GAATACACCCGACAGCAACCC R: CCGAGCTGTGGTATAACATAC	(GT) ₁₇	N/A*	N/A*	N/A*
Hal 13	F: CCACCTAGTAAGGAGCTTTGG R: CTTGTGTTTGTGCGAGATG	(CA) ₁₇	N/A*	N/A*	N/A*
Hal 6	F: CATCCAAACTATTCAAGCGCTA R: AGAGCAGGTGTCTTTTCAGAGC	(CAA) ₄ ‡	1	180 bp	62 °C
Hal 7	F: TCCGAAGGTGCATGCAGTAG R: GGGATGTGCAAGAAATCTACC	(GT) ₁₃	11	150 bp	60 °C
Hal 10	F: CATGCACCGTGTGAATCAG R: ACCCCACCAAGCTTACCAGTG	(CA) ₁₂	1	225 bp	64 °C
Hal 14	F: GTCGCACTCTCTTGGACAC R: CAACACTTTCAGGATGCTC	(AGG) ₄ ‡	1	235 bp	58.4 °C

*Locus comes from Busch et al., 2005; all other loci come from Hailler et al., 2005
 † Could not interpret results
 ‡ Complex repeat structure

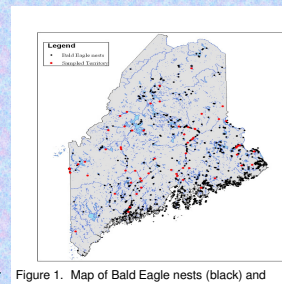


Figure 1. Map of Bald Eagle nests (black) and sampled territories (red) in Maine.

Results

- 84% of the samples came from Maine (Figure 1), 8% from New York, and 8% from New Hampshire
- All of the markers amplified, but 4 out of 10 were polymorphic
 - Each polymorphic marker contained between 2 and 5 alleles (Table 2)
 - The number of eagles analyzed at each locus varied due to problems with primers
 - Hal 5 yielded problematic peak patterns, especially when moved to the automated genotyping system. Patterns were difficult to interpret as homozygous or heterozygous (Figures 2 and 3). As a result, allele frequencies at this locus were difficult to interpret.
 - Hal 9 was not in Hardy Weinberg Equilibrium.
 - Hal 3, Hal 5, and IEAAAG05 were in HW Equilibrium.
- Juvenile sex ratio: 51.6% males, 48.4% females (n=91 eagles)

Discussion

These preliminary results indicate that cross-species amplification was successful in Bald Eagles, and therefore this technique can be used to find other polymorphic loci for future studies in site fidelity, territoriality, and gene flow. Three of the loci were in Hardy Weinberg Equilibrium: Hal 3, Hal 5, and IEAAAG05, and one was not, Hal 9. Samples sizes were quite small for Hal 9 and the IEAAAG05 loci (see below) and this may have affected the Hardy Weinberg test for Hal 9, since this locus also had numerous alleles (Table 2). Fluorescently labeled forward primers for both Hal 5 and IEAAAG05 are being resynthesized because results from these loci were inconsistent (see below).

Amplification of the loci used in this study was finicky. A change of 0.2°C in the annealing temperature often resulted in no PCR product. Also, Hal 5 and Hal 9 required specialized Taq and an increase in the concentration of MgCl₂ for successful amplification. This may be because the primers developed for a different species do not perfectly match the Bald Eagle DNA template or because the intervening sequence is a challenging for standard Taq to amplify. We intend to sequence some of these loci to determine if redesigning primers will increase our success.

Conversion from manual polyacrylamide gel to a capillary electrophoresis proved to be challenging. We believe most of the problems were caused by faulty fluorescently labeled primers. As a result, sample sizes are not the same for each locus because of inconsistency in calling alleles (Table 2). Hal 5 produced different banding patterns on the manual gels (Figure 2) compared to the capillary system (Figure 3), which made it very difficult to interpret. Despite these problems, the capillary electrophoresis system is still a much more efficient way to perform genotype analyses. It is more expensive than manual gels, but many more samples can be analyzed in a fraction of the time

Sexing was also successful, using primers developed to amplify a section of the sex chromosomes in loons. These were used because they had a more visible difference in the banding than the ones which were developed for falconiformes (Itoh et al., 2001). However, both sets did amplify and produced visibly different banding in males and females.

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Adult eagle



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Locus	n	Allele	Frequency
Hal 3	99	1	0.640
		2	0.360
Hal 5*	46	1	0.402
		2	0.511
		3	0.087
Hal 9	27	1	0.148
		2	0.259
		3	0.111
		4	0.426
		5	0.056
IEAAAG05	27	1	0.660
		2	0.340

Table 2. Sample size (n) and total allele frequencies for the four polymorphic loci. * Allele frequencies vary for this locus because interpretation was problematic (Figure 2).