A GENETIC ANALYSIS OF BALD EAGLES IN THE PACIFIC NORTHWEST: RETAINED GENETIC DIVERSITY FOLLOWING A LARGE-SCALE POPULATION BOTTLENECK

by

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ABSTRACT

Bald eagle (*Haliaeetus leucocephalus*) populations in the continental United States experienced a dramatic population decline during the twentieth century. Populations across the species' range have largely recovered, thanks in part to the ban of DDT, rapidly enacted conservation measures, and reintroduction efforts. Using six microsatellite loci, I tested the genetic variation of bald eagles across six states in the Pacific Northwest. Genetic analysis revealed that, despite undergoing a population-wide decline, the bald eagles in the locations sampled outside of Idaho did not exhibit the characteristics typically associated with a genetic bottleneck (i.e., a reduced number of alleles and a heterozygosity excess) and likely persisted through the DDT-era with stable genetic variability. Furthermore, there was no significant genetic structuring in the Pacific Northwest samples outside of Idaho, suggesting a panmictic population across the area. The bald eagle's long generation time may act as an intrinsic buffer against the loss of genetic diversity and aid in retaining genetic variability across its range. The retention of genetic diversity in these isolated populations likely reduced the effective time of the bottleneck and contributed to the genetic similarity of the populations sampled in this research.

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CHAPTER ONE: INTRODUCTION

Introduction

Natural populations with sufficient genetic variation have the ability to respond to changes in their environment. These responses can include changes in morphology, behavior, and life history. Genetic variation may also be essential for long-term population persistence (Lande and Shannon 1996). However, the ability to elicit these types of evolutionary responses depends on the genetic variation present in the gene pool.

Species with small population sizes, particularly populations that are isolated from each other, are often exposed to higher rates of extinction because they are prone to losses of genetic variation (Saccheri et al. 1996). Loss of genetic variation in these small populations can lead to reduced short-term viability (Saccheri et al. 1996, Hailer et al. 2006) and lead to the long-term persistence of reduced genetic variability (Leberg 1990, Luikart et al. 1998, Whitlock 2000). Moreover, the risk of inbreeding is also elevated in small populations. Inbreeding can lead to an alteration or reduction of a population's genetic variation, can generate elevated levels of gametic phase disequilibrium, and increase levels of homozygosity among individuals in a population (Crow and Kimura 1970, Leberg 1993, Rumball et al. 1994, Saccheri et al. 1999). For populations with high genetic load (i.e., recessive deleterious alleles), increased levels of homozygosity can lead to a reduction in the population's fitness (Crow and Kimura 1970, Rumball et al. 1994).

One of the demographic events through which genetic variation within natural populations can be lost includes population bottlenecks. A population bottleneck occurs when the size of a population is severely and temporally reduced as a result of environmental pressures such as habitat destruction or fragmentation (Leberg 1992). Natural populations that have undergone a bottleneck can be severely affected by random genetic drift (i.e., the stochastic loss of alleles over time) (Wright 1931). This process reduces the overall amount of genetic variation. Bottlenecks result in a rapid loss of low frequency alleles and an increase in abundance of mid- and high-frequency alleles (Luikart et al. 1998). The amount of genetic diversity lost during a bottleneck event depends upon the size and duration of the bottleneck event, and the rate of population growth following the bottleneck (Nei et al. 1975).

Founder events are another demographic process that leads to the establishment of new, small populations in areas previously devoid of that species. Often these new populations arise from only a few migrants from a neighboring source population. During an event such as this, allelic richness is often reduced compared with the source population (Chakraborty and Nei 1977, Luikart et al. 1998). In addition, the random sampling of alleles from the source population can alter allele frequencies in the new population and may result in the fixation or complete loss of certain alleles (Chakraborty and Nei 1977, Maruyama and Fuerst 1985, Leberg 1992).

Typical of large raptor species, Bald Eagles (*Haliaeetus leucocephalus*) possess several life history characteristics that place them at a high risk of population bottleneck events and potentially local extinctions/extirpations (Purvis et al. 2000, Brown et al. 2007). As a high trophic-level predator, Bald Eagles occur at low population densities,

have low reproductive rates, high nest site and mate fidelity, low fecundity levels, and high levels of juvenile philopatry (Buehler 2000, Martinez-Cruz et al. 2004, Perkins 2006).

Bald Eagle populations across the conterminous United States began declining in the early 1900s due to habitat fragmentation and direct human persecution (Sprunt and Ligas 1964, Buehler 2000, Perkins 2006). In the mid-century, the decline was exacerbated by the use of toxic chemicals and pesticides such as dichloro-diphenyl-trichloroethane (DDT) and other organochlorides. These chemicals had well-documented adverse effects on the size of natural populations and communities of raptor species (Brown et al. 2007). The use of DDT as an insecticide became widespread worldwide during the 1940s and 1950s. DDT was effective at preventing the transmission of insect-borne diseases to humans (Carson 1962), and its derivatives are persistent organic pollutants that accumulate in the environment. DDT concentrations are highest in high trophic-level predators due to the process of bioaccumulation (EPA 1975).

Although the use of DDT in the United States was banned in 1972, the number of Bald Eagles in the lower 48 states continued to severely decline into the 1970s. In 1978, the Bald Eagle was issued protection under the Endangered Species Act (ESA). This protection, combined with the ban on DDT and successful captive breeding programs, led to the recovery of Bald Eagle populations in the continental United States over the past three decades (Weidensaul 1996). In 2007, the Bald Eagle was de-listed from the ESA. Despite the recovery of this species, the genetic signatures of this prolonged bottleneck event may persist in Bald Eagle populations around the continental United States.

In the Pacific Northwest, a few populations of Bald Eagles in the Greater Yellowstone Ecosystem (GYE) of Wyoming and Montana, and populations in central Oregon, are believed to have persisted through the DDT era (F. Isaacs, pers. comm.). However, with no published molecular studies regarding the genetic structure of these populations, it is impossible to determine if there is in fact a persistent signature of a genetic bottleneck.

An analysis of southern Idaho populations of Bald Eagles, however, revealed the characteristics of a recent bottleneck: high levels of gametic phase disequilibrium and heterozygosity excesses (Perkins 2006). Both of these characteristics, combined with high levels of inbreeding and significant genetic population structuring, indicate that the populations in southern Idaho experienced a recent, severe population bottleneck. Populations in neighboring states (Wyoming, Montana, and Oregon) may also have retained the signatures of such an event.

Microsatellites are useful genetic markers in population genetics studies because of their high-length polymorphisms. Allelic state can easily be genotyped by fragment length analysis (Anmarkrud et al. 2008). These markers are especially useful when dealing with threatened or high-risk species because they can be analyzed via noninvasive sampling of tissue (e.g., shed feathers) (Taberlet et al. 1996, Luikart et al. 1998). Genetic change can be readily monitored because microsatellites are codominant, hypervariable, and allow for numerous loci to be scored simultaneously (Paetkau et al. 1995).

The overall objective of this research was to determine, using microsatellite markers, if Bald Eagles in the Pacific Northwest exhibit both 1) a reduced number of

alleles and 2) a heterozygosity excess, which are the genetic characteristics typical of a population that has experienced a genetic bottleneck. Data collected from Idaho Bald Eagle populations by Perkins (2006) were utilized to determine levels of connectivity between populations in the Pacific Northwest (i.e., levels of gene flow that influence the genetic structuring of populations) and to determine if localities neighboring Idaho may have served as source populations for recent founder events, as was suggested by Perkins (2006).

CHAPTER TWO: METHODS

Field Sampling

In this study, feather samples were collected from known breeding territories in Bald Eagle populations across four states: Alaska, Oregon, Wyoming, and Montana. Volunteers from each state collected feathers during the 2008 and 2009 Bald Eagle breeding seasons (Figure 1). In addition, Perkins (2006) previously collected Bald Eagle feathers, and the data generated by Perkins (2006) are included here.

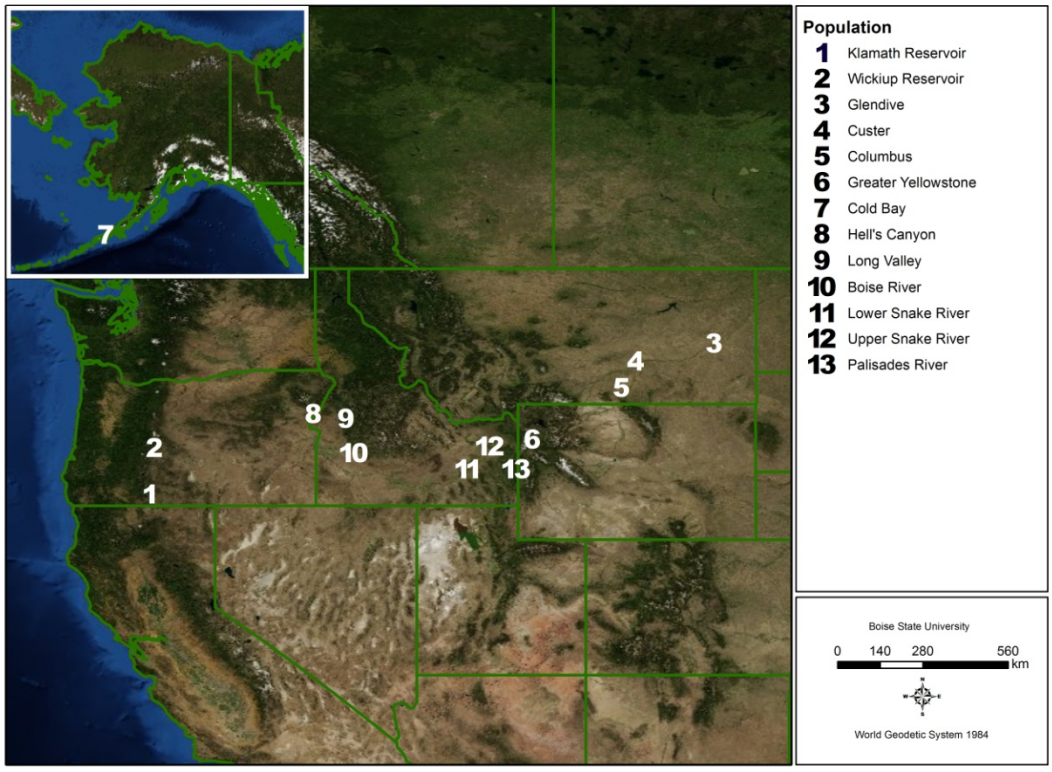

Figure 1 Bald Eagle Populations Sampled During the 2008 and 2009 Breeding Seasons. Populations 8-13 were previously analyzed in Perkins (2006).

The feathers were collected 20-30 m from the base of an occupied Bald Eagle nest tree or perch tree. Shed feathers have proven to be suitable for microsatellite analysis and represent a noninvasive sampling method (Luikart et al. 1998, Horváth et al. 2005).

Feathers were stored in plastic Ziploc bags or in 8.5 x 11 in. manila envelopes and shipped to the lab for analysis. In the instances that shipping whole feathers was not possible, feathers were clipped and only the tip of the feather was stored. Feathers were stored in the lab in Ziploc bags with trace amounts (>1 g) of silica gel and stored in the laboratory at room temperature until DNA extraction (Perkins 2006).

Blood samples of 50-60 day-old nestlings (n = 27) were taken from the brachial vein in the Montana and Wyoming populations. Blood samples were stored in a Longmire buffer solution (Longmire et al. 1997) and shipped to the lab for storage at 4° C.

DNA Extraction

DNA was extracted from the shafts of the collected feathers using QIAGEN (Valencia, California) DNeasy kits and followed manufacturer's protocol. To genotype individuals, I used six polymorphic loci developed for Bald Eagles by Tingay et al. (2006). Polymerase chain reaction (PCR) amplifications were performed in 10 uL volumes using 1 uL of genomic DNA, 1 uL of forward primer, 1 uL of reverse primer (only one primer was fluorescently labeled), 0.5 uL 1% BSA (0.05%), 0.2 uL PCR nucleotides (0.2 mM), 2 uL 5x NH_4 reaction buffer, 0.4 uL $MgCl_2$ (1.0 mM), 0.05 Taq polymerase (0.025 units), and 3.85 uL dd$H_2$0. The volume of genomic DNA was increased to 2 uL for individual samples that failed to produce amplified products that resulted in bands that were score-able.

Polymerase chain reaction amplification was carried out on an MJ Research PTC-100 thermocycler (Bio-Rad, Hercules, California). After the initial denaturing step of 95° C for 2 min, 34 cycles were performed. Each cycle consisted of a 30 s segment at 95° C, 30 s of annealing temperature (according to Tingay et al. 2006, variable annealing temperatures were utilized [see Perkins 2006]), followed by an extension step of 1 min, 30 s at 72° C. Lastly, a 2 min extension step was used at 72° C following the annealing step of the final PCR cycle.

Microsatellite Genotyping

Polymerase chain reaction products were mixed with loading dye and spun down by centrifuge for 10 sec at maximum speed (14,000 rpm). Following dye addition, samples were loaded into a polyacrylamide gel; each gel was 0.25 mm x 25 cm and 6.5% polyacrylamide gel. Amplified PCR products were then electrophoresed alongside of a 20-400 bp size standard using a LI-COR 4300 LR automated sequencer (LI-COR, Lincoln, Nebraska). Genotyping gels were visualized and scored using LI-COR e-seq software.

To genetically identify two parental birds for each nest, I surveyed and compared the different genotypes of the feathers collected under the nests. When genotypes at a site did not match, they were assumed to be separate birds. When two unique genotypes were identified, they were determined to be the nesting pair of birds for that site. If samples from nesting sites were unable to be identified to two birds, the sample site was removed from analysis.

Statistical Analysis

Population samples were hierarchically arranged based on the geographic region in which they were collected (Table 1). Within each region, the feathers were divided into their respective geographic populations for analysis. The program Micro-Checker 2.2.3 was used to test for null alleles and scoring errors due to large allelic dropout and stutter peaks (van Oosterhout et al. 2004). Microsatellite loci were tested for linkage disequilibrium, as described by Lewis and Zaykin (2001), using the program GDA v. 1.1. GDA also allowed estimates of population structure to be made using pairwise estimates of F_{st}. RST CALC v. 2.2 (Goodman 1997) was used to obtain pairwise estimates of R_{st}.

The number of alleles, number of polymorphic loci, and the number of private alleles within each population were determined using the program GENEPOP version 4.0 (Raymond and Rousset 1995b). GENEPOP was also used to calculate estimates of F_{is} (Wright's F/inbreeding coefficient) and to determine whether each locus conformed to Hardy-Weinberg equilibrium (HWE). Within GENEPOP, the observed and expected heterozygosity for each locus were also calculated. A Markov chain method was used to estimate the exact P-value without bias as described by Guo and Thompson (1992). Standard Markov parameters (dememorization number = 1000, number of batches = 100, number of iterations per batch = 1000) as suggested by Raymond and Rousset (1995a) were used in this analysis. Allelic richness was calculated using the procedure described by Kalinowski (2005) in HP-RARE 1.1.

Table 1 Regions of Bald Eagle Populations and Number of Individuals and Territories Sampled. Acronyms for Populations Here Are Used in Subsequent Tables and Figures.

Region	Population	Population Number	Acronym	Latitude	Longitude	# Territories Sampled	N
Oregon	Klamath Reservoir	1	KL	N 42° 21.3'	W 121° 54.1'	8	16
	Wickiup Reservoir	2	WK	N 43° 43.2'	W 121° 47.3'	9	18
Montana	Glendive	3	GD	N 46° 48.9'	W 105° 14.5'	3	6
	Custer	4	CS	N 46° 4.4'	W 107° 42.9'	3	6
	Columbus	5	CO	N 45° 36.4'	W 109° 11.3'	2	4
Wyoming	Greater Yellowstone	6	GYS	N 43° 40.4'	W 110° 42.2'	6	10
Alaska	Cold Bay	7	CB	N 55° 11.2'	W 162° 40.1'	5	10
Southwest Idaho	Hell's Canyon	8	HC	N 44° 42.4'	W 117° 03.0'	4	8
	Long Valley	9	LV	N 44° 34.9'	W 116° 06.0'	15	24
	Boise River	10	BR	N 43° 34.3'	W 115° 51.3'	2	3
Southeast Idaho	Lower Snake River	11	LSR	N 43° 05.5'	W 112° 31.7'	5	10
	Upper Snake River	12	USR	N 43° 39.3'	W 111° 50.1'	6	12
	Palisades River	13	PR	N 43° 14.1'	W 111° 06.6'	5	8

Pairwise migration rates were obtained using BAYESASS v.1.3 (Wilson and Rannala 2003). Using Bayesian inference to identify individuals that are potential immigrants from source populations, BAYESASS is able to estimate the percentage of individuals within a population that came from various source populations. This provides reliable estimates of migration between populations because BAYESASS does not assume constant population sizes, evolutionary equilibrium, or symmetrical migration (Wilson and Rannala 2003).

To test for potential situations of isolation by distance, the web-based program IBD v3.6 (Jensen et al. 2005) was used. This program introduced a matrix containing parameters describing genetic differentiation between each pair of populations (i.e, Fst/1-Fst) as well as a matrix containing Euclidean geographic distance (in kilometers) between each population pair.

Gene flow (N_m) estimates were not included as it was suspected that several of the populations, especially those in Idaho, would be in violation of HWE (Perkins 2006). In situations where violation of equilibrium is expected, F_{ST} and R_{ST} estimators of N_m can be misleading because the influence of drift becomes confounded in the estimates of N_m (McCauley 1993, Bossart and Prowell 1998, Hutchison and Templeton 1999).

To test the assumption that a breeding population of birds constitutes a true population, the program STRUCTURE 2.3.1 (Pritchard et al. 2000) was utilized. The program STRUCTURE implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers (Pritchard et al. 2000) and is useful in determining the correct number of populations in a study area and in assigning individuals to populations. Ten simulations were carried out for each K value

ranging from 1 – 6 when not including Perkins (2006) data, or K values from 1-13 when the data were included. Correlated allele frequencies and an admixture model were assumed as suggested by Pritchard et al. (2000). Analyses were carried out with 10,000 iterations, following a burn-period of 10,000 iterations.

Bottleneck Analysis

The program BOTTLENECK v. 1.2.02 (Piry et al. 1999) was used to test for recent bottlenecks within populations in each state sampled and within the regions sampled. This program is particularly useful in testing for heterozygosity excess in a population; an excess in heterozygosity is a classic signature of populations that have undergone a recent bottleneck (Luikart et al. 1998). BOTTLENECK assumes that when Ne is reduced, the alleles in the population are lost at a more rapid rate while the heterozygosity of the population experiences slower decline. Heterozygosity is more closely related to breeding dynamics than alleles are, and declines at a slower rate.

Wilcoxon sign-rank tests were used to test for heterozygosity excess in populations compared to drift-migration equilibrium expectations under a two-phase mutation model (TPM). This model incorporated a 95% single-step mutation model (SSM) with a variance of 12 as recommended for microsatellite loci by Piry et al. (1999). For the most accurate results, sample sizes are recommended to be no less than 10 (Piry et al. 1999). Because of this, the Glendive, Custer, and Columbus populations in Montana and the Boise River population in Idaho were not tested. Lastly, populations were grouped together by region and were tested for recent population bottlenecks as suggested by Piry et al. (1999).

CHAPTER THREE: RESULTS

Results

The total number of alleles per locus across the study area ranged from 4 to 11, while the mean number of alleles per locus in a population spanned a range of 2.93 (Hle6A11) to 5.57 (Hle6H10) (Table 2). Allelic frequencies for all loci and populations across the study area are given in Appendix A and Appendix B. Pairwise comparisons across 105 pairs of all six loci revealed 5 pairs exhibiting significant levels of gametic phase disequilibrium (GPD) (Table 3). The pairs were found in Oregon (n = 2), Alaska (n = 1), Wyoming (n = 1), and Montana (n = 1); however, the loci in disequilibrium in Montana exhibited marginal significance (P = 0.0537). The overall number of loci in GPD was not substantially higher than what could be expected by chance alone (5%, or 5.25 pairs). Further analyses were conducted under the assumption that no loci were physically linked and that all were independent; this was done because no pairs of loci exhibiting GPD were consistent across the study area and were most likely the result of demographic events occurring in the history of specific populations.

Table 2 PCR Primers and Conditions for the Six Microsatellite Loci (Tingay et al. 2006) Used to Analyze Genetic Structure of Bald Eagles (Including Idaho Populations). Forward and Reverse Primers are Designated by F and R, respectively. Ta (°C) Is the Annealing Temperature of Each Primer.

Locus	Primer Sequences (5' – 3')	Ta (°C)	MgCl2 (mM)	# Alleles	Mean # Alleles/Locus	Observed Allele Size Range (bp)	EMBL Accession Number
Hle6A11	F:CCCCTTATCCCAGGTGCTAT R:GGAAATAAGAAGCACACCGAGT	55	2	4	2.93	194-200	AJ620434
Hle0E12	F:CTAATGGTCCTGAGGGCAAA R:TTCATGTGCCAGCTGACCT	60	2	8	3.86	153-171	AJ620427
Hle6F02	F:TGGGACTCCAAATCCAAGTC R:GGATAACTGGCACTGGAGGA	60	1	8	3	223-237	AJ620437
Hle6H10	F:GCCTCACAGTGCCATTACCT R:CCTTTCCTGGGGGATTAAAA	59	1	11	5.57	204-224	AJ620440
Hle0B10	F:ATGATGGTGTTGTGGGTGAA R:CAGGCTGTCCCATTTCAACT	60	2	7	3.71	180-192	AJ620420
Hle0E05	F:CAAGACACCAATCTCCCCACATC R:GGCTCGCTCTCACACAGAAGG	65	2	8	4	225-239	AJ620425

Table 3 Statistically Significant Linkage Disequilibrium Among 23 of 210 Loci Pairs. Calculations Performed in GDA 1.1 (Lewis and Zaykin 2001) and ARLEQUIN 2.000 (Schneider et al. 2000).

Region	Population	Loci in Disequilibrium	P-value
Oregon	WK	Hle0E12-Hle6H10	0.0388
		Hle6H10-Hle0E05	0.0431
Montana	CO	Hle0B10-Hle0E05	0.0537*
Wyoming	GYS	Hle0B10-Hle0E05	0.035
Alaska	CB	Hle0B10-Hle0E05	0.0113

*marginally significant

Regional means of allelic diversity ranged from 2.72 (Montana) to 4.17 (Oregon), and rarefied allelic richness ranged from 1.87 (Montana) to 2.08 (Oregon) (Table 4). Oregon, Montana, Wyoming, and Alaska had relatively high levels of observed heterozygosity (0.729, 0.623, 0.617, and 0.667, respectively) and in all cases there was no significant differences between observed and expected heterozygosities (all populations were in HWE).

Table 4 Estimates of Each Population's Average Genetic Diversity. AD = Allelic Diversity, AR = Allelic Richness Rarefied to Three Alleles/Locus Using the Program HP-Rare 1.1 (Kalinowski 2005), % P=Percent Polymorphic Loci, H_o = Mean Observed Heterozygosity (* = Statistically Significant Heterozygote Deficiency (P=.001). According to HW Global Tests in GENEPOP 4.0), H_e = Mean Expected Heterozygosity, F_{is} = Mean Inbreeding Coefficient (Weir and Cockerham 1984), W=P-values from Wilcoxon Tests of Heterozygosity Excess Performed by BOTTLENECK 1.2.02 (* = Statistically Significant (P=0.05) Departure from Aallelic Diversity/Heterozygosity Proportions Expected by TPM Mutation-Drift Equilibrium).

Region	Population	N	AD	AR	%P	H_o	H_e	F_{is}	# Private Alleles	W
Southwestern ID	HC	8	4.17	2.17	1	0.396*	0.749	0.474	1	0.0391*
	LV	24	5.83	2.26	1	0.449*	0.718	0.365	3	0.0781
	BR	3	3.83	2.26	1	0.389*	0.711	0.433	0	†
Mean			**4.61**	**2.23**	**1**	**0.411**	**0.726**	**0.424**	**2.33**	
Southeastern ID	LSR	10	4	2.22	1	0.467*	0.718	0.357	2	0.0156*
	USR	12	4.5	2.19	1	0.403*	0.692	0.456	2	0.0078*
	PR	8	4.6	2.18	1	0.417*	0.676	0.381	1	0.5781
Mean			**4.37**	**2.2**	**1**	**0.429**	**0.695**	**0.398**	**1.67**	
Oregon	KL	16	4	2.04	1	0.738	0.627	-0.185	1	0.2188
	WK	18	4.33	2.11	1	0.72	0.656	-0.099	0	0.0781
Mean			**4.17**	**2.075**	**1**	**0.729**	**0.641**	**-0.142**	**0.5**	
Montana	GD	6	2.5	1.87	1	0.667	0.548	-0.244	0	†
	CS	6	3.17	1.92	1	0.667	0.563	-0.206	0	†
	CO	4	2.5	1.82	1	0.625	0.518	-0.25	0	†
Mean			**2.72**	**1.87**	**1**	**0.623**	**0.543**	**-0.233**	**0**	
Wyoming	GYS	10	3.5	1.89	1	0.617	0.548	-0.133	0	0.7188
Alaska	CB	10	2.5	2	1	0.667	0.595	-0.129	0	0.5

† randomization tests not conducted due to limited sample size

When comparing the results of populations from OR, WY, MT, and AK with the previously reported results from Idaho populations (Perkins 2006), populations had much lower levels of observed heterozygosity (0.411 for populations from southwestern Idaho, and 0.429 for populations from southeastern Idaho (Table 4). After Fisher's exact tests were performed across all loci, all Idaho populations were determined to deviate from HWE due to significant levels of heterozygosity deficiency (P = 0.001).

Mean inbreeding coefficients for all populations corresponded inversely with the levels of observed heterozygosity (i.e., populations with high observed heterozygosity had low inbreeding coefficients). Idaho populations had high F_{is} values compared with populations from the rest of the study area, but were similar to each other (0.424 southwest, 0.398 southeast) (Table 4). Other populations in the study displayed values and ranging from -0.233 (Montana) to -0.129 (Alaska) (Table 4), indicating higher observed heterozygosity values compared to the values for expected heterozygosity.

BOTTLENECK revealed evidence of recent population bottlenecks in the HC, LV, LSR, and USR populations – all of which are in Idaho (Table 4). No other populations had significant heterozygosity excess levels indicative of a recent bottleneck. Wilcoxon sign-rank tests were also insignificant when populations were grouped by regions and treated as one (West Idaho [P = 0.4211], East Idaho [P = 0.6563], Oregon [P = 0.719], Montana [P = 0.219], Wyoming [P = 0.719], and Alaska [P = 0.5000]).

Pairwise comparisons of F_{st} and R_{st} (Weir and Cockerham 1984) revealed no significant differentiation between populations within the same geographic regions (for example, KL and WK in Oregon did not significantly differ from each other). However,

populations from Idaho indicated significant genetic differentiation when compared to Oregon, Montana, Alaska, and Wyoming populations (Table 5).

Southeastern Idaho populations exhibited significant genetic differentiation when compared to the populations in southwestern Idaho. Genetic structuring was most evident when comparisons were made across long geographic distances. For example, comparisons between LV in the southwest and LSR in the southeast exhibited statistically significant differentiation (Table 5).

The Alaskan population displayed significant genetic structuring when compared to the eastern and western Idaho populations, as well as most of the populations in Montana. However, when comparisons were made with the Oregon or Wyoming populations, there was insignificant genetic differentiation, this despite the vast geographic distance separating these populations.

In Wyoming, there was no significant structuring between the nearest populations in Montana (CU, CO) or Idaho (PR). Comparisons made with Oregon populations and both east and west Idaho did however indicate genetic structuring.

In Idaho, migration rates estimated by Bayesian inference revealed that migration between eastern and western populations was uncommon but within region migration did occur (Table 6). For example, in western Idaho, the estimated percentage of individuals in the HC population derived from the LV population was 14%. The LV population in the west and USR population in the east had the lowest proportions of migrants from other populations (0.0471 and 0.0355, respectively) and most likely served as the source populations for those regions.

Table 5 Pairwise Values of Fst (Above Diagonal) and Rst (Below Diagonal) Between All Populations. Significant Values (P ≤ 0.05) Are Indicated in Bold.

		Oregon		Montana			Wyoming	Alaska	Southwestern Idaho			Southeastern Idaho		
		KL	WK	GD	CU	CO	GYS	CB	HC	LV	BR	LSR	USR	PR
Oregon	KL	-	0.0022	0.0069	0.0617	-0.0055	**0.0695**	0.0405	**0.1959**	**0.1602**	**0.1764**	**0.0855**	**0.1235**	**0.116**
	WK	0.0036	-	0.0274	0.0712	0.0091	**0.043**	0.0153	**0.1712**	**0.1426**	0.1601	**0.061**	**0.1203**	**0.1003**
Montana	GD	-0.0485	-0.0147	-	0.0611	0.0072	**0.1412**	**0.1073**	**0.2145**	**0.1758**	0.1909	**0.1069**	**0.1414**	0.1393
	CU	0.0538	0.1226	0.0463	-	0.0688	0.1425	**0.1386**	**0.2353**	**0.1968**	0.2319	0.1425	**0.1192**	0.1074
	CO	-0.0747	-0.0406	-0.0837	0.0482	-	0.1031	0.081	0.221	**0.1893**	0.2063	0.111	**0.1228**	0.1329
Wyoming	GYS	**0.1668**	**0.1641**	**0.186**	0.0543	**0.1374**	-	0.0277	**0.2086**	**0.1827**	0.2377	**0.1102**	**0.1936**	0.1065
Alaska	CB	0.0883	0.1142	0.0899	-0.0127	0.0812	-0.0105	-	**0.1977**	**0.175**	0.2068	**0.103**	**0.175**	**0.1208**
Southwestern Idaho	HC	**0.236**	**0.2102**	**0.2314**	**0.2783**	0.1613	**0.2508**	**0.2528**	-	-0.0294	-0.123	0.0511	**0.073**	0.0099
	LV	**0.2643**	**0.2224**	**0.2465**	**0.3202**	0.1829	**0.3027**	**0.2839**	-0.0349	-	-0.0549	**0.0566**	**0.087**	0.0499
	BR	**0.2554**	0.242	0.2479	0.298	0.1533	**0.2805**	0.2618	-0.0797	-0.0266	-	0.0006	-0.0278	-0.0142
Southeastern Idaho	LSR	**0.2567**	**0.2407**	**0.2536**	**0.2762**	**0.2591**	**0.2611**	**0.2545**	**0.2866**	**0.2753**	**0.2913**	-	0.0244	-0.013
	USR	**0.352**	**0.3346**	**0.3476**	**0.372**	**0.3539**	**0.359**	**0.3507**	**0.3795**	**0.3684**	**0.3867**	-0.043	-	0.0087
	PR	**0.2372**	**0.2192**	**0.2329**	**0.2606**	**0.2391**	**0.2452**	**0.2373**	**0.2684**	**0.2555**	0.2736	-0.0565	-0.0379	-

Table 6 Estimated Migration Rates of Bald Eagles Across Populations in the Pacific Northwest and Alaska. Estimates Obtained Using BAYESASS v.1.3 (Wilson and Rannala 2003). Rates Are Estimated as the Proportion of Individuals in Column Populations that are Derived from Other Populations. Values ≥ 0.05 are Indicated in Bold. Italics Along the Diagonal Indicate Non-Migrant (Native) Individuals.

		Oregon		Montana			WY	AK	Southwestern Idaho			Southeastern Idaho		
		KL	WK	GD	CU	CO	GYS	CB	HC	LV	BR	LSR	USR	PR
Oregon	KL	*0.6847*	0.0016	0.015	0.0144	0.0167	0.011	0.0102	0.0135	0.0033	0.0173	0.0113	0.0026	0.0141
	WK	**0.2484**	*0.979*	**0.1247**	0.1237	**0.0883**	**0.1747**	**0.1894**	0.012	0.0154	0.016	0.0262	0.0025	0.0259
	GD	0.0063	0.0017	*0.7077*	0.0157	0.0183	0.0103	0.0101	0.0129	0.0025	0.0163	0.0087	0.0032	0.0115
Montana	CU	0.0058	0.0018	0.0496	*0.7086*	0.0164	0.0101	0.0104	0.012	0.0023	0.0148	0.009	0.0027	0.0115
	CO	0.0064	0.0016	0.0157	0.0156	*0.724*	0.011	0.01	0.0148	0.0025	0.0156	0.0085	0.0029	0.0106
Wyoming	GYS	0.006	0.0021	0.015	0.0153	0.018	*0.6974*	0.0107	0.0119	0.0024	0.0172	0.0103	0.0027	0.0114
Alaska	CB	0.0057	0.0016	0.0151	0.0134	0.017	0.01	*0.6373*	0.0114	0.0028	0.0162	0.0113	0.0028	0.0134
Southwestern Idaho	HC	0.0062	0.0018	0.0156	0.016	0.0161	0.0104	0.0104	*0.7101*	0.0025	0.0155	0.0095	0.0028	0.0117
	LV	0.0061	0.0017	0.0146	0.0152	0.0179	0.0206	0.0102	**0.1412**	*0.9529*	**0.05**	**0.0518**	0.0031	0.0481
	BR	0.0061	0.0017	0.0148	0.0154	0.016	0.0107	0.0099	0.0139	0.0028	*0.7557*	0.01	0.0031	0.0119
Southeastern Idaho	LSR	0.0058	0.0019	0.016	0.0148	0.0173	0.0117	0.0094	0.0133	0.0036	0.017	*0.7679*	0.004	0.0288
	USR	0.0063	0.0018	0.0152	0.0157	0.0175	0.0114	0.011	0.0189	0.0041	0.0311	**0.0623**	*0.9645*	**0.0805**
	PR	0.0062	0.0017	0.0155	0.0163	0.0167	0.0105	0.011	0.0143	0.0028	0.0712	0.0131	0.0031	*0.7205*

Oregon exhibited a similar situation as Idaho in which one population (WK) appeared to be a source population for the other (KL). The proportion of migrants from other populations in WK was only 0.021 and approximately 25% of KL were migrants from WK. This was the only migrant source for the Oregon populations as no out-of-region migrants significantly contributed to KL. The WK population also was a source for 12% of the CU and GD populations and 17% of the GYS population.

When all populations were pooled by region and treated as one unit, Oregon, west Idaho, and east Idaho exhibited no significant migration between regions (Table 7). Despite the close geographic locale, migration values indicate that east Idaho did not receive or contribute any migrants. Similarly, Oregon and west Idaho did not contribute any significant level of migrants. Wyoming appeared to be comprised of migrants from both Oregon and west Idaho populations, while Montana's only significant source of migrants appear to have been drawn from Oregon populations.

Table 7 Estimated Migration Rates of Bald Eagles Across Five Regions in the Pacific Northwest (Alaska Excluded). Estimates Obtained Using BAYESASS v. 1.3 (Wilson and Rannala 2003). Rates Are Estimated as Proportion of Individuals in Column Populations That Are Derived from Other Populations. Values >.05 Are Highlighted in Bold.

	Oregon	Montana	Wyoming	Southwestern Idaho	Southeastern Idaho
Oregon	*0.9896*	**0.2855**	**0.244**	0.0204	0.0083
Montana	0.0024	*0.6854*	0.0157	0.0043	0.0073
Wyoming	0.0026	0.0099	*0.6953*	0.0044	0.0095
Southwestern Idaho	0.0024	0.0092	**0.2428**	*0.9612*	0.012
Southeastern Idaho	0.003	0.0099	0.0208	0.0096	*0.9627*

Results from IBD v3.6 revealed no significant correlation between geographic distance and genetic separation of the Oregon, Montana, and Wyoming populations (Mantel Test, P = 0.8402, R = 0.2100) (Figure 2). However, when Idaho populations were included in the analysis, significant correlation between genetic separation and geographic distance was detected (Mantel Test, P = 0.0140, R = 0.3089), which suggests the presence of isolation by distance (Figure 3).

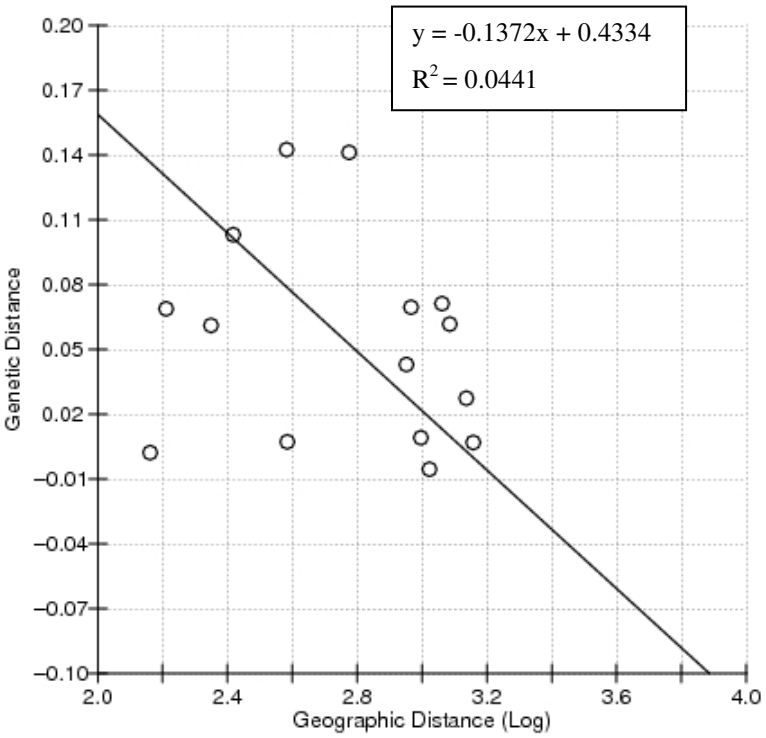

Figure 2 Mantel Test Showing Correlation between Genetic Separation and Geographic Distances (Jensen et al. 2005) between All Pairwise Comparisons of Pacific Northwest Bald Eagle Populations (Perkins [2006] Data Not Included). (P = 0.8402, R = 0.2100).

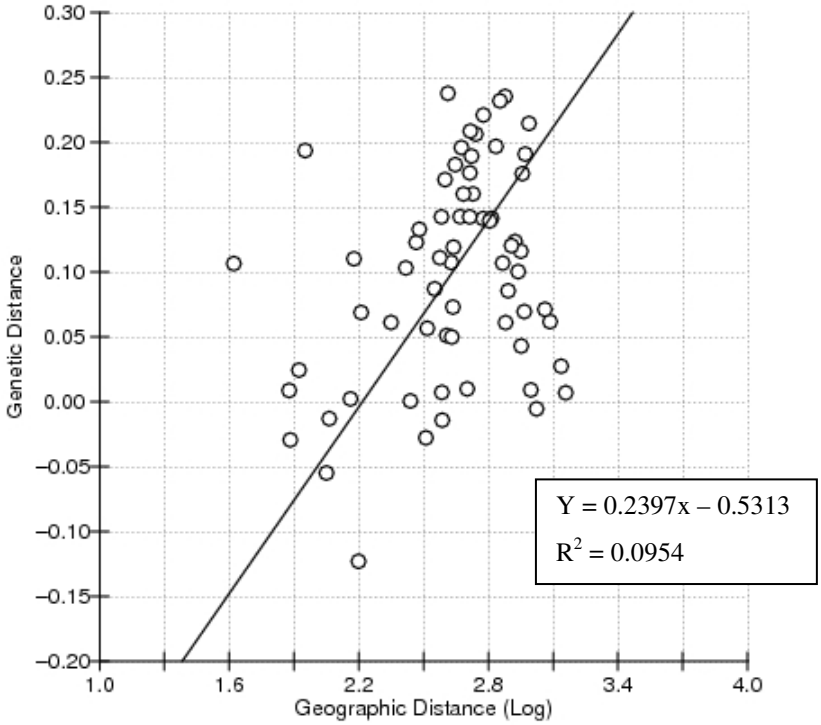

Figure 3 Mantel Test Showing Correlation between Genetic Separation and Geographic Distances (Jensen et al. 2005) between All Pairwise Comparisons of Pacific Northwest Bald Eagle Populations (Perkins [2006] Data Included). (P = 0.0140, R = 0.3089).

The clustering analysis implemented in STRUCTURE 2.3.1 detected only one distinct cluster within the Pacific Northwest Bald Eagle populations (Figure 4). While probabilities for all K values approached approximately the same value (Ln P(D) = -841.0), the 10 iterations for K = 1 were closely clustered and exhibited the smallest range of probability values (-841.0 to -843.2). These results appear to agree with pairwise comparisons of populations in the Pacific Northwest (Table 5). Only the GYS population of Wyoming exhibited any significant levels of genetic structuring when comparing pairwise F_{st} values across the Pacific Northwest. This suggests one panmictic Bald Eagle

population in the Pacific Northwest; however, limited sample sizes and sampling ranges may have contributed to this conservative estimate.

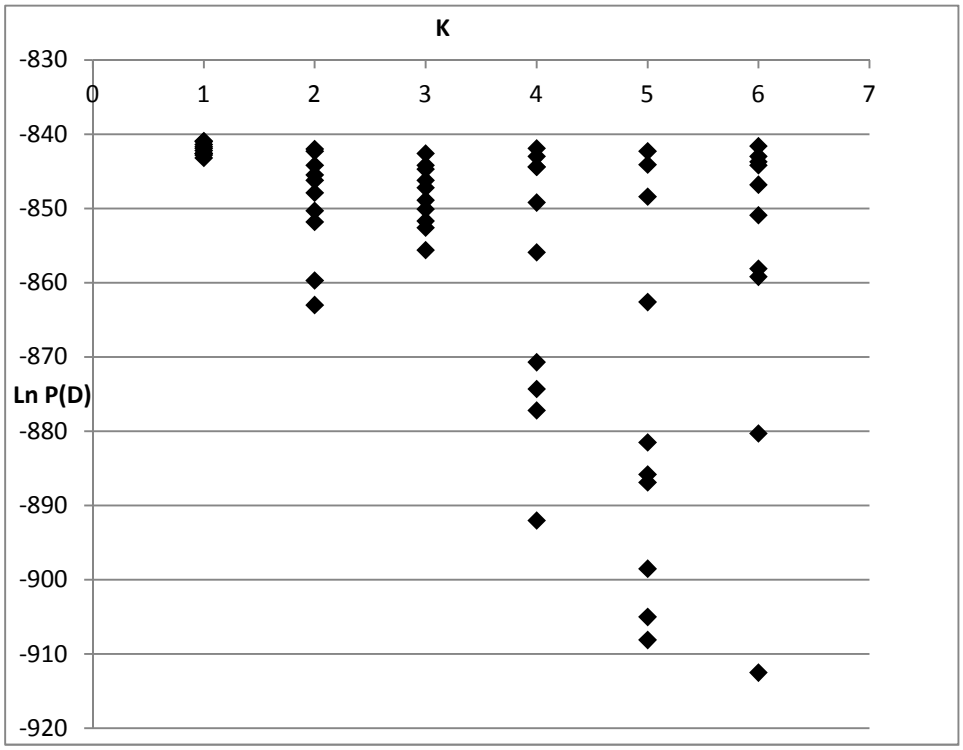

Figure 4 Bayesian Clustering Analysis of Bald Eagle Populations in the Pacific Northwest (Perkins [2006] Excluded). For Each Value of K, 10 Simulations Were Carried Out to Obtain Probability Values (y-axis).

When Idaho populations were included in the STRUCTURE clustering, six distinct clusters were detected (K = 6) (Figure 5). It appears that the Idaho populations introduced sufficient diversity to this analysis to begin to tease out some genetic differences among Bald Eagle populations in the Pacific Northwest.

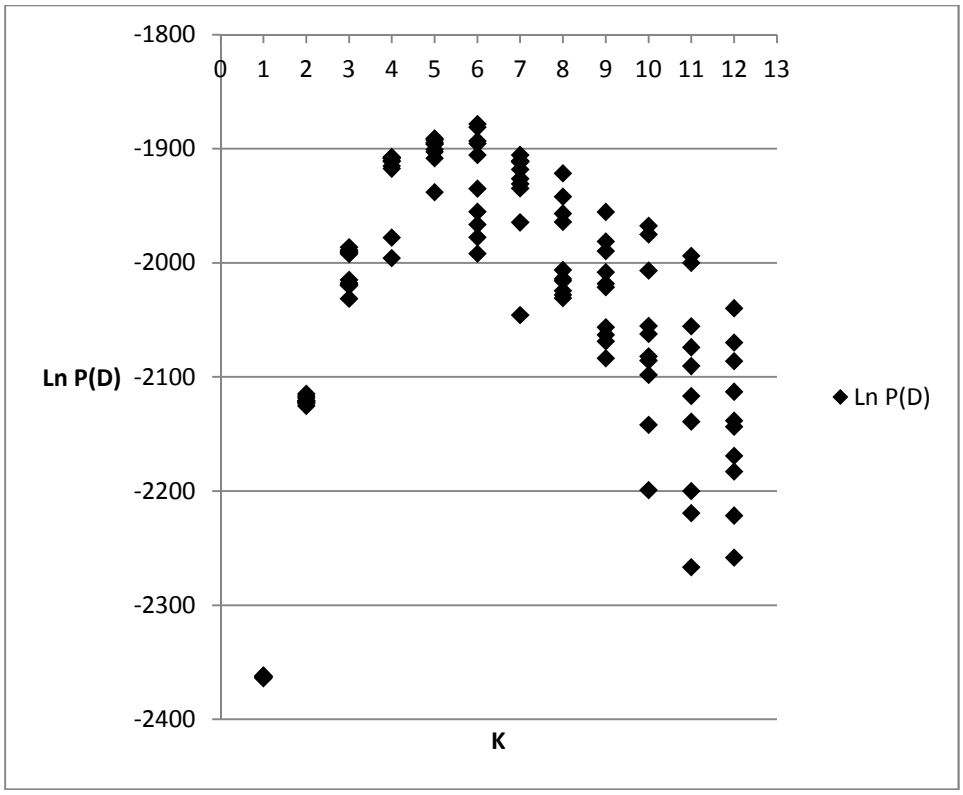

Figure 5 Bayesian Clustering Analysis of Bald Eagle Populations in the Pacific Northwest (Perkins [2006] Included). For Each Value of K, 10 Simulations Were Carried Out to Obtain Probability Values (y-axis).

CHAPTER 4: DISCUSSION

Discussion

The results of this study provide necessary information to assess wide-scale genetic patterns among populations of Bald Eagles in the Pacific Northwest. For many populations, genetic information is provided for the first time. Despite experiencing a continent-wide decline in numbers, Bald Eagles in the regions sampled in the Pacific Northwest appear to have persisted through the DDT era with apparently stable population sizes (Harmata et al. 1999, Stinson et al. 2001, F. Isaacs pers. comm.) and genetic variability (Table 5).

Bald Eagles in the Pacific Northwest exhibited Ho = 0.67, whereas raptor species that have undergone recent reductions in population size typically show reduced levels of heterozygosity: peregrine falcon (*Falco peregrinus*; Ho = 0.45; Nesje et al. 2000), Spanish imperial eagle (*Aquila adalberti*; Ho = 0.52, Martinez-Cruz et al. 2004), Bonelli's eagle (*Hieraaetus fasciatus*; Ho = 0.48; Mira et al. 2005), and Bald Eagle in Idaho (Ho = 0.42; Perkins 2006). Elevated levels of gametic phase disequilibrium are also expected in recently bottlenecked populations (Slatkin 1994, Kruglyak 1999, McVean 2002), this has been observed in the Mexican pinyon (*Pinus maximartinezii*; 14.5% maternal and 18.2% pollen loci pairs in GPD; Ledig et al. 1999), bowhead whales (*Balena mysticetus*; 16% of pairs in GPD; Rooney et al. 1999), and Bald Eagles in Idaho

(17% of pairs in GPD; Perkins 2006). In the populations from the Pacific Northwest and Alaska analyzed in this study, GPD levels of Bald Eagles did not exceed 5% (Table 5).

Unlike all other populations sampled in the Pacific Northwest, Idaho populations exhibited all of the characteristics typical of a recently bottlenecked population (Perkins 2006). The lower levels of genetic diversity and higher levels of GPD detected within Idaho populations of Bald Eagles appears to stem from several factors: population bottlenecks, elevated levels of inbreeding, and founder events that resulted in genetically distinct and structured populations (Nei et al. 1975, Slatkin 1994, Saccheri et al. 1996, Hutchison and Templeton 1999, McVean 2002, Perkins 2006).

Bottleneck Analysis

The results from BOTTLENECK indicate that none of the other populations sampled in the Pacific Northwest experienced a recent population bottleneck. This is supported by the lack of detectable heterozygosity excesses or deficiencies (Table 6). Most published studies that test for an expected population bottleneck using microsatellite markers report statistical support for the bottleneck (i.e., Glenn et al. 1999, Rooney et al. 1999, Nesje et al. 2000, Le Page et al. 2000, Whitehouse and Harley 2001, Bellinger et al. 2003, Perkins 2006,). My study provides one of the few microsatellite studies that tested for an expected population bottleneck (as reported in Idaho by Perkins [2006]) and failed to resolve statistical support for such an event in the Pacific Northwest. As expected in Alaska, no significant population bottleneck was detected. These results must be interpreted carefully as only 10 individuals were analyzed and may not provide an accurate assessment for the region's true evolutionary history (Piry et al. 1999).

If a population experiences a genetic bottleneck and subsequently recovers, the recovered population often exhibits high inbreeding levels (Frankham et al. 2001). With estimated F_{is} values in the Pacific Northwest ranging from -0.250 to -0.099, combined with high levels of observed and expected heterozygosities and long generation times in Bald Eagles, it is unlikely that a bottleneck event went undetected due to subsequent growth and recovery.

All populations sampled in Idaho exhibited significant heterozygosity deficiencies (Table 6). BOTTLENECK indicated that 3 of the 5 populations sampled had undergone a recent, severe population bottleneck (the BR population could not be tested due to a lack of sample size). The lack of evidence for the occurrence of a recent genetic bottleneck in the PR population was largely consistent with historic information that this population persisted at a relatively large size throughout the DDT era (Greater Yellowstone Ecosystem Working Team 1983). When Idaho populations were merged into two regional groups, eastern and western Idaho, BOTTLENECK did not detect the signature of a significant bottleneck. This may potentially be explained by regional and temporally isolated reductions in effective population sizes rather than a range-wide synchronized reduction in Idaho (Perkins 2006). Levels of GPD detected in Idaho were consistent with the pattern expected in recently bottlenecked populations that have experienced subsequent inbreeding (Slatkin 1994); inbreeding levels in Idaho ranged from 0.357 (LSR) to 0.474 (HC) (Table 6). Significant GPD was detected in 18 of 105 (17%) pairwise comparisons among all six loci (Perkins 2006). The fact that GPD has persisted at such high levels suggests that isolation and elevated levels of inbreeding have played important roles in shaping the genetic diversity within these populations.

Intrinsic Buffer Against Loss of Genetic Diversity

Bald Eagles possess several life history characteristics, such as long maturation periods, long life expectancy (average life span is about 16 years; Harmata et al. 1999), high nest site fidelity, high mate fidelity, strong juvenile philopatry, and low fecundity (Buehler 2000, Perkins 2006), which typically place them at high risk for bottleneck events and local extinctions. However, Hailer et al. (2006) has proposed that several of the characteristics large eagles possess, specifically long-life expectancies and associated long generation times, act as an intrinsic buffer against the loss of genetic diversity, thus producing a shorter effective time of an experienced bottleneck. Despite passing through documented demographic bottlenecks, microsatellite analysis of White-tailed Eagle (*Haliaeetus albicilla*) populations in Europe revealed high levels of genetic diversity retained within populations, without sustained migration among regions (Hailer et al. 2006). A similar trend was reported in Canadian Peregrine Falcons (White et al. 2002); comparisons of historic and contemporary samples indicated no decline in mitochondrial DNA nucleotide diversity, this despite a documented population decline because of DDT exposure (Brown et al. 2007).

With the high genetic diversity reported in the Pacific Northwest (Table 6), it appears that genetic diversity within populations was retained in part due to the long generation time of the Bald Eagle. The long life span of the Pacific Northwest Bald Eagles minimized genetic drift and acted as an intrinsic buffer against the rapid loss of genetic diversity. While the decline of the species across the continent is documented (Sprunt 1969), few analyses of the species have documented its evolutionary history. Further sampling and genetic analysis of the species across its entire range will help to

determine if the Bald Eagle's long generation time has retained genetic diversity across the continent.

Isolation by Distance Patterns

Isolation by distance is the tendency of an individual to find a mate(s) from populations that are in close proximity and not from populations that are expected to be more distant (Wright 1943). As a result of this trend, populations in close geographic proximity are more genetically similar than populations separated by large geographic distances (Wright 1943). Typically, isolation by distance will occur in subdivided populations, when sub-populations exchange genes at a rate dependent upon the geographic distance separating the two, or when the dispersal of gametes and/or zygotes is spatially restricted (Wright 1943).

The lack of evidence for isolation by distance in the Pacific Northwest samples (P = 0.8402; Figure 2) is surprising as several of the populations are separated by large distances (KL is approximately 1439 km from the GD population; Figure 1) and Bald Eagles are highly philopatric with strong nest-site fidelity. However, there is not always a direct association between the spatial distribution of populations and the spatial distribution of genetic diversity (Alcaide et al. 2009). An example of this was presented in Koopman et al. (2007). The authors found that boreal owls (*Aegolius funereus*) in North America were not partitioned into distinct subpopulations (F_{st} = 0.004), despite the fact that the species depends on spruce-fir forests (a habitat type that is in patchy distributions across the continent). Assignment results from STRUCTURE failed to assign individual owls to their population of origin and indicated that boreal owls instead formed a single panmictic population (Koopman et al. 2007). These results suggested that

the gene flow, even between the most distant and disconnected boreal owl habitat patches, was sufficiently high to genetically homogenize the subpopulations (Koopman et al. 2007).

Although N_m was not estimated, estimates of genetic differentiation provided by F_{st} and R_{st} supported the results from IBD. Only a few pairwise F_{st} and R_{st} comparisons between populations in the Pacific Northwest exhibited significance, (Table 7), and these comparisons all involved the GYS population, perhaps suggesting that this population may be more genetically distinct from the rest of the study area. Nonetheless, pairwise F_{st} and R_{st} comparisons combined with the lack of an isolation by distance pattern suggest that the Pacific Northwest Bald Eagles exhibit characteristics typical of an admixed population rather than several unique regional populations. A similar trend of genetic uniformity and admixture was observed in the Eurasian Kestrel (*Falco tinnunculus)* (Alcaide et al. 2009). No significant evidence for an isolation by distance pattern was observed, ($P = 0.84$) and the Eurasian kestrel exhibited little genetic differentiation between sample sites.

Tests in IBD that included the Idaho populations indicated a significant pattern of isolation by distance ($P = 0.0140$; Figure 3). In Idaho, the persistence of high GPD and inbreeding levels support the notion that these populations are isolated and genetically structured from each other and from the rest of the Pacific Northwest. Perkins (2006) suggested that Idaho populations were recently established due to a series of founder events in the area. The House Finch (*Carpodacus mexicanus*) exhibited a similar trend as described in Hawley et al. (2006). The House Finch is native to the western United States, but has established stable populations in the eastern United States through a series

of small founder events in the 1940s. Much like the Idaho Bald Eagles, the recently founded eastern population of House Finches exhibited significantly lower H_o values compared to the western population (P = 0.032), and the eastern House Finches also exhibited stronger isolation by distance trends than the source populations (P = 0.005 in introduced population, P = 0.801 in native population) (Hawley et al. 2006).

Population Structure

Similar to IBD results and pairwise Fst and Rst comparisons, STRUCTURE suggested a pattern of admixture in the Pacific Northwest populations of Bald Eagles when Idaho was not included. Bayesian clustering analysis most strongly supported a K-value of 1 (Figure 4), suggesting that the regions sampled comprised one single population and not genetically distinct populations as was assumed prior to analysis. Pairwise comparisons of migration rates between previously assumed populations revealed that all populations with the exception of WK and CU received or contributed significant levels of migrants across state and regional boundaries (Table 8). For example, the GYS population in Wyoming was comprised of approximately 17% of migrants from the WK population in Oregon. This pattern is consistent with the findings of no isolation by distance patterns and the pattern of panmixia across the study area.

Typically, avian species nesting at northern latitudes exhibit southward movement from their nesting territories (Dunstan 1973, Reese 1973, Dunstan et al. 1975, Postupalsky 1976, Gerrard et al. 1978, Griffin et al. 1980). Bald Eagle migration in the Pacific Northwest does not always follow that trend however, as birds from Washington, Oregon, and California have been documented travelling north to coastal British Columbia, Canada and southeastern Alaska for several weeks following breeding. Birds

remained in these areas for several weeks and returned to the Pacific Northwest in January or late February (Servheen and English 1979, Watson and Pierce 1998). Bald Eagles in the Greater Yellowstone Ecosystem (GYE) have been documented travelling as far west as the California and Washington coasts in the autumn (Harmata et al. 1999), and Bald Eagles of the Glacier National Park ecosystem have been documented as travelling south to eastern Idaho and northern Utah (McClelland et al. 1994).

These documented movement patterns allow for the social interaction of birds from across the Pacific Northwest during the non-breeding periods of the year, but they do not explain the lack of genetic structure between the regions. While they may interact in the winter months, they are not exchanging genes. Recruitment of migrants into these wintering regions is unlikely as Bald Eagles exhibit philopatry to their nest-site and natal regions (Buehler 2000); Harmata et al. (1999) recorded five of eight radio PTT marked juveniles in the GYE returning in the general vicinity of their natal site after successive westward winter migrations.

STRUCTURE analysis that included all Pacific Northwest populations and all Idaho populations indicated Idaho populations were strongly isolated and structured (Figure 5). The populations in Idaho provided enough variability to the overall analysis to identify six potential K's in the entire Pacific Northwest. However, only two of the clusters are clearly separated and resolved (southeast Idaho and southwest Idaho) (Figure 5); this may be representative of an artifact caused by admixture between populations in each region. The Idaho clusters also contained the only populations in the study area that exhibited a recent genetic bottleneck. High levels of inbreeding following the bottleneck,

combined with the geographic isolation of the populations and limited migration rates into these study areas likely resulted in these genetically distinct populations.

It is surprising the addition of two populations to the Pacific Northwest STRUCTURE analysis led to the identification of six potential K's in the study area. The results, with the exception of the two clusters in Idaho, do not fit the geographic pattern that is expected based on the Bald Eagle's life history characteristics. Instead, the clusters are distributed in an unusual pattern that could be explained as an artifact of the sampling type (e.g., shed feather analysis), allelic frequencies in Idaho, or the number of microsatellite loci used in the study (here six loci were used, comparable studies have used 47 [Johnson et al 2009]; 26 [Hailer et al. 2006]; 11 [Brown et al. 2007] and nine [Alcaide et al. 2009]).

Perhaps a more logical explanation of why six K's appeared in STRUCTURE after adding Idaho populations is that Idaho populations were founded by populations not sampled in this study. The notion that the Idaho populations were established through a series of founder events from neighboring regions (i.e., Oregon, Wyoming, Montana) was rejected based on the results of this study. Idaho populations exhibited no significant migration rates to or from potential source populations (Table 8, Table 9) and genetic distance estimates clearly separated Idaho populations from all others (Table 7). Alternative source population(s) could have introduced new alleles into Idaho not found elsewhere in the Pacific Northwest, and could have led to the emergence of subtle differences in STRUCTURE for the Pacific Northwest samples that were not detected in my initial STRUCTURE analysis (Figure 4). This explanation is further supported by the fact that Idaho populations possess nine private alleles whereas the rest of the study area

in the Pacific Northwest only possesses one (Table 6). It is possible that an unsampled source population (potentially from Canada, Wyoming, Montana, or northern Utah/Nevada) for the founding populations in Idaho contained alleles that are otherwise rare throughout the Pacific Northwest

Contributing Scenarios

It appears that during the DDT era Bald Eagle populations in the Pacific Northwest experienced isolated and temporary reductions in size, as opposed to range-wide decline as observed for other raptor species (Roberts and Green 1983, Safford and Jones 1997, Brown et al. 2007). Molecular data obtained from Bald Eagle nesting sites in the states surrounding Idaho failed to indicate any evidence of a recent genetic bottleneck, while sites within Idaho exhibited characteristics of a genetic bottleneck in three of the five populations.

The fact that there are several pockets of sustained, genetically variable populations of Bald Eagles in the Pacific Northwest indicates that a range-wide decline was not the case for this species. It cannot be said with certainty that the Pacific Northwest as a whole did not experience a bottleneck, only that the six populations sampled in this study persisted through the DDT era with sufficiently large populations sizes. These results are supported by historical data (Greater Yellowstone Ecosystem Working Team 1983, Melquist 1987, Beals and Melquist 1995, Perkins 2006). The high genetic diversity reported in the Pacific Northwest populations of Bald Eagles (Table 6) appears to have been retained as a consequence of the long generation time of the Bald Eagle. Such long generation times have been shown to minimize genetic drift and act as

an intrinsic buffer against the rapid loss of genetic diversity (Hailer et al. 2006, Brown et al. 2007).

In Idaho, significant heterozygote deficiencies, GPD levels, and high inbreeding coefficients indicate Bald Eagles in this region have not yet recovered from historic demographic events. The level of genetic diversity in southern Idaho Bald Eagles is comparable to populations of other raptors that also experienced population bottlenecks: peregrine falcons (4.41 alleles/locus, $H_O = 0.45$; Nesje et al. 2000), Spanish Imperial Eagles (4.49 alleles/locus, $H_O = 0.52$; Martinez-Cruz et al. 2004), and Bonelli's Eagle (*Hieraaetus fasciatus*; 4 alleles/locus $H_O = 0.48$; Mira et al. 2005). Post-bottleneck genetic variability within populations is subject to the various and unique combinations of demographic events and evolutionary forces acting within individual populations. Significant population structure among regions in Idaho indicates current levels of gene flow between the regions is not yet sufficient to overcome the reduction in genetic diversity associated with small population sizes.

Whereas, populations outside of Idaho appear to be at an evolutionary equilibrium: all exhibit low levels of inbreeding, and genetic differentiation and migration levels suggest a pattern of panmixia. Further sampling of these areas may begin to detect new, rare alleles and define geographically distinct populations.

Several isolated demographic events associated with Bald Eagles in Idaho have uniquely shaped the structure of these populations, and several scenarios could have shaped the genetic diversity of populations from this region. One such scenario is that the Idaho populations persisted at low population levels throughout the DDT era and subsequently recovered through inbreeding, leading to genetically unique and structured

populations. The conclusion that a population carries low genetic diversity because of a bottleneck is often only an inference. To truly grasp the evolutionary history, other sources of data must be gathered and compared (Dinerstein and McCracken 1990). Johnson et al. (2009) showed through microsatellite analysis that the critically endangered Madagascar Fish Eagle (*Haliaeetus vociferoides*) has maintained a small effective population size for hundreds to thousands of years and that the low genetic diversity of this species was not the result of a recent bottleneck.

This scenario appears unlikely for Bald Eagles in Idaho as the historical records of these birds in this region is well documented; with the exception of the PR population, all populations were established after 1968 (Greater Yellowstone Ecosystem Working Team 1983, Melquist 1987, Beals and Melquist 1995, Perkins 2006), which was nearing the end of the DDT era.

Another potential explanation for this unexplained panmixia is that Bald Eagle populations from outside of Idaho (Wyoming, Montana, and Oregon) are from long-established populations and are largely in natural environments (i.e., not reliant on habitats created by humans, such as reservoirs). The only exception is the WK population, which is near a reservoir that was created in 1949. Idaho populations are almost entirely dependent upon human-made reservoirs, which may suggest that the Bald Eagles in Idaho occupy an ecologically distinct habitat type.

The most likely scenario explaining the genetic diversity of Bald Eagles in Idaho is that these populations exhibit low observed heterozygosity, and elevated inbreeding which are the characteristics of recently founded populations (Wright 1943, Nei et al. 1975, Slatkin 1994, Saccheri et al. 1996, Alcaide et al. 2009). Additionally, the unique

genetic diversity of Idaho Bald Eagle populations (e.g., private alleles) suggests that the source population of these founders has not yet been samples. A study of founder events in Canadian moose (*Alces alces*) populations found a reduction in observed heterozygosity ranging from 14-30% in recently founded populations (Broders et al. 1999). This reduction in heterozygosity is consistent with comparisons between Idaho populations and the other Pacific Northwest populations (Table 6). Potential founder regions sampled here were from well-established regions in the states surrounding Idaho and were not necessarily the nearest populations in a geographical sense. With the GYE population in such close geographic proximity to many of the southeastern Idaho populations, it was surprising to find that the migration between the regions was almost non-detectable (Table 8, Table 9). This suggests that founder locations are orientated north-south as opposed to east-west, as tested here. Founders of the southern Idaho populations could have originated from northern Idaho or from northern Nevada or Utah where no genetic information on the species has been documented. It is also possible that potential founder populations in the Pacific Northwest were not included in this study.

Conclusion and Future Directions

The Idaho Bald Eagle population confounds the already complex population structure of Bald Eagles in the Pacific Northwest. Despite being in close geographic proximity to many of the populations sampled across the region, Idaho populations remained genetically distinct from the rest of the region. The Idaho populations were also the only populations to show signs of a recent population bottleneck. While these populations are suspected to have originated from recent founder events, none of the

sampled populations from the Pacific Northwest could resolve the source of these founders.

When excluding Idaho, the Bald Eagles in the Pacific Northwest appear to represent a panmictic population. Large distances separated many of the populations, yet no genetic differentiation was evident among populations in the region. Furthermore, there was no evidence of a recent population bottleneck, despite the documented decline of the species due to the use of DDT and organochlorides.

Additional analysis of Bald Eagles across North America may help clarify the evolutionary forces and demographic events shaping the genetic diversity of these Pacific Northwest populations. Larger sample sizes across the species' continental range may reveal that the Pacific Northwest region only includes a small portion of the species' geographic distribution. If this is the case, it may validate my conclusion of panmixia in the Pacific Northwest, but also reveal that Bald Eagle populations across North America are genetically structured over a larger spatial scale. An increased sample size may also help identify potential source populations for the individuals founding these Idaho populations. This may help to resolve the large-scale biogeographic history of this emblematic species.

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APPENDIX

Allele Frequencies by Locus

Table A.1 Allele Frequencies by Locus as Performed in GENEPOP 4.0 (Raymond and Rousset 1995b). Western and Eastern Idaho Populations are not Included. Number of Sampled Individuals in Each Population is Given in Parentheses. Bolded values indicate a private allele.

		Oregon		Montana			Wyoming	Alaska
Locus	**Allele(bp)**	**KL(16)**	**WK(18)**	**GD(6)**	**CS(6)**	**CO(4)**	**GYS(10)**	**CB(10)**
Hle6A11	194	0.0313	0	0	0	0	0	0
	196	0.4839	0.3056	0.75	0.05	0.625	0	0.15
	198	0.4375	0.5	0.25	0.5	0.375	0.65	0.8
	200	0.0625	0.1944	0	0	0	0.35	0.05
Hle0E12	153	0	0	0	0	0	0	0
	159	0	0	0	0	0	0	0
	161	0	0	0	0	0	0	0
	163	0	0	0	0	0	0.1	0
	165	0.0313	0.0833	0	0	0	0	0
	167	0.1563	0.1667	0.0833	0.1667	0.25	0.1	0.25
	169	0.5	0.5	0.6667	0.6667	0.625	0.75	0.5
	171	0.3125	0.25	0.25	0.1667	0.125	0.05	0.25
Hle6F02	223	0	0	0	0	0	0	0
	225	0	0	0	0	0	0	0
	227	0	0	0	0	0	0	0
	229	0	0	0	0	0	0	0
	231	0	0.0278	0	0	0	0	0
	233	0.375	0.3889	0.4167	0.1667	0.125	0.55	0.5
	235	0.5938	0.5833	0.5833	0.8333	0.875	0.45	0.45
	237	0.0313	0	0	0	0	0	0.05
Hle6H10	204	0	0	0	0	0	0	0
	206	0	0	0	0	0	0	0.05
	208	0	0.0278	0	0	0	0	0.05
	210	0	0.0556	0.1667	0.0833	0	0	0.2
	212	0.125	0.1111	0	0.4167	0.25	0.45	0.2
	214	0.1875	0	0	0	0	0.1	0.05
	216	0.4688	0.3889	0.5833	0.4167	0.5	0.45	0.25
	218	0.125	0.1389	0	0	0	0	0.1
	220	0.0938	0.2778	0.25	0.0833	0.25	0	0
	222	0	0	0	0	0	0	0.1
	224	0	0	0	0	0	0	0
Hle0B10	180	0	0	0	0	0	0	0

Locus	Allele(bp)	Oregon KL(16)	Oregon WK(18)	Montana GD(6)	Montana CS(6)	Montana CO(4)	Wyoming GYS(10)	Alaska CB(10)
	182	0	0	0	0	0	0.05	0
	184	0	0.0294	0	0.0833	0	0.1	0
	186	0.5	0.3824	0.4167	0.3333	0.625	0.4	0.35
	188	0.1	0.1471	0	0.0833	0	0.05	0.15
	190	0.4	0.3824	0.5833	0.4167	0.375	0.35	0.45
	192	0	0.0588	0	0.0833	0	0.05	0.05
Hle0E05	225	0	0	0	0	0	0	0
	227	0	0	0	0	0	0	0
	229	0.0625	0.1667	0	0	0	0.1	0.15
	231	0.5313	0.5556	0.5	0.0833	0.75	0.7	0.75
	233	0.1563	0.1667	0.3333	0.6667	0.125	0.15	0.1
	235	0.2188	0.0278	0.1667	0.25	0.125	0.05	0
	237	0	0.0833	0	0	0	0	0
	239	**0.0313**	0	0	0	0	0	0

Table A.2 Allele Frequencies by Locus as Performed in GENEPOP 4.0 (Raymond and Rousset 1995b). Number of Sampled Individuals in Each Population is Given in Parentheses. Bolded values indicate a private allele

Locus	Allele(bp)	Southwestern Idaho			Southeastern Idaho		
		HC(8)	LV(24)	BR(3)	LSR(10)	USR(12)	PR(8)
Hle6A11	194	0.312	0.375	0.167	0	0	0.062
	196	0.404	0.375	0.667	0.4	0.583	0.375
	198	0.25	0.214	0.167	0.3	0.333	0.5
	200	0	0.036	0	0.3	0.083	0.062
Hle0E12	153	**0.062**	0	0	0	0	0
	159	0	**0.018**	0	0	0	0
	161	0.125	0.071	0	0	0	0.062
	163	0.312	0.268	0.333	0	0	0.062
	165	0.125	0.321	0	0.15	0.167	0
	167	0	0.018	0	0	0	0
	169	0.375	0.268	0.333	0.6	0.583	0.812
	171	0	0.036	0.333	0.25	0.25	0.062
Hle6F02	223	0	**0.071**	0	0	0	0
	225	0	0	0	0	0	**0.062**
	227	0	0.107	0.167	0	0	0
	229	0	0	0	0	**0.083**	0
	231	0.5	0.286	0.5	0.2	0.5	0.312
	233	0.5	0.464	0.333	0.45	0	0.375
	235	0	0.071	0	0.3	0.417	0.25
	237	0	0	0	0.05	0	0
Hle6H10	204	0	**0.018**	0	0	0	0
	206	0.062	0.018	0	0	0	0
	208	0.62	0.038	0	0	0.042	0.062
	210	0	0	0.167	0	0	0
	212	0.188	0.125	0.167	0	0.167	0.312
	214	0.062	0.071	0.167	0.1	0.292	0.188
	216	0.188	0.125	0.167	0.2	0.083	0.125
	218	0.062	0.143	0.167	0.4	0.292	0.062
	220	0.125	0.125	0.167	0.1	0.125	0.062
	222	0.25	0.179	0	0.15	0	0.188
	224	0	0	0	**0.05**	0	0
Hle0B10	180	0	0	0	0	**0.208**	0
	182	0	0	0	0.4	0.125	0.25
	184	0.688	0.554	0.833	0.25	0.375	0.375

	186	0.188	0.214	0	0.25	0.125	0.312
	188	0.125	0.161	0.167	0.1	0.125	0
	190	0	0.054	0	0	0.042	0
	192	0	0.018	0	0	0	0
Hle0E05	225	0	0	0	0.05	0.042	0
	227	0.188	0.278	0.333	0	0.333	0.125
	229	0.5	0.407	0.333	0.4	0.292	0.5
	231	0.25	0.204	0.333	0.35	0.125	0.125
	233	0	0.074	0	0.1	0.167	0.188
	235	0	0.037	0	0	0.042	0.062
	237	0.062	0	0	0	0	0
	239	0	0	0	0	0	0