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Evaluating the Taxonomic Status of the Great White Heron (*Ardea Herodias Occidentalis*) Using Morphological, Behavioral and Genetic Evidence.

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**EVALUATING THE TAXONOMIC STATUS OF THE GREAT WHITE HERON
(*ARDEA HERODIAS OCCIDENTALIS*) USING MORPHOLOGICAL,
BEHAVIORAL AND GENETIC EVIDENCE**

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The School of Forestry, Wildlife, and Fisheries

by

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ABSTRACT

The Great White Heron was originally described as a distinct species (*Ardea occidentalis*), but is currently considered to be the white morph of a polymorphic Great Blue Heron subspecies (*A. herodias occidentalis*). Great White and Great Blue heron breeding ranges overlap in the Florida Keys (USA), providing an opportunity to evaluate the degree to which these two taxa are reproductively isolated. The current classification leads to predictions of no significant size difference (H_01), random mate choice (H_02), and no genetic divergence (H_03) between sympatric white and blue herons. Sympatric Great White and Great Blue herons did not differ significantly in size at any of seven morphological variables. However, the power of these tests was low, and patterns of mean values were consistent with the hypothesis of a hybrid origin for Great Blue Herons breeding in the Florida Keys. Mate choice was not random with respect to plumage color. I observed more white/white and blue/blue pairs and fewer mixed pairs than expected in a randomly mating population. This positive assortative pattern suggests that prezygotic reproductive barriers exist within the Florida Keys population. However, mixed pairs occur and may provide an opportunity for gene flow between white and blue herons within the Florida Keys or between the Florida Keys breeding population and other Great Blue Heron populations. Allele frequencies at 12 nuclear microsatellite loci differed significantly between the Florida Keys breeding population and Great Blue Herons breeding on the nearby Florida peninsula. These data suggest that the Florida Keys population is distinct from other Great Blue Heron populations and that there are barriers to gene flow between the Florida Keys Great White Heron population and nearby Great Blue

Heron populations. The Great White Heron appears to be a good biological species and a review of its taxonomic status is merited. Recruitment from Great Blue Heron populations does not appear to be an important factor in maintaining the Great White Heron population. Effective conservation, therefore, will require understanding and managing the small Florida Keys Great White Heron population as an isolate.

INTRODUCTION

Conservation biology has emerged as a discipline that seeks to preserve biological diversity by recognizing evolutionary processes as the foundation for conservation decisions (Soulé and Wilcox 1980, Frankel and Soulé 1981). The goal of conservation biology is to conserve evolutionary potential. Barriers to gene flow between populations and subsequent genetic differentiation due to natural selection or genetic drift are the fundamental processes of speciation and, thus, the origin of biological diversity. Species are the cornerstone of some of the most powerful environmental legislation ever written (e.g. U.S. Endangered Species Act, Convention on International Trade in Endangered Species) and remain a galvanizing force behind most conservation efforts. However, geographically and genetically isolated populations are of particular interest to the conservation biologist because these populations have the greatest potential for speciation (Meffe and Carroll 1994).

Many species definitions have been proposed (e.g. biological species, evolutionary species, phylogenetic species, morphological species, recognition species, cohesion species, ecological species, genealogical species), but no single definition works well for all biological organisms. The biological species concept (BSC) defines species as groups of actually or potentially interbreeding populations that are reproductively isolated from other such groups (Dobzhansky 1937, Mayr 1942). Although the BSC is one of the most widely used definitions, it has limitations. It does not apply to organisms that reproduce asexually or to fossil taxa. It is often difficult to apply to plants because hybridization is common (even between genera), and many phylogeneticists reject the BSC because it does not necessarily

yield monophyletic species (Quicke 1993, Futuyma 1998). Highly differentiated populations that never interbreed or undifferentiated populations that interbreed freely rarely present problems for the BSC. However, there are many cases where the level of “actual” or “potential” interbreeding is ambiguous and the BSC offers no concrete rules to govern what level renders two populations conspecific. Many find this to be a weakness, but it is precisely those cases that present a challenge to the BSC where we may find the most compelling examples of speciation in progress. Any species concept can become mired in trivial phenotypic differences among populations. The criterion of reproductive isolation anchors the BSC to the evolutionary processes that split a single lineage into two.

Analysis of species with significant among-population variation can provide insights into evolutionary processes such as adaptation and speciation (Mayr 1942) and identify unique populations that may require attention as conservation priorities. Many bird species exhibit geographic variation among populations and are divided into subspecies based on differences in size, plumage, and geographic distribution. For example, seven Great Blue Heron (*Ardea herodias*) subspecies are recognized in North America (AOU 1957, AOU 1973). Six are composed entirely of individuals with dark plumage (collectively, Great Blue Heron). Only *A. h. occidentalis* contains individuals with all white plumage (Great White Heron). The taxonomic status of the Great White Heron has been debated for over a century. Unlike the Great Blue Heron, which is widely distributed throughout North America, the Great White Heron is restricted to south Florida (USA) and parts of the Caribbean (Stevenson and Anderson 1994). It is extremely rare in the Caribbean (Raffaele et al. 1998) and the largest

known breeding population (approximately 850 breeding pairs) occurs in the Florida Keys (Powell and Bjork 1996). The debate surrounding the Great White Heron's taxonomic status, and the vulnerability of this Florida Keys population to natural catastrophic events (e.g. hurricanes) and habitat degradation from human activities motivated my research, which seeks to understand the relationships between Great White Heron and Great Blue Heron populations.

The Great White Heron was originally described as a distinct species, *Ardea occidentalis*, but is currently considered to be the white morph of a polymorphic Great Blue Heron subspecies, *A. herodias occidentalis* (AOU 1973). Support for the current classification stems largely from limited observations of interbreeding between white and blue herons in the Florida Keys and the existence of a putative hybrid (Würdemann's Heron), which has plumage that is clearly intermediate between a Great White Heron and a Great Blue Heron. Typical Great Blue Heron plumage includes a white forehead, white crown and white cheeks (which contrast sharply with a black crest and black occipital plumes), a black breast and belly streaked with white, and deep bluish-gray wings and back. Würdemann's Herons vary considerably, but they are generally distinguished from Great Blue Herons by an all white head (or mostly white head streaked with gray or black), white or gray occipital plumes, white breast and belly streaked with gray or black, and pale bluish-gray wings and back. Würdemann's Heron is not found in other North American Great Blue Heron populations. It is widely believed to be a hybrid, but has also been regarded as a distinct species (*A. würdemanni*), a light color phase of the Great Blue Heron, or a dark color phase of the Great White Heron (Holt 1928).

Würdemann's Heron is not directly addressed in the 1973 revision of the Great White Heron's taxonomic status:

Ardea occidentalis is considered conspecific with *A. herodias*, a polymorphic species in the West Indies and Caribbean area; the population of the Florida Keys, on additional morphological grounds, is entitled to subspecific rank with the name *A. herodias occidentalis*...

...The English species name remains Great Blue Heron, with "Great White Heron" available for the white morph. (AOU 1973)

Although vague, the classification criteria appear to be based primarily on geography rather than phenotype. Würdemann's Heron would, presumably, be considered *A. h. occidentalis* because of its geographic distribution (Florida Keys).

Not only is the status of the Würdemann's Heron unclear, but accounts conflict concerning which blue form predominates in the Florida Keys. Holt (1928) and Mayr (1956) independently examined museum skins of herons collected in breeding condition from the Florida Keys. They identified all blue individuals as Würdemann's Herons and concluded that few, if any, Great Blue Herons from the Florida peninsula (belonging to the subspecies *A. h. wardi*) breed in the Florida Keys. In contrast, Stevenson and Anderson (1994) stated that there are "apparently no published reports of the nesting of 2 Würdemann's Herons," implying that Great Blue Herons breeding in the Florida Keys are something other than Würdemann's Herons (presumably *A. h. wardi*).

White-dark polymorphism is relatively common in herons. Mock (1978) identified six species of dichromatic herons (including the Great Blue Heron) in which adults are either white or dark. A seventh species, the Grey Heron (*A. cinerea*), is dark throughout most of its range (Europe, Asia and Africa), but contains an isolated

population of “nearly white” individuals. This population is restricted to small islands off the coast of Mauritania and has been given subspecific rank (*A. c. monicae*). *A. herodias* and *A. cinerea* are closely related allopatric species. They have continent wide distributions and, with two exceptions (*A. h. occidentalis* and *A. c. monicae*), are monochromatic throughout their range. These exceptions may be the result of similar evolutionary events (geographic isolation followed by adaptation to local conditions and phenotypic divergence) and possess attributes that distinguish them from the other five dichromatic herons.

The Great Blue Heron differs from the other dichromatic herons in at least two respects. First, although the ratio of white to dark individuals varies among populations within dichromatic species, both color phases are generally present within any given population. Great White Herons are almost never found in other North American Great Blue Heron populations. Second dichromatic species contain few intermediates. Individuals are usually either all white or uniformly dark. The Florida Keys population exhibits a wider range of intermediate plumages than any of the other dichromatic herons (Mock 1978), and others have suggested that most breeding Great Blue Herons in Florida Bay have intermediate plumage (Holt 1928, Mayr 1956).

The absence of white individuals in other North American Great Blue Heron populations and the wide range of blue phenotypes in the Florida Keys population are more consistent with a hypothesis that south Florida is a contact zone between two previously isolated taxa rather than a hypothesis that south Florida contains a truly dichromatic subspecies of the Great Blue Heron (Mayr 1956, Mock 1978, Lazell 1989). The Great White Heron may have diverged from the Great Blue Heron,

perhaps in the Caribbean, during a Pleistocene interglacial when much of the Florida peninsula was submerged (Mayr 1956, Shinn 1988, Lazell 1989). As sea level subsided, movement of Great Blue Herons onto the emerging Florida peninsula and westward expansion of the Great White Heron into the Florida Keys produced a contact zone where these two taxa currently interbreed. The plumage polymorphism is probably maintained by immigration of Great Blue Herons into the Florida Keys and subsequent hybridization with Great White Herons

The importance of mixed pairs and the existence of putative hybrids may have been overemphasized. A key question is whether white and blue individuals interbreed freely or only rarely (Mayr 1956). The current classification implies that reproductive barriers have not accrued between Great White and Great Blue herons, but there is little evidence to support or refute this assumption. Despite a lively historical debate regarding the Great White Heron's taxonomic status (Holt 1928, Mayr 1956, Meyerriecks 1957, Lazell 1989, Stevenson and Anderson 1994), little attention has been paid to the relationships between sympatric white and blue herons. Great White Heron and Great Blue Heron breeding ranges overlap in the Florida Keys. This sympatry provides an opportunity to study the degree to which these two taxa are reproductively isolated (if at all).

The challenges imposed on south Florida's ecosystems by a growing human population provide compelling reasons to study the Great White Heron, regardless of how we ultimately classify it (full species or subspecies). The Great White Heron is one of the world's many small endemic populations that persist in remnants of ecosystems increasingly influenced by surrounding urban, agricultural and recreational

landscapes. It is entirely dependent on troubled south Florida ecosystems, including Florida Bay, the Florida Keys, and the Florida Everglades. Because of its narrow geographic distribution, this population is vulnerable to natural catastrophic events (e.g. hurricanes) and habitat loss or deterioration resulting from human activities. The discontinuous variation between Great White Herons in the Florida Keys and Great Blue Herons on the nearby Florida peninsula suggests the presence of reproductive barriers between these taxa. If reproductive barriers exist between Great White Herons and Great Blue Herons, conservation of the Great White Heron population will require that it be viewed and managed as an isolate. For example, if Great White Heron numbers decline, recruitment from other Great Blue Heron populations would not be a viable management option. A greater understanding of the relationships between Great White Herons and Great Blue Herons, therefore, is essential to formulate an appropriate management scheme for the conservation of this unique Great White Heron population.

As a first step toward answering the question of whether reproductive barriers exist between these two taxa, I collected morphological, behavioral, genetic data sets to evaluate the relationships between Great White Heron and Great Blue Heron populations. My objectives were to clarify the Great White Heron's taxonomic status and provide information that may guide conservation efforts. The current classification leads to predictions of no significant size differences (H_01), random mate choice (H_02), and no genetic divergence (H_03) between sympatric white and blue herons. I collected morphometric data from museum specimens to test H_01 and monitored breeding Great White and Great Blue herons in Florida Bay (Monroe

County, USA) to test H_02 . I compared allele frequencies at 12 nuclear microsatellite loci and tested for genetic differentiation between Great White Heron and Great Blue Heron populations (H_03). Together, these data suggest that the Florida Keys breeding population is distinct from other Great Blue Heron populations and that there are barriers to gene flow between the Florida Keys breeding population and nearby Great Blue Heron populations. Although reproductive isolation does not appear to be complete, I believe that the Great White Heron is a good biological species and that these data provide sufficient evidence to merit a review of the Great White Heron's taxonomic status. Recruitment from Great Blue Heron populations does not appear to be an important factor in maintaining the Great White Heron population. Effective conservation, therefore, will require understanding and managing the Great White Heron population as an isolate.

CHAPTER 1. Analysis of seven morphological variables measured from museum skins of Great White and Great Blue herons

INTRODUCTION

Although biological species are defined by reproductive isolation, morphology is often the yardstick by which inferences about species or subspecies boundaries are made. The Great Blue Heron has been divided into several subspecies, which are distinguished by differences in size, plumage, and geographic distribution. Although authors disagree about how many subspecies should be recognized (AOU 1957, AOU 1973, Hancock and Elliott 1978, Eckert 1981, del Hoyo et al. 1992), most classifications identify three subspecies in eastern North America (Figure 1.1). The nominate race, *Ardea herodias herodias*, breeds throughout the mid-Atlantic states (USA) to Nova Scotia (Canada) and west to northern Montana (USA) and southern Alberta (Canada). *Ardea herodias wardi* is distributed throughout the southeastern and south-central United States, including the southern Florida peninsula. *Ardea herodias occidentalis* is restricted to south Florida (Florida Bay and the Florida Keys). *Ardea h. herodias* and *A. h. wardi* are composed entirely of individuals with dark plumage ("blue"), while Florida's *A. h. occidentalis* population contains white and blue individuals. Other investigators have documented size differences among *A. h. herodias*, *A. h. wardi* and white *A. h. occidentalis* (Ridgway 1878, Oberholser 1912, Holt 1928, Mayr 1956, Zachow 1983), but little attention has been given to morphometric comparisons of white and blue herons from within Florida's *A. h. occidentalis* breeding population. Considering the intensity of the Great White Heron species debate over the years, this information gap is remarkable.

Figure 1.1: Distribution of the three Great Blue Heron subspecies found in eastern North America

Holt (1928) calculated an index of proportion, culmen length divided by tarsus length, for Great Blue Herons collected on the Florida peninsula (*A. h. wardi*) and Great White Herons (*A. h. occidentalis*). He reported that this index “sharply separates” the two (“the index for *wardi* falling always definitely below that of *occidentalis*”), but did not provide supporting quantitative data. Mayr (1956)

presented another index, bill length divided by wing length, for *A. h. wardi* and white *A. h. occidentalis*. Mayr acknowledged that white *occidentalis* differed from *wardi* by an average longer bill, but noted much overlap in the values of this index ($\bar{X}_{wardi} = 29.7$, $n = 14$; $\bar{X}_{occidentalis} = 31.6$, $n = 11$). A t-test of these data show this index is significantly different between the two groups ($H_0: \mu_{wardi} = \mu_{occidentalis}$, $t = 2.84$, $df = 23$, $p = 0.007$). In one of the most thorough treatments, Zachow (1983) found significant size differences among northern Great Blue Herons (*A. h. herodias*), Great Blue Herons from the Florida peninsula (*A. h. wardi*), and Great White Herons (*A. h. occidentalis*). Among these three groups, northern Great Blue Herons were the smallest, Great Blue Herons from the Florida peninsula were intermediate in size, and Great White Herons were the largest.

Despite evidence of size differences among *A. h. herodias*, *A. h. wardi*, and white *A. h. occidentalis*, only one investigator has directly compared white and blue herons within the Florida Keys breeding population at any morphometric variable. Occipital plumes, which are normally elongated in adult Great Blue Herons, are generally reduced or absent in adult Great White Herons. Holt (1928) measured the longest occipital plume in a small series of Great Blue Herons from the Florida peninsula and Great White Herons and intermediate Würdemann's Herons from the Florida Keys. Among females, Great Blue Herons from the Florida peninsula had the longest plumes ($\bar{X}_{Blue} = 154$ mm, $n = 4$), plumes from Würdemann's Herons were intermediate in size ($\bar{X}_{Würdemann} = 137$ mm, $n = 5$), and Great White Herons had the shortest occipital plumes ($\bar{X}_{white} = 87.7$ mm, $n = 11$). Among males, occipital plumes of Great White Herons and Würdemann's Herons did not differ in length ($\bar{X}_{white} =$

109.2 mm, n = 11; $\bar{X}_{\text{Würdemann}} = 109$ mm, n = 5), but both were shorter than plumes of Great Blue Herons from the Florida peninsula ($\bar{X}_{\text{Blue}} = 181$ mm, n = 18).

Zachow's (1983) analysis of skeletal measurements showed that Great Blue Herons from the Florida peninsula were smaller than Great White Herons. Holt's occipital plume data exhibit the opposite trend (Great Blue herons from the Florida peninsula have longer occipital plumes than Great White Herons) and suggest that morphometric comparisons may reveal significant size differences between white and blue herons breeding in the Florida Keys. Together these data suggest that a morphometric comparison of Great White and Great Blue herons from the Florida Keys may provide insight into the origin of blue herons breeding in this population.

Extracting appropriate raw data from existing literature, however, is not possible because no other comparable morphological measurements have been published for these two groups (Palmer 1962 and Appendix A). I attempted to fill this information gap by measuring seven morphological variables from museum skins of Great Blue Herons, Great White Herons, and intermediate Würdemann's Herons. My objectives were to test for size differences between white and blue herons within the Florida Keys breeding population and, if size differences exist, to determine whether they are consistent with the hypothesis of a hybrid origin for the intermediate Würdemann's Heron. If size differences exist, then this would suggest that Great White Herons and Great Blue Herons within the Florida Keys are not a randomly mating population. The hybrid origin hypothesis would be supported if Würdemann's Herons are intermediate in size at all variables measured, regardless of whether an individual variable is larger in Great Blue Herons or in Great White Herons.

METHODS

Data Collection

I measured seven morphological variables (length of exposed culmen, depth of bill at base, length of tarsus, wing chord, length of tail, length of middle toe, and length of longest occipital plume) from museum skins of herons in the following groups: northern Great Blue Herons (B-N), Great Blue Herons from the Florida peninsula (B-FP), and Great White and Great Blue herons from the Florida Keys breeding population (B-FK, W-FK). The first five variables are commonly reported for most birds, the length of the middle toe is often reported for herons and other large birds, and existing data suggest that the longest occipital plume may vary among Great White Heron and Great Blue Heron populations (Holt 1928).

Ardea h. herodias (B-N) is migratory. Winter ranges overlap with *A. h. wardi* (B-FP) and other subspecies to the south (B-FK and W-FK) or west (Palmer 1962, Eckert 1981). Departures from northern breeding grounds generally begin in mid-September; returns begin in early February and continue through April (Palmer 1962). To minimize the possibility of including winter migrants, I restricted the B-FP sample to Great Blue Herons collected in Florida (excluding the Florida Keys: Monroe County, FL) during summer, which I defined as 01 April through 30 September. Selection criteria for B-FK and W-FK included Great Blue Herons collected during summer in Monroe County, Florida, and Great White Herons and intermediate Würdemann's Herons regardless of collection date. My selection criteria for B-N included Great Blue Herons collected within the *A. h. herodias* subspecies breeding range regardless of collection date.

I used a ruler to measure the longest occipital plume to the nearest millimeter, digital calipers to measure depth of bill at base to the nearest 0.1 mm (Baldwin et al. 1931, Proctor and Lynch 1993), a ruler with an upright stop at the zero point to measure the wing chord to the nearest 0.5 mm (Palmer 1962, Proctor and Lynch 1993), and dividers and a ruler to measure the remaining variables to the nearest 0.5 mm (Baldwin et al. 1931, Proctor and Lynch 1993). For each individual, I measured each variable at least twice and averaged these measurements to obtain the values reported in Appendix B.

Because males are larger than females, I analyzed them separately and omitted birds whose sex was unknown. I did not have a large enough sample of juvenile birds within any group to adequately test for size differences between adult and juvenile birds. Therefore, I omitted juvenile birds from subsequent analysis rather than pool them with adults. Only seven adult Great Blue Herons from the Florida peninsula (3 males, 3 females, 1 unknown) met my selection criteria. Raw data for these seven herons are included in Appendix B, but I dropped them from subsequent statistical analyses because of the small sample sizes.

Statistical Analysis

I analyzed males and females separately for each variable within each group (B-N, B-FK, and W-FK for males; B-FK and W-FK for females). For each variable, I calculated descriptive statistics (mean, variance, standard deviation, etc.) and tested ANOVA and t-test assumptions of normality and homogeneity of variance. I used the W test for normality (Shapiro and Wilk 1965) to determine whether observations for each variable within each group were normally distributed. For females, I used an F-

test (Sokal and Rohlf 1995) to test for homogeneity of variance between groups ($H_0: \sigma^2_{B-FK} = \sigma^2_{W-FK}$). For males, I used Bartlett's test for homogeneity of variance (Sokal and Rohlf 1995) to test among groups ($H_0: \sigma^2_{B-N} = \sigma^2_{B-FK} = \sigma^2_{W-FK}$). I used appropriate parametric or nonparametric statistics to test for differences between or among means (H_0 females: $\mu_{B-FK} = \mu_{W-FK}$, H_0 males: $\mu_{B-N} = \mu_{B-FK} = \mu_{W-FK}$). Flow charts for statistical procedures are shown in Appendix C.

RESULTS

I measured seven morphological variables from a total of 101 Great White and Great Blue herons (Appendix B). After excluding juvenile birds, birds of unknown sex, and groups with inadequate sample sizes, my final data set contained 75 herons: eight male northern Great Blue Herons (B-N), 26 (14 male, 12 female) Great Blue Herons from the Florida Keys breeding population (B-FK), and 41 (24 male, 17 female) Great White Herons from the Florida Keys breeding population (W-FK). I did not find any specimens collected from the Florida Keys that met my selection criteria and had "typical" Great Blue Heron plumage (white cheek and crown, black crest and occipital plumes, black breast and belly streaked with white, and deep bluish-gray wings and back). The B-FK group, therefore, contains only intermediate Würdemann's Herons (head all white or white streaked with gray or black, white breast and belly streaked with gray or black, and pale bluish-gray wings and back).

Results of tests for normality and homogeneity of variance are provided in Appendix D (males) and Appendix E (females). Descriptive statistics and tests concerning the difference between means are presented in Table 1.1 (males) and Table 1.2 (females).

Table 1.1: Mean (in millimeters), standard error (SE), and sample size (n) of seven morphological variables measured from museum skins of adult male herons and p-values for tests of the difference between means.

Variable	Group ^a	Mean	SE	n	p-value ^b		
Culmen	B-N	145.3	1.6	08	N	I	
	B-FK	158.2	2.0	14	I	<0.001	
	W-FK	160.2	1.0	22	W	<0.001	0.293
Depth of Bill	B-N	30.4	0.4	08			
	B-FK	32.2	0.4	14	I	0.002	
	W-FK	32.5	0.3	22	W	<0.001	0.463
Tarsus	B-N	183.1	2.0	08			
	B-FK	201.9	2.7	14	I	<0.001	
	W-FK	206.8	2.4	24	W	<0.001	0.174
Middle Toe	B-N	109.6	2.0	08			
	B-FK	114.7	1.1	14	I	0.037	
	W-FK	116.9	1.2	24	W	0.002	0.220
Wing	B-N	490.7	3.2	08			
	B-FK	490.2	3.8	14	I	0.935	
	W-FK	490.3	3.0	20	W	0.947	0.981
Tail	B-N	180.9	1.4	08			
	B-FK	178.3	2.1	14	I	0.370	
	W-FK	181.6	1.3	24	W	0.798	0.139
Occipital plume	B-N	192.6	5.6	8			
	B-FK	113.4	10.0	14	I	<0.001	
	W-FK	98.2	10.5	20	W	<0.001	0.281

^a B-N = N = Great Blue Herons collected in northeastern and north-central United States, B-FK = I = intermediate Würdemann's Herons collected in the Florida Keys (Monroe County, FL), W-FK = W = Great White Herons collected in the Florida Keys (Monroe County, FL).

^b $H_0: \mu_1 = \mu_2$, $t^* = (\bar{X}_1 - \bar{X}_2) / \sqrt{MSE(1/n_1 + 1/n_2)}$, $df = df_{\text{error}}$, Bonferroni correction for multiple comparisons = $0.05/3 = 0.017$

Among males, $\bar{X}_{B-N}$ differed significantly from both $\bar{X}_{B-FK}$ and $\bar{X}_{W-FK}$ at five of the seven variables - length of exposed culmen, depth of bill at base, length of tarsus, length of middle toe, and length of longest occipital plume (Table 1.1. Figure 1.2). Wing chord and tail length did not differ among groups. $\bar{X}_{B-FK}$ and $\bar{X}_{W-FK}$ did not differ significantly at any of the seven variables in either males or females (Table

1.1 and Table 1.2). Although not significantly different, mean values for these two groups followed a consistent pattern (Figure 1.2). In males and females, $\bar{X}_{W-FK}$ was larger than $\bar{X}_{B-FK}$ at all variables except longest occipital plume (where $\bar{X}_{B-FK} > \bar{X}_{W-FK}$). $\bar{X}_{B-FK}$ was intermediate between $\bar{X}_{B-N}$ and $\bar{X}_{W-FK}$ at five of the seven variables - length of exposed culmen, depth of bill at base, length of tarsus, length of middle toe, and length of longest occipital plume.

Table 1.2: Mean (in millimeters), standard error (SE), and sample size (n) of seven morphological variables measured from museum skins of adult female herons. Test statistic (TS), degrees of freedom (df) and p-value for two-tailed tests concerning the difference between means ($H_0: \mu_{B-FK} = \mu_{W-FK}$).

Variable	Group ^a	Mean	SE	n	TS	df	p > TS
Culmen	B-FK	148.4	1.8	12	0.21 ^b	25.5	0.82
	W-FK	149.1	2.8	17			
Depth of Bill	B-FK	30.2	0.6	12	108 ^c	12, 17	0.81
	W-FK	30.3	0.5	17			
Tarsus	B-FK	187.7	1.8	12	0.81 ^b	21.4	0.43
	W-FK	191.5	4.3	17			
Middle Toe	B-FK	105.5	1.3	12	1.02 ^d	27	0.32
	W-FK	107.6	1.5	17			
Wing	B-FK	466.0	3.2	11	0.85 ^b	23.1	0.40
	W-FK	471.3	5.4	16			
Tail	B-FK	172.3	1.6	12	0.68 ^d	27	0.50
	W-FK	174.3	2.2	17			
Occipital plume	B-FK	113.2	12.6	11	117 ^c	11, 15	0.07
	W-FK	81.5	6.3	15			

^a B-FK = intermediate Würdemann's Herons collected in the Florida Keys (Monroe County, FL), W-FK = Great White Herons collected in the Florida Keys (Monroe County, FL).

^b Two sample t-test assuming unequal variances (Ott 1993), test statistic = t'

^c Mann-Whitney test (Zar 1984), test statistic = U

^d Two sample t-test assuming equal variances (Ott 1993), test statistic = t

Figure 1.2: Mean values ($\pm$ 95% CI) for seven morphological variables measured from museum skins of northern Great Blue Herons (B-N), Great Blue Herons from the Florida Keys (B-FK), and Great White Herons from the Florida Keys (W-FK).

DISCUSSION

Previous studies have documented significant size differences among the three Great Blue Heron subspecies found in eastern North America (Holt 1928, Mayr 1956, Zachow 1983). Northern Great Blue Herons (*A. h. herodias*) are the smallest, Great White Herons from the Florida Keys (*A. h. occidentalis*) are the largest, and southern Great Blue Herons (*A. h. wardi*) are intermediate in size (Zachow 1983). Clinal variation in size among these widely distributed subspecies is not unexpected. However, the current classification presumes that Great White and Great Blue herons in the Florida Keys interbreed freely. Finding size differences between these sympatric taxa would indicate that they are not a randomly mating population. Comparable measurements of white and blue herons from within south Florida's *A. h. occidentalis* breeding population have been published for only one morphological variable (longest occipital plume). I compared seven morphological variables from museum skins of adult Great White Herons and Great Blue Herons from within the Florida Keys breeding population (*A. h. occidentalis*). My objectives were to test for size differences between white and blue herons within the Florida Keys breeding population and, if size differences exist, to determine whether they are consistent with the hypothesis of a hybrid origin for the intermediate Würdemann's Heron.

The plumage of Great Blue Herons from the Florida Keys (B-FK) varied, but all appeared to possess at least some plumage characteristics of intermediate Würdemann's Herons (head all white or mostly white streaked with gray or black, white breast and belly streaked with gray or black, and pale bluish-gray wings and back). The phenotypic composition of this sample may accurately reflect resident

Great Blue Herons within the Florida Keys breeding population, but it may also be an artifact of my selection process. To reduce the chances of including migrants from northern populations, I excluded birds with “typical” Great Blue Heron plumage collected during winter. This may have biased the B-FK sample against resident herons with typical Great Blue Heron plumage. Furthermore, some adult Great White Herons migrate from the Florida Keys to the southern Florida peninsula during non-breeding summer months (Powell and Bjork 1990). If this is true of Great Blue Herons breeding in the Florida Keys, then this would also reduce the chances of finding a museum skin from the Florida Keys with “typical” Great Blue Heron plumage.

I found no significant size differences between Great Blue Herons from the Florida Keys (B-FK) and Great White Herons from the Florida Keys (W-FK) at any of the seven morphological variables (Table 1.1 and Table 1.2). However, because sample sizes were small, the power of these tests was low ($1-\beta < 0.30$ for all tests of $H_0: \mu_{B-FK} = \mu_{W-FK}$). Although B-FK and W-FK did not differ significantly at any variable, the low power of these tests and a consistent pattern in mean values suggests that larger sample sizes might reveal significant differences at some of these variables.

In males, B-FK and W-FK differed significantly from northern Great Blue Herons (B-N) at five of the seven variables - length of exposed culmen, depth of bill at base, length of tarsus, length of middle toe, and length of longest occipital plume (Table 1.1). Although B-FK and W-FK did not differ significantly, there was a pattern in the mean values of these five variables (Figure 1.2). $\bar{X}_{B-FK}$ was intermediate between $\bar{X}_{B-N}$ and $\bar{X}_{W-FK}$ regardless of whether the variable was larger in B-N

(longest occipital plume) or W-FK (culmen, depth of bill, tarsus, and middle toe).

This pattern is consistent with the hypothesis that intermediate Würdemann's Herons are the result of hybridization between Great White Herons and the smaller Great Blue Herons from the Florida peninsula. This interpretation might be supported more strongly if I had been able to obtain larger sample sizes and demonstrate these patterns in a larger number of independent variables (the four hard bony structures - culmen, depth of bill, tarsus, and middle toe - are almost certainly not independent).

The absence of samples from the Florida peninsula is unfortunate. Zachow's (1983) morphometric evidence from skeletons suggests that Great Blue Herons from the Florida peninsula are smaller than Great White Herons, but that these differences may be small compared to the differences between these two groups and northern Great Blue Herons. Adequate morphometric samples from the Florida peninsula would, perhaps, provide stronger inferences regarding the affinities of Great Blue Herons breeding in the Florida Keys.

CHAPTER 2. Mate choice between sympatric Great White and Great Blue herons

INTRODUCTION

Biological species are defined by reproductive isolation. Even phenotypically distinct populations may be considered conspecific if gene flow between them can occur freely. Reproductive barriers reduce or prevent gene flow between taxa and play an important role in the formation and maintenance of reproductive isolation between species. Reproductive barriers are categorized as either prezygotic (occurring before the formation of a hybrid zygote) or postzygotic (inviability of hybrid zygotes or reduced fertility of hybrid offspring). Behavioral isolation (potential mates encounter each other but do not mate) is an important prezygotic barrier to gene flow between sympatric taxa (Futuyma 1998). Positive assortative mating, a consequence of behavioral isolation, is one line of evidence often used to diagnose reproductive isolation between sympatric taxa.

Support for the Great White Heron's subspecies status stems largely from limited observations of interbreeding between sympatric white and blue herons, and impressions that mate choice is random with respect to plumage color (Holt 1928, Mayr 1956, Meyerriecks 1957). Mated pairs of white and blue herons have been reported, as have nests containing mixed broods (Holt 1928, Meyerriecks 1957, Bancroft 1969, McHenry and Dyes 1983). These observations of mixed pairs may have carried too much weight in the decision to reclassify the Great White Heron as a subspecies. A key question is whether white and blue individuals interbreed freely or only rarely (Mayr 1956).

There are no published data that support a hypothesis of random mating with respect to plumage color. Meyerriecks (1957) interpreted his observations of a small number of mated pairs as support for a random mating hypothesis, but cautioned against accepting this hypothesis without further study. Robertson (1978) reported that mixed pairs occur “about” one order of magnitude lower than expected from a randomly mating population, but gave no details of methodology. Powell’s unpublished surveys reportedly support Robertson’s hypothesis of positive assortative mating (Powell and Bjork 1996).

I tested the hypothesis that sympatric Great White Herons and Great Blue Herons pair randomly with respect to plumage color by monitoring nests in Florida Bay (Monroe County, Florida, USA). Rejection of this hypothesis would suggest that prezygotic reproductive barriers currently exist between Great White Herons and Great Blue Herons breeding in the Florida Keys. I also recorded nestling phenotypes to see if these data would provide insight into the genetic basis of color inheritance. This is the first study to conduct observations on a large number of nests in sufficient detail to confirm the phenotypes of both members of a mated pair and their offspring. This allowed me to test the random mate choice hypothesis and make inferences regarding the genetic basis of the plumage polymorphism found in the Florida Keys population.

METHODS

Natural History and Study Area

Great Blue Herons are distributed widely throughout North America and are also found in parts of Central America and the Caribbean. The Great White Heron is

restricted almost entirely to south Florida, Cuba, Jamaica, and off the coasts of Venezuela and the Yucatan (Powell and Bjork 1996). Great White Herons are extremely rare in the Caribbean (Raffaele et al. 1998), and south Florida supports the largest known breeding population. Approximately 850 pairs breed in the shallow marine and coastal mangrove environments of Florida Bay and the Florida Keys (Powell and Bjork 1996). Although some non-breeding birds do move to freshwater wetlands on the southern Florida peninsula during the non-breeding season, this Great White Heron population is essentially non-migratory, and many birds spend the entire year within the Florida Keys ecosystem (Powell and Bjork 1990).

Figure 2.1: Map of south Florida, including Florida Bay and the Florida Keys.

Florida Bay is a large shallow estuary, which receives freshwater input from the Florida Everglades. It is open to the Gulf of Mexico on its western boundary and lies between the Florida Everglades to the north and the Florida Keys to the south and

east (Figure 2.1). The “outer Keys” refer to the portion of the Florida Keys that extend beyond Florida Bay’s western boundary (a line drawn approximately between Cape Sable on the southwestern Florida peninsula and Long Key in the Florida Keys). Florida Bay is composed of a series of shallow basins separated by an network of shoals, mudflats, and hundreds of small mangrove islands. Great White Herons build nests on these islands within Florida Bay and on small mangrove islands along the outer Keys. They rarely breed on the Florida peninsula or on the main Keys themselves (Robertson 1978). Not only do Great Blue Herons nest on islands within Florida Bay and along the outer Keys, but they also breed in a variety of environments on the Florida peninsula.

Florida Bay’s population breeds asynchronously. Nests can be found at any time of year, but peak breeding activity coincides with south Florida’s dry season, approximately October through April. Although it is difficult to distinguish males and females in the field, the breeding cycle provides many opportunities to observe both members of a mated pair together at their nest. Shared duties and characteristic behaviors between mates make it possible to assign pair status with confidence (Meyerriecks 1960, Mock 1976, Butler 1992).

Data Collection and Analysis

To test the random mate choice hypothesis (H_0 : sympatric Great White Herons and Great Blue Herons pair randomly with respect to plumage color), I studied breeding Great White Herons and Great Blue Herons in Florida Bay during the peak of the 1998-1999 breeding season (October through February). I used high quality optical equipment to observe nests from a distance (Leica 8x44 binoculars or

Swarovski 60x spotting scope) and monitored each nest until I observed adults engaged in activities that positively identified them as a pair (e.g. switching incubation duties). For each blue adult, I attempted to determine whether its plumage was characteristic of a “typical” Great Blue Heron or of an “intermediate” Würdemann’s Heron. However, for reasons discussed below, adult phenotype is reported here as either white or blue.

I used the number of white and blue adults from the sample of observed nests to estimate the proportion of white and blue individuals in the breeding population. I used this estimate to generate expected values for each of the three pairing categories (white/white, white/blue, blue/blue). I used a X^2 goodness of fit test for the difference between observed and expected values (Sokal and Rohlf 1995), subtracting one degree of freedom for the total sample size and one degree of freedom because sample frequencies were used to generate expected values.

Whenever possible, I recorded nestling phenotypes in nests where adult phenotypes were known. I could not discern any differences in plumage among blue nestlings, even upon close inspection (I handled nestlings to take blood and feather samples for genetic analysis). This made it impossible to infer whether adult phenotype would be characteristic of a “pure” Great Blue Heron or of an “intermediate” Würdemann’s Heron. Nestling color, therefore, was recorded as either white or blue.

RESULTS

I determined adult plumage color at 114 nests from 14 islands within Florida Bay during the 1998-1999 breeding season. White and blue individuals were clearly

distinguishable. Among blue adults a continuum of phenotypes ranged from those with plumage indistinguishable (under field conditions) from other North American Great Blue Herons (white cheek and crown, black crest and occipital plumes, black breast and belly streaked with white, and deep bluish-gray wings and back) to obvious intermediates (all white head or mostly white with streaks of gray or black, white breast and belly streaked with gray or black, and pale bluish-gray wings and back). If I define, for a moment, three somewhat arbitrary plumage categories (blue herons at one end of the blue plumage continuum, intermediate herons at the other end of the blue plumage continuum, and white herons), all pair combinations were observed and all combinations produced viable offspring.

Although some blue adults had plumage that was clearly intermediate and others had plumage that was indistinguishable from “typical” Great Blue Herons, the continuum of blue phenotypes made it extremely difficult to devise any meaningful criteria to categorize blue adults as either “blue” or “intermediate.” Adult phenotype, therefore, is reported as either white or “blue” (Table 2.1). I rejected the random mating hypothesis using a X^2 goodness of fit test for the difference between observed and expected values ($X^2=31.32$, $df=1$, $p<0.001$).

Table 2.1: Observed (O) and expected (E) values for each pairing category expressed as number of mated pairs and percent of total.

		# of Mated Pairs		% of Total	
Adult Phenotypes		O	E ^a	O	E ^a
White	White	83	73	73	64
White	Blue	17	36	15	32
Blue	Blue	14	5	12	4

^a Expected values assume $p(\text{white}) = 0.8$, $q(\text{blue}) = 0.2$, and adults pair randomly with respect to plumage color

Table 2.2 contains nestling and adult phenotype data for two breeding seasons: 1997-1998 and 1998-1999. I found only white nestlings in nests where both adults were white. Mixed pairs produced broods with all blue offspring, all white offspring, and mixed offspring. Blue/blue pairs produced broods that were either all blue or mixed. I did not find any blue/blue pairs with all white offspring, however the number of nests in this category is small and does not preclude the possibility that two blue parents could produce a brood of all white offspring.

Table 2.2: Number and color of offspring in nests for which adult phenotypes are known

Adult phenotypes		Number of nests	Number of offspring	
			White	Blue
White	White	51	113	0
White	Blue	14	15	13
Blue	Blue	14	6	25

DISCUSSION

Support for the Great White Heron's subspecies status stems largely from limited observations of interbreeding between Great White Herons and Great Blue Herons and from impressions that mate choice is random with respect to plumage color. However, there are no published data that support a random mate choice hypothesis. I tested the hypothesis that sympatric Great White Herons and Great Blue Herons pair randomly with respect to plumage color by monitoring nests in Florida Bay (Monroe County, Florida, USA). I observed more white/white and blue/blue pairs and fewer mixed pairs than expected from a randomly mating population ($X^2=31.32$, $df=1$, $p<0.001$).

My data are consistent with Robertson's (1978) thesis of positive assortative mating, but the number of mixed pairs that I observed was approximately half that

expected from a randomly mating population rather than the tenfold reduction reported by Robertson (WBR). Robertson counted 563 white and 380 blue herons at nests during four surveys of Florida Bay and, therefore, expected about 48% of the pairs to be mixed. He did not provide the number of pairs observed in each category, but said the number of mixed pairs was about one order of magnitude lower than expected (presumably about 5%). Not only does the proportion of observed to expected values for mixed pairs differ between our data (HLM - about 1:2; WBR - about 1:10), but our estimates of the relative proportions of white to blue herons in the Florida Bay breeding population also differ (HLM - about 4:1, WBR - about 3:2).

These estimates suggest that either the proportion of blue herons breeding in Florida Bay fluctuates over time, or one (or both) of our estimates is (are) incorrect. Robertson's surveys were done at two to four month intervals from June 1959 through May 1960. Recent aerial surveys (Gawlik 1998) show that peak activity is essentially the same for white and blue herons in Florida Bay (although the Great White Heron season is more protracted) and breeding Great White Herons outnumber breeding Great Blue Herons at any given time (Figure 2.2). Great Blue Heron nests comprised between zero and 26% of the total nests during any given month. My estimate of relative proportions of white to blue herons in the Florida Bay breeding population (80% white, 20% blue) is consistent with these recent surveys. If my data and Robertson's data are both accurate, the discrepancy between our data sets invites the following hypothesis. As Great Blue Herons become rare within the Florida Bay breeding population, the probability that a Great Blue Heron will pair with a Great White Heron increases, but remains below the level expected from a randomly mating

population. This hypothesis is consistent with mate choice theory that predicts the degree of “choosiness” exhibited by courting animals will be influenced by the availability of potential mates (Crowley et al. 1991, Nuechterlein and Buitron 1998).

Figure 2.2: Great White Heron and Great Blue Heron nests detected in Florida Bay during aerial surveys from October 1995 through October 1997 (Data from Gawlik 1998).

Although the pattern of mate choice in Florida Bay is assortative, the mechanism producing this pattern is unknown. The non-random pattern does not necessarily imply that these herons use plumage color as a criterion for mate choice. Other factors (habitat preference, timing of breeding, sex ratios, historical geographic distribution, etc.) may also influence patterns of mate choice. These factors may function at several spatial or temporal scales and could either inhibit or promote mixed pairs. For example, subtle differences in habitat preference (nest sites or foraging habitat) could segregate Great White Herons and Great Blue Herons during the breeding season. This might manifest itself on small scales (within individual islands) or on larger scales (among islands). If Great White Herons and Great Blue Herons

occupy different habitats and mate choice is restricted primarily to birds within a preferred habitat, the probability of encountering potential mates of the opposite phenotype is reduced and there will be fewer mixed pairs than expected from a randomly mating population.

Conversely, there may be factors that facilitate mixed pairing even if these herons prefer mates with like phenotype. In Florida Bay pairs nest singly or in loose association with other breeding birds, but rarely in the dense colonies typical of other Great Blue Heron populations. Even large islands generally have fewer than thirty nesting pairs at any given time and most have fewer than ten (Gawlik and Ogden 1996). Breeding is asynchronous. Not all birds acquire the visible signs of breeding condition (brilliant soft part coloration and elongated neck, back and occipital plumes) simultaneously, and nests at different stages of the breeding cycle (egg, nestling, fledgling) are commonly found in close proximity (pers. obs.). Great blue herons are relatively rare in the Florida Bay breeding population. If mate choice occurs on a small spatial scales (within individual islands or among closely spaced islands), some blue herons may not encounter a suitable mate of the preferred phenotype. These birds may pair with the less desired phenotype rather than give up an opportunity to mate during a particular breeding season. Thus, some mixed pairs could occur even if Great White Herons and Great Blue Herons have strong preferences for mates with like phenotype.

The positive assortative pattern of mate choice with respect to plumage color suggests that prezygotic reproductive barriers exist between Great White Herons and Great Blue Herons in Florida Bay. Without a more detailed understanding of some of

the variables that influence mate choice, it is difficult to assess the nature of the reproductive isolation between these two taxa. The assortative pattern may reflect historical influences (e.g. geographic distribution) and may break down over time. Conversely, “mis-mating” may incur significant fitness consequences and reproductive isolation may be reinforced over time.

My data from Florida Bay also provide some insight into the genetic basis of the plumage polymorphism observed in the Florida Bay population. Some ornithologists have suspected that two white adults are capable of producing blue offspring (Mayr 1956, Meyerriecks 1957). Mayr (1956) proposed a model in which a dominant allele conferred white plumage and modifier genes were responsible for producing the intermediate plumage of the Würdemann’s Heron. However, I found only white offspring in nests where both parents were white (Table 2.2). If plumage color is determined primarily at a single locus and white is dominant, then the probability of observing this sample is extremely low unless most pairs (>88%) have at least one member homozygous for the dominant white allele (Table 2.3). Given the assortative pattern of mate choice and my estimate of the ratio of white to blue herons in the breeding population (4:1), it is conceivable that most white herons are homozygous for a dominant white allele and that this sample is statistically likely. However, I found both blue and white offspring in nests where both parents were blue and, if plumage color is controlled primarily at a single locus, two blue parents can produce white offspring only if white plumage is a recessive trait and both parents are heterozygotes. Furthermore, as Table 2.4 illustrates, the proportion of white offspring found in nests of blue/blue pairs ($19.4 \pm 13.9\%$) is within the range expected under the

hypothesis that white plumage is recessive (0-25%), and differs significantly from the expected value for the hypothesis that white plumage is dominant (0%).

Table 2.3: Probability of producing 113 white offspring (Table 2.2) from white/white pairs given different relative proportions of genotype crosses and assuming white plumage is dominant.

Relative proportions of genotype crosses ^a		Probability of producing a single white offspring	Probability of producing 113 white offspring
Ww x Ww	WW x Ww or WW x WW		
1.00	0.00	0.75	7.62×10^{-15}
0.20	0.80	0.95	0.003
0.12	0.88	0.96	0.010
0.08	0.92	0.98	0.102
0.00	1.00	1.00	1.00

^a WW and Ww = white, ww = blue

Table 2.4: Possible genotype crosses and percent white offspring expected from these crosses for two hypotheses of color dominance (assuming color is controlled by a single locus) compared with the percent of white offspring ($\pm$ 95% CI) observed in nests with known parental phenotypes

Parental phenotypes	WHITE DOMINANT		WHITE RECESSIVE		% white offspring observed
	Possible genotype crosses	% white offspring expected	Possible genotype crosses	% white offspring expected	
White/White	WW x WW WW x Ww Ww x Ww	75-100	bb x bb	100	100
White/Blue	WW x ww Ww x ww	50-100	bb x Bb bb x BB	0-50	53.6 ($\pm$ 18.5)
Blue/Blue	ww x ww	0	Bb x Bb BB x Bb BB x BB	0-25	19.4 ($\pm$ 13.9)

Although nestling and adult phenotype data indicate that white plumage behaves as a recessive trait, the single locus hypotheses for the inheritance of plumage color presented in Table 2.4 ignore the range of blue phenotypes found in the Florida Bay population. A number of hypotheses can be constructed to explain these

intermediate plumages (e.g. incomplete dominance at a single locus or additive alleles at more than one locus). However, testing any of these hypotheses would require examining large numbers of offspring from known crosses and the ability to determine what their adult phenotype will be. Both are beyond the reach of my data.

Regardless of whether the allele conferring white plumage is dominant or recessive, it appears to be unique to the Florida Keys population. White individuals are rarely found in other North American Great Blue Heron populations and there is only one published observation of a white nestling outside south Florida (McHenry and Dyes 1983). The lack of white individuals in other Great Blue Heron populations suggests that most North American Great Blue Herons do not carry an allele conferring white plumage. This implies that there is little emigration of either white or blue individuals from the predominantly white Florida Bay population to other Great Blue Heron populations.

Inferences regarding emigration from other Great Blue Heron populations to the Florida Bay population are harder to make. Although there were fewer mixed pairs than expected from a randomly mating population, the number that I observed (17 out of 114) is not trivial. These may provide an avenue for gene flow between white and blue herons in Florida Bay or between the Florida Bay breeding population and other Great Blue Heron populations. Because there is a continuum of blue phenotypes, it was not possible to confidently identify the origin of an adult blue heron. It is, therefore, difficult to use this mate choice data to make inferences about the role of emigration from other Great Blue Heron populations to Florida Bay. I will

address this question in chapter four, by exploring patterns of genetic differentiation among six Great White and Great Blue heron populations.

CHAPTER 3. Isolation of microsatellite loci in *Ardea herodias*

INTRODUCTION

Microsatellites have become the marker of choice for evaluating patterns of genetic differentiation among populations of recently diverged taxa. Microsatellites are non-coding nuclear sequences composed of tandemly arrayed repeat units. These repeat units are short, generally 1-6 base pairs (bp) long. Microsatellites evolve through gain or loss of repeat units caused by slippage and mis-alignment during DNA replication (Schlotterer and Tautz 1992, Schlotterer and Pemberton 1994). Alleles at a locus, therefore, differ in length and are easily resolved by electrophoresis (Queller et al. 1993). Microsatellites have several advantages over other nuclear markers (Queller et al. 1993, Sunnucks 2000). They are co-dominant (heterozygotes can be distinguished from both classes of homozygotes), single-locus (a single "gene" is amplified), and presumably neutral (an assumption of many population genetic analyses). Microsatellites evolve rapidly and are particularly well suited for genetic analyses at the level of populations or individuals and, thus, provide a resolution not possible with other more slowly evolving co-dominant nuclear markers (e.g. allozymes).

The primary disadvantage of microsatellites is a technical one. Although rapid evolution makes them ideal for genetic analysis at lower taxonomic levels (species, subspecies, populations, and individuals), it also means that microsatellite loci are often species-specific and require an initial investment of time and money to develop in new species. Relative to other genetic markers, they are difficult and expensive to isolate - particularly in birds, in which microsatellites are relatively rare (Primmer et

al. 1997, Longmire et al. 1999). Traditional isolation techniques use radioactively labeled oligonucleotide probes to screen large libraries of bacterial clones (genomic DNA fragments from the target organism inserted into plasmid vectors, which are then introduced into bacterial cells and replicated along with the bacterial DNA). These techniques have met with limited success in birds (Tarr and Fleischer 1998).

Enrichment protocols employ an initial “screen” of genomic DNA fragments to increase the proportion of microsatellite repeats in the insert library prior to cloning (Armour et al. 1994, Fleischer and Loew 1995). I used an enrichment technique (Hamilton et al. 1999) to isolate 60 *Ardea herodias* microsatellite loci and used 12 of these loci to document patterns of genetic differentiation among Great White Heron and Great Blue Heron populations (Chapter 4).

METHODS

I isolated microsatellite markers using an enrichment technique that employs a subtractive hybridization to increase the proportion of microsatellite repeats in the genomic DNA insert library prior to cloning (Hamilton et al. 1999). Briefly, streptavidin-coated magnetic beads and biotinylated oligonucleotide repeats retain single-stranded genomic DNA fragments containing repeat sequences. Linker sequences, ligated to genomic DNA, provide a PCR (polymerase chain reaction) priming site (to recover double-stranded DNA) and contain restriction sites to create compatible ends for cloning. Detailed descriptions of this type of enrichment protocol can be found elsewhere (Fischer and Bachmann 1998, Hamilton et al. 1999). Except where indicated, I used the reagent concentrations and reaction conditions suggested by Hamilton et al. (1999) in the expanded protocol, referenced therein.

I used *Sau3AI*, *NheI* and *HhaI* to digest *A. herodias* genomic DNA and I conducted separate hybridization reactions for four different biotinylated oligonucleotide repeat motifs: (CA)₁₅, (TC)₁₅, (AGC)₁₀, and (CATA)₇CA. I used *NheI* to digest linker sequences and ligated the repeat enriched library into pUC19 plasmid DNA digested with *XbaI*. I transformed plasmids into *E. coli* (Life Technologies, Library Efficiency[®] DH5 α [™]) and grew the *E. coli* overnight at 37 °C on an LB agar medium with 100 mg/L ampicillin and 20 mg/L X-gal for blue/white screening of bacterial colonies (Sambrook et al. 1989). I omitted the chemiluminescent screen and used pUC19 primers to amplify *A. herodias* DNA inserts directly from bacterial colonies. Each 50 μ L reaction volume contained 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 1.5 mM MgCl₂, 0.2 mM each dNTP, 0.5 μ M each pUC19 forward and reverse primers (forward: 5'- CCC AGT CAC GAC GTT GTA AAA CG-3', reverse: 5'- AGC GGA TAA CAA TTT CAC ACA GG-3'), and 1.0 unit *Taq* polymerase. DNA was added by lightly touching a sterile toothpick to a bacterial colony and swirling the toothpick into the reaction mix. I used a Hybaid Omn-E thermal cycler for all reactions and the following thermal profile: an initial denaturing step at 95 °C for 5 min; 30 cycles of 94 °C for 60 s, 55 °C for 30 s, 72 °C for 30 s; rapid thermal ramp to 40 °C. A 5 μ l aliquot of the reaction mixture was visualized under ultraviolet light after electrophoresis through a 2% agarose gel stained with ethidium bromide. For successful reactions (those with a distinct band 300-1000 bp long), I cleaned the remaining PCR product with a QIAquick PCR Purification Kit (QIAGEN) and eluted clean product from the QIAGEN mini column with 30 μ L sterile ddH₂O. I used 2 μ L of clean product in subsequent sequencing reactions.

I used an ABI PRISM[®] Big Dye[™] Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer Applied Biosystems) to sequence PCR products. Each 10 μ L reaction volume contained 2 μ L PCR product (clean), 2 μ L BigDye ready reaction mix, and 3.2 pmol (final concentration) pUC19 primer (either forward or reverse). I used a Hybaid Omn-E thermal cycler (with hot lid) for the cycle sequencing reaction. The thermal profile for all reactions was 25 cycles of 96 °C for 30 s, 50 °C for 15 s and 60 °C for 4 min; the cycle sequencing reaction was followed by a rapid thermal ramp to 40 °C. I used the kit's ethanol/sodium acetate precipitation protocol to remove unincorporated dye terminators and sent dried precipitated sequencing products to the Louisiana State University Museum of Natural Science (LSUMNS), where the precipitated sequencing products were re-suspended in a loading buffer and electrophoresed through a polyacrylamide gel on an ABI PRISM 377. Gels were scored using Sequencher 3.1 (Gene Codes Corporation, Ann Arbor, Michigan). Output for each sequence was provided by LSUMNS in the form a text file and an electropherogram. I checked each electropherogram for accuracy of base pair assignments and repeated PCR amplification and sequencing reactions for PCR products that produced ambiguous electropherograms. In most cases, I sequenced both strands to obtain unambiguous electropherograms on both sides of the microsatellite.

I used Oligo Analyzer 2.0 (Integrated DNA Technologies, <http://www.idtdna.com>) to design PCR primers for sequences that contained a microsatellite with nine or more repeat units and sufficient flanking sequence on both sides of the microsatellite. A 19 bp M13 forward primer (5'- CAC GAC GTT GTA AAA CGA C

-3') was added to the 5' end of the forward primer of each primer pair. This primer sequence, labeled with an infrared dye (IRD), is included in PCR amplifications, where it is incorporated into the PCR product. This allows for infrared fluorescence detection. I screened for polymorphism by genotyping 30 Great Blue Herons (10 each from three populations) and 10 Great White Herons. I also attempted to amplify these microsatellites in 1-2 individuals in each of 3 closely related species - *A. alba*, *A. cinerea*, and *A. cocoi*. Each 10 μ L PCR volume contained 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.05 μ M forward and reverse primers, 0.04 μ M IRD labeled M13 primer (LI-COR), 1.0 unit *Taq* polymerase, and 20-200 ng DNA template. I used a Hybaid Omn-E thermal cycler and the following touchdown thermal profile: an initial denaturing step at 95 °C for 5 min; X cycles of 94 °C for 60 s, Y °C for 30 s, 72 °C for 30 s; rapid thermal ramp to 40 °C. X and Y equal 3 and 61, then 3 and 58, and finally 27 and $T_m - 5$ °C (see Table 3.1 for melting temperatures). PCR products were visualized on a LI-COR 4200-2 after electrophoresis through a 25 cm x 0.25 mm 6% acrylamide gel. Images were analyzed with Gene ImagIR™ software (LI-COR).

RESULTS

Sixty sequences contained microsatellites with four or more repeat units (Appendix F). I designed PCR primers for 28 loci that had nine or more repeats. Twenty-six primer pairs amplified products of the appropriate length (two failed to amplify any product). Seventeen produced PCR products that could be reliably scored (Table 3.1). Fifteen of these 17 were polymorphic in *A. herodias*. Two were apparently monomorphic in *A. herodias* (based on genotypes from 40 or more

individuals), but exhibited size variation among all the taxa scored. The remaining nine primer pairs produced ambiguous banding patterns (poor amplification, confusing stutter bands, or multiple products). These, presumably, could be improved by redesigning primers and/or optimizing PCR conditions.

DISCUSSION

Typically, birds have relatively few microsatellites (Primmer et al. 1997, Longmire et al. 1999). Isolating these markers using traditional methods has proven to be inefficient and expensive for many organisms and has met with limited success in birds (Fischer and Bachmann 1998, Tarr and Fleischer 1998). The enrichment technique (Hamilton et al. 1999) was an efficient and relatively inexpensive method for creating a library of DNA fragments from *A. herodias* enriched for microsatellites. Attempts to isolate CATA and ACG microsatellites did not produce many clones containing these repeat units. Because ACG was reported to be the most abundant microsatellite repeat in the Brown-headed Cowbird (Longmire et al. 1999), I suspect this failure is due to sub-optimal hybridization temperatures rather than a lack of these repeats in *A. herodias*. The loci reported here are the first microsatellite markers developed for any heron species. The ability to amplify polymorphic products in closely related species suggests that these markers may also be useful in other herons, particularly within the genus *Ardea*.

Table 3.1: *Ardea herodias* microsatellite loci and results of cross-species amplification in closely related taxa.

Locus	Repeat ^a	Size (bp) ^b	# of alleles ^c	n ^d	Amplified in ^e :			Primer sequences (5' to 3')	T _m (°C)
					<i>al</i>	<i>ci</i>	<i>co</i>		
Ah 205	(AC) ₁₂	256	05	282	x ^f	x	x	F: CTGTGGAAGCAAGGCTACCC R: GTCCTCATTGTAGGCTGATTCTTGG	58.9 59.7
Ah 208	(CA) ₁₀	207	01	40	x ^f	x ^f	x ^f	F: GCTAATAACACCCAGTGTGGACC R: GACCCTGTACATACACTTCTAAAACCC	59.0 58.7
Ah 209	(AC) ₁₆	214	10	270	np	np	x ^f	F: GAAACACATCAGTGCAAGAGCAG R: AGTTAAGGAACAAATGTTTGGAAGGAATG	58.1 59.3
Ah 210	(CA) ₁₁	179	02	40	x ^f	x	x	F: ACGGGAACGTTTCAAAAATTTAAGATGTG R: ACGTTTCTATGGCTCAGAAACTGG	59.0 58.7
Ah 211	(CA) ₁₃	152	10	291	x ^f	x	x	F: GCTCATCAGGAGTTGAATCTGGC R: TCTGTCATTTCAGCAATGGACC	59.2 56.3
Ah 212	(CA) ₉	173	01	247	x ^f	x ^f	x	F: TCAGGCTAACTTTGGGCAAAGC R: AGCCCACTTTCATGACTTGCAG	59.7 59.4
Ah 217	(CA) ₁₀	178	04	286	x	np	x ^f	F: GCTCAGGCTCTGCTTTGTCTAC R: CACAGATTCAAAACAAGCACCATGC	59.0 59.3
Ah 320	(AC) ₁₃	188	05	289	x	x	x	F: TTAGGAGCAAGATTTTAAAGAAGGTGC R: AAGTGCTGGGTCATACTGGAATAG	57.7 58.0
Ah 341	(AC) ₁₂	160	03	287	x ^f	x ^f	x ^f	F: GGTAATGATTCTGATTTACCACTGAGGG R: ATGTGTTATCATATCTGGTCTTCACAGC	58.9 58.7
Ah 343	(AC) ₁₇	228	09	288	x	x ^f	np	F: CATTGCTTAACTTCTGAAGAAAC R: CTTGACCCAGCATTGTGAATAAACTG	58.6 59.0
Ah 414	(AC) ₂₂	240	12	287	x	np	np	F: CATTCCAGCTGCTCTTCATTCTTG R: GGCAAAAGCAACTAGGGGC	57.9 57.7
Ah 421	(CA) ₁₅	182	05	263	x ^f	x ^f	x ^f	F: CCCGTTCCACGCTGCTC R: GCCTGCTCACCGAGTGC	59.2 59.2
Ah 517	(TC) ₁₅	176	09	277	np	np	x	F: TTTTCCATCATGCTTCCATCAATACG R: GGCACAAACCAGTAGAGCAATATAATC	57.8 57.8

Table 3.1 continued

Locus	Repeat ^a	Size (bp) ^b	# of alleles ^c	n ^d	Amplified in ^e :			Primer sequences (5' to 3')	Tm (°C)
					<i>al</i>	<i>ci</i>	<i>co</i>		
Ah 522	(TCTTC) ₂₅	260	18	37	x	x	nd	F: TTGTGGGACTAAACAGTGAAGCAG R: CAAAGCTGATTTAAAGATGTTCCATCCC	58.9 58.5
Ah 526	(TC) ₂₇	256	18	267	x	np	x	F: GAATGGGGAAGAGAACTGAAAGAGC R: CACTGCTCAGGGACTGGC	59.2 58.5
Ah 536	(AC) ₁₄	130	07	273	x ^f	x ^f	x ^f	F: CCCTGGTTTAGATCACATGATGGAG R: CTGGGCAACCTGTTCCATCT	58.6 58.4
Ah 630	(TC) ₁₂	122	04	286	x	np	x	F: TCCTCCTTCACAATGCTACTTGC R: CGGCAGGCAGTATTATTTTCAGTGG	58.7 59.6

^a sequenced clone

^b length of sequenced allele includes a 19bp M13 primer extension

^c number of alleles found in *A. herodias*

^d number of individuals genotyped

^e *al* = *A. alba* (n=1), *ci* = *A. cinerea* (n=1), *co* = *A. cocoi* (n=2), np = no product, nd = no data

^f amplified additional alleles not found in *A. herodias*

CHAPTER 4. Large and small scale geographic patterns of genetic differentiation among Great White and Great Blue heron populations

INTRODUCTION

Genetic divergence between parapatric or sympatric populations suggests the presence of significant barriers to gene flow. Genetic differentiation is a consequence of reproductive isolation. Barriers to gene exchange between populations and subsequent genetic differentiation due to natural selection, mutation, or genetic drift are the fundamental processes of speciation. Interbreeding between populations counteracts these processes. Migration (movement of individuals between populations and subsequent interbreeding) is a powerful homogenizing force, and very few migrants are required to prevent genetic divergence due to random genetic drift (Hartl and Clark 1989).

Mate choice data from Florida Bay suggest that Great White Herons and Great Blue Herons pair selectively with respect to plumage color (Chapter 2). I observed more white/white and blue/blue pairs and fewer mixed pairs than expected in a randomly mating population. This positive assortative pattern suggests that reproductive barriers exist within the Florida Bay breeding population. However, mixed pairs did occur providing an opportunity for gene flow not only between Great White and Great Blue Herons within the Florida Bay population, but also between the Florida Bay population and other Great Blue Heron populations (e.g. Great Blue Herons breeding nearby on the southern Florida peninsula). To evaluate large and small scale geographic patterns of genetic differentiation among Great White and Great Blue Heron populations, I compared allele frequencies at 12 microsatellite loci

for six *A. herodias* groups representing four Great Blue Heron subspecies - *occidentalis*, *wardi*, *herodias*, and *fannini* (Figure 4.1).

Figure 4.1: Distribution of the four North American Great Blue Heron subspecies that were sampled to evaluate geographic patterns of genetic variation at microsatellite loci

The nominate race, *A. h. herodias*, breeds throughout the mid-Atlantic states (USA) to Nova Scotia (Canada) and west to northern Montana (USA) and southern Alberta (Canada). *Ardea h. wardi* is distributed throughout the southeastern and south-central United States, including the southern Florida peninsula. *Ardea h. occidentalis* is restricted to extreme south Florida (Florida Bay and the Florida Keys).

Ardea h. fannini is narrowly distributed along the Pacific coasts of Washington (USA), British Columbia (Canada), and southeastern Alaska (USA); it is allopatric with the other three subspecies and probably rarely interbreeds with any of these. *Ardea h. herodias* and *A. h. wardi* are parapatric. The former is migratory (especially in the northern portion of its range), and many individuals have winter ranges that overlap with *A. h. wardi* and *A. h. occidentalis* (Palmer 1962, Eckert 1981). The degree to which these two subspecies interbreed has not been investigated. *Ardea h. wardi* and *A. h. occidentalis* are also parapatric. Although the intermediate Würdemann's Heron is commonly believed to be a "hybrid" between these two subspecies, the degree to which they intergrade is also unknown.

I defined six *A. herodias* groups within these for subspecies: Great Blue Herons from the Pacific Northwest (B-PNW), northern Great Blue Herons (B-N), Great Blue Herons from the southern Florida peninsula (B-FP), Great Blue Herons from Florida Bay (B-FB), Great White Herons from Florida Bay (W-FB), and Great White Herons from the outer Keys (W-OK). My primary objectives were to determine if allele frequencies differed significantly between Great White Herons and Great Blue Herons breeding in Florida Bay (W-FB and B-FB) and whether either (or both) differed from Great Blue Herons breeding on the nearby Florida peninsula (b-FP). I also wanted to compare these differences (if any) with those found among other populations of allopatric and parapatric Great Blue Heron subspecies. Finally, I looked for evidence of genetic structure within the Great White Heron population by comparing samples collected along the outer Keys (W-OK) with those collected in Florida Bay (W-FB).

METHODS

Sample Collection and Preparation

The number of individuals genotyped in each of the six *A. herodias* groups is presented in Table 4.1. *Ardea h. fannini* (B-PNW) tissues from adult Great Blue Herons collected in Washington were provided by the Burke Museum. *Ardea h. herodias* (B-N) tissues from adult Great Blue Herons collected in Minnesota, Wisconsin, and Illinois were provided by the Bell Museum of Natural History and the Field Museum of Natural History.

Table 4.1: *A. herodias* groups used to examine large scale geographic patterns of genetic differentiation at microsatellite loci and the number of individuals genotyped in each group.

Group	Subspecies	n	Comments
B-PNW	<i>fannini</i> ^a	11	Great blue heron tissues collected in Washington (B-PNW = blue, Pacific Northwest).
B-N	<i>herodias</i>	30	Great blue heron tissues collected in MN, WI and IL (B-N = blue, northern).
B-FP	<i>wardi</i>	23	Great blue heron tissues collected in Dade County, FL (B-FP = blue, Florida peninsula).
B-FB	<i>occidentalis</i>	35	Great blue heron tissues collected in Monroe County, FL (B-FB = blue, Florida Bay).
W-FB	<i>occidentalis</i>	77	Great white heron tissues collected in Monroe County, FL (W-FB = white, Florida Bay).
W-OK	<i>occidentalis</i>	37	Great white heron tissues collected in Monroe County, FL (W-OK = white, outer Keys)

^a putative subspecies based on locality data

Although the *A. h. fannini* tissues are not identified to subspecies, it is unlikely that they contain individuals from other subspecies. Some could be migrants from more northern populations (especially those collected in the fall), but these migrants (although not local breeders) would be members of the same subspecies. I collected blood or feathers from nestlings for the *A. h. wardi* (B-FP) and *A. h. occidentalis* (W-FB, B-FB, W-OK) samples. I collected *A. h. wardi* tissues in the southern portion this

subspecies' range (Water Conservation Area 3A, Dade County, FL), where individuals are unlikely to interbreed directly with *A. h. herodias*. Many adult Great Blue Herons in south Florida during winter and early spring are non-breeding winter migrants. To minimize the possibility of including migrants from northern subspecies, I collected feathers from nestlings (Marsden and May 1984). By collecting tissues from nestlings, I ensured that the B-FB and B-FP samples were representative of local breeding populations. Sample details are provided in Appendix G.

I used a sterile 1 mL syringe and 25 gauge needle (Gaunt and Oring 1997) to collect blood from the tibio-tarsal vein. Approximately 0.1 mL of blood was mixed with 1.0 mL 10% EDTA anticoagulant/preservative buffer. I allowed red blood cells (RBCs) to settle overnight, removed and discarded the plasma/EDTA supernatant, re-suspended the RBCs in 1 mL 10% EDTA, and refrigerated the samples at 4 °C. Feathers were refrigerated at 4 °C during the field season and then stored at -80 °C.

I isolated genomic DNA from each tissue sample (muscle, feather, or blood) with a DNeasy Tissue Kit (QIAGEN). For muscle samples, I used approximately 50 mg of tissue, followed the kit's extraction protocol for animal tissues, and eluted DNA from the QIAGEN mini column with 150 µL of 10 mM Tris-HCl (pH 8.3). For feathers, I cut approximately 5 mm from the root of the feather shaft, added 3 mg dithiothreitol (DTT) to the initial lysis solution, incubated this lysis solution overnight, and eluted DNA from the QIAGEN mini column with 50 µL of 10 mM Tris-HCl (pH 8.3). For blood samples I added 50-100 µL of the RBC/EDTA suspension to the initial lysis solution and eluted DNA from the QIAGEN mini-column with 50 µL of 10 mM Tris-HCl (pH 8.3).

Selection of Microsatellite Loci

I used a number of criteria to select loci for genetic analyses. First, I omitted loci with too little or too much variation. Monomorphic loci provide no information about genetic differentiation among populations. Hypervariable loci often have very low allele frequencies spread among many alleles. Thus, large sample sizes are needed to detect small differences in allele frequencies among populations. Second, tests for population differentiation assume independent loci. I tested this assumption for all pairs of variable loci with GENEPOP 3.3 - an updated version of GENEPOP 1.2 (Raymond and Rousset 1995). GENEPOP creates a contingency table of the observed genotype combinations for all pairs of loci in each population. The null hypothesis (independence of rows and columns) is that genotypes at one locus are independent from genotypes at another locus. A Markov chain method (Guo and Thompson 1992) is used to obtain an unbiased estimate of the exact type I error probability for all pairs of loci in each population (1000 batches, 10,000 iterations per batch, 10,000 dememorization steps), and Fisher's combined probability test (Sokal and Rohlf 1995) is used as a global test for each pair of loci across populations.

Third, a sex-linked locus could produce a false rejection of the null hypothesis when testing for genetic differentiation (H_0 : no difference in allele frequencies among populations) if a bias in sex ratios exists within any of the heron populations sampled. To guard against this possibility I determined the sex of a subset of *A. herodias* samples (n=201) and examined allele frequency data for evidence that any of the loci were sex-linked. If a locus is present only on the "W" chromosome, then all females (the heterogametic sex, ZW) will be homozygotes, and the locus will not amplify in

males (ZZ). If a locus is present only on the “Z” chromosome, then it will amplify in both males and females, but all females will be homozygotes.

I used a PCR-based sex identification protocol (Griffiths et al. 1996, Griffiths et al. 1998) to amplify homologous regions of two CHD (chromo-helicase-DNA-binding) genes located on the avian “Z” and “W” sex chromosomes. The CHD-Z gene occurs in both males (ZZ) and females (ZW), but the CHD-W gene is unique to females (Griffiths and Tiwari 1995, Griffiths and Korn 1997). PCR primers anneal to conserved regions and amplify across a less conserved intron. PCR products are digested with *Hae*III, which cuts a 65 bp fragment from the CHD-Z gene but does not cut the CHD-W gene (Griffiths et al. 1996). Females, therefore, have two bands and males have one band after the restriction enzyme digest (the small 65 bp fragment cut from the CHD-Z gene is usually not visible on an agarose gel but is not relevant for sex determination).

I used PCR primers P2 and P8 (Griffiths et al. 1998) to amplify homologous regions of the two CHD genes. Each 10 μ L PCR volume contained 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 1.5 mM MgCl₂, 200 μ M dNTPs, 1 μ M each P2 and P8 primer, 1.25 units *Taq* polymerase, and 20-200 ng DNA template. I used a Hybaid Omn-E thermal cycler and the thermal profile recommended by Griffiths et al. (1998). In *A. herodias*, this amplified a fragment just under 400 bp. PCR products were digested with five units *Hae*III (New England Biolabs) and a 1x final concentration of the restriction buffer supplied with the enzyme (37 °C, 1 hr). Restriction digests were visualized under ultraviolet illumination after electrophoresis through a 2% agarose

gel stained with ethidium bromide. Females had two bands (approximately 400 bp and 335 bp), and males had one band (approximately 335 bp).

Finally, many tests for population differentiation also assume within population Hardy-Weinberg (HW) equilibrium. To determine which tests for population differentiation would be appropriate (those assuming HW equilibrium or those that do not) I used TFPGA 1.3 (Miller 1997) to test for deviations from HW expectations for each locus within each of the *A. herodias* groups (H_0 : genotype frequencies do not deviate from HW expectations). Because many loci had expected genotype values less than one, I used exact tests (conventional Monte Carlo method, 20 batches, 10,000 permutations per batch, 1,000 initial dememorization steps, *a priori* $\alpha = 0.05$), which are preferred over large sample goodness of fit tests (e.g. Chi-square or G-tests) when sample sizes are small or some genotypes have low expected values (Guo and Thompson 1992).

Descriptive Statistics and Exact Tests For Population Differentiation

I calculated allele frequencies and unbiased heterozygosity estimates (Nei 1978) for each *A. herodias* group at each locus using FSTAT 2.9.1 (Goudet 2000) and TFPGA (Miller 1997) respectively. I used FSTAT to calculate pairwise F_{ST} and R_{ST} values. F_{ST} and R_{ST} (an F_{ST} analog often calculated for microsatellite data) are common descriptive statistics used to evaluate population genetic structure and are reported here to allow comparison with other studies. I used FSTAT to perform exact tests for population differentiation between all pairs of the 6 *A. herodias* groups (H_0 : No difference in allele frequencies between groups; *a priori* $\alpha = 0.05$). I chose an

analysis that does not assume HW equilibrium within groups and applied a Bonferroni correction for multiple comparisons (corrected $\alpha = 0.0033$).

RESULTS

Selection of Microsatellite Loci

I used 12 of the 17 loci (Table 3.1) for genetic analyses. I omitted Ah 208 and Ah 212 because they appeared to be monomorphic across the *A. herodias* groups being compared, and Ah 210 because it showed extremely low variation across groups. I omitted locus Ah 522 because it was too variable (I found 18 alleles in only 37 heron samples and, presumably, would have found many more). Although it would be an excellent marker for studies requiring identification of individuals (e.g. assigning paternity), this locus is composed almost entirely of rare alleles (allele frequencies generally <0.1), which makes detection of any patterns of population differentiation extremely difficult.

I tested for independence of the remaining 13 loci and rejected the null hypothesis (genotypes at one locus are independent from genotypes at another locus) in 3 of 78 pairwise tests (Appendix H). All 3 involved the same microsatellite locus - Ah 211 vs. Ah 341 ($p = 0.023$, $df = 10$), Ah 211 vs. Ah 526 ($p = 0.031$, $df = 8$), and Ah 211 vs. Ah 630 ($p = 0.007$, $df = 10$). Although I would expect, by chance, to reject approximately four of 78 tests at the 0.05 significance level, and only one of the contrasts approaches the rejection criteria if a Bonferroni correction for multiple comparisons is applied ($0.05/78 = 0.0064$), I adopted a conservative approach and omitted locus Ah 211 from tests for population differentiation. I found no evidence

that any of the loci were sex-linked. All loci amplified in males ($n = 111$) and females ($n = 110$), and heterozygous males and females were found at all loci.

I tested for deviations from HW equilibrium within each of the six *A. herodias* groups at 12 microsatellite loci. I rejected the null hypothesis in 8 of 72 tests (H_0 : observed genotype frequencies do not deviate from HW expectations, $\alpha = 0.05$). One locus in W-FB (Ah 205, $p = 0.0111$), one locus in B-FP (Ah 209, $p = 0.0097$), two loci in W-OK (Ah 517, $p = 0.0166$; Ah 536, $p = 0.0375$) and four loci in B-N (Ah 414, $p = 0.0450$; Ah 517, $p = 0.0074$; Ah 526, $p = 0.0377$; Ah 536, $p = 0.0077$) appeared to deviate from HW expectations. There was no evidence of deviation from HW expectations at any of the 12 loci in the B-FB and B-PNW groups. Only two loci (Ah 571 and Ah 536) deviated from HW expectations within more than one *A. herodias* group, suggesting that null alleles (unamplified alleles caused by point mutations in flanking sequences that prevent PCR primers from annealing) are not an issue for any of these loci. I would expect to reject, by chance, only about four of 72 tests at the 0.05 significance level. Thus, these tests provide evidence that some of the loci in some of the *A. herodias* groups (particularly the B-N group) are not in HW equilibrium.

Descriptive Statistics and Tests of Population Differentiation.

I genotyped a total of 213 *A. herodias* individuals at 12 microsatellite loci (Appendix I). Allele frequencies and sample sizes for each locus in each of the *A. herodias* groups are provided in Appendix J. In general, heterozygosity estimates (Table 4.2) were high in all six *A. herodias* groups (average unbiased heterozygosity over all loci ranged from 0.540 in the W-OK group to 0.671 in the B-FP group) and

ranged from 0 (group B-N at Ah 414) to 0.913 (group B-FP at Ah 526). F_{ST} and R_{ST} values (Table 4.3) reveal genetic structure among the six *A. herodias* groups at both large and small geographic scales. All pairwise exact tests for population differentiation (H_0 : No difference in allele frequencies between groups) were significant (Table 4.4). Allele frequencies of Great White Herons breeding in Florida Bay (W-FB) differed significantly from those of Great Blue Herons breeding in Florida Bay (B-FB). Both W-FB and B-FB differed significantly from Great White Herons breeding along the outer Keys (W-OK). All three of these groups differed significantly from Great Blue Herons breeding on the Florida peninsula (B-FP) less than 80 km north of Florida Bay. Large scale geographic structure was also evident in the B-PNW and B-N comparisons.

Table 4.2: Nei's (1978) unbiased heterozygosity estimate (H) for six *A. herodias* groups at each of 12 microsatellite loci and averaged over all loci.

Locus	B-PNW		B-N		B-FP		B-FB		W-FB		W-OK	
	H	n	H	n	H	n	H	n	H	n	H	n
Ah 205	0.44	10	0.50	30	0.58	19	0.31	35	0.34	76	0.10	37
Ah 209	0.85	10	0.62	28	0.74	20	0.73	34	0.72	71	0.66	35
Ah 217	0.37	11	0.64	30	0.67	18	0.68	35	0.60	77	0.57	35
Ah 320	0.38	11	0.54	30	0.57	21	0.67	35	0.64	77	0.70	37
Ah 341	0.37	11	0.56	30	0.47	22	0.43	35	0.36	74	0.38	37
Ah 343	0.65	11	0.83	30	0.82	22	0.76	35	0.77	77	0.64	36
Ah 414	0.77	11	0.83	27	0.77	22	0.78	35	0.78	77	0.82	37
Ah 421	0.44	10	0.00	10	0.32	20	0.03	35	0.06	77	0.16	35
Ah 517	0.78	11	0.82	30	0.82	17	0.77	34	0.78	74	0.71	35
Ah 526	0.90	09	0.90	29	0.91	17	0.86	31	0.84	70	0.77	35
Ah 536	0.71	11	0.68	30	0.78	15	0.77	32	0.74	74	0.76	37
Ah 630	0.25	11	0.47	30	0.56	19	0.43	34	0.41	77	0.20	36
All	0.57		0.62		0.67		0.60		0.58		0.54	

Abbreviations: W - Great White Heron, B - Great Blue Heron, PNW - Pacific Northwest, N - north-central United States, FP - Florida peninsula, FB - Florida Bay, OK - outer Keys, n - number of individuals genotyped

Table 4.3: Pairwise F_{ST} values across all loci (above diagonal). Pairwise R_{ST} values across all loci (below diagonal).

Population ^a	B-PNW	B-N	B-FP	B-FB	W-FB	W-OK
B-PNW		0.0736**	0.0885**	0.1231**	0.1221**	0.1861**
B-N	0.022		0.0203**	0.0547**	0.0675**	0.1108**
B-FP	0.039	0.016		0.0346**	0.0510**	0.0872**
B-FB	0.120	0.045	0.023		0.0066	0.0246**
W-FB	0.102	0.058	0.043	0.001		0.0262**
W-OK	0.161	0.083	0.059	0.030	0.027	

^a W - Great White Heron, B - Great Blue Heron, PNW - Pacific Northwest, N - north-central United States, FP - Florida peninsula, FB - Florida Bay, OK - outer Keys

** 99% CI does not include zero (confidence intervals were not calculated for R_{ST} estimates)

Table 4.4: Combined probabilities for exact tests of population differentiation (α after Bonferroni correction for multiple comparisons = 0.0033).

Population ^a	B-PNW	B-N	B-FP	B-FB	W-FB
B-N	<0.001				
B-FP	<0.001	<0.001			
B-FB	<0.001	<0.001	<0.001		
W-FB	<0.001	<0.001	<0.001	0.003	
W-OK	<0.001	<0.001	<0.001	<0.001	<0.001

^a W - Great White Heron, B - Great Blue Heron, PNW - Pacific Northwest, N - north-central United States, FP - Florida peninsula, FB - Florida Bay, OK - outer Keys

DISCUSSION

Analysis of allele frequencies at 12 microsatellite loci in six *A. herodias* groups revealed large and small scale geographic patterns of genetic differentiation (Tables 4.3 and 4.4). I found significant differences in allele frequencies among all four of the *A. herodias* subspecies compared - *A. h. fannini* (B-PNW), *A. h. herodias* (B-N), *A. h. wardi* (B-FP), and *A. h. occidentalis* (W-FB, B-FB, and W-OK). I also found evidence of genetic subdivision within the Florida Keys *A. h. occidentalis* population. Great White Herons and Great Blue Herons breeding in Florida Bay (W-FB and B-

FB) differed significantly from each other, and both differed significantly from Great White Herons breeding along the outer Keys (W-OK). Although the W-FB vs. B-FB comparison was significant (Table 4.4), F-statistics indicate that the difference between these two groups is very small relative to the other comparisons (Table 4.3).

F_{ST} and R_{ST} values, which are measures of the consequence of population subdivision, are comparable to those found among isolated populations or allopatric subspecies of other birds (Goostrey et al. 1998, Tarr et al. 1998, von Segesser et al. 1999). However, comparisons with other species are less important than the patterns of genetic differentiation exhibited among the allopatric, parapatric, and sympatric *A. herodias* groups studied. We expect to find genetic divergence between allopatric groups, but do not necessarily expect to find genetic differentiation among sympatric groups unless there are reproductive barriers between them.

Table 4.5: Nei's (1978) unbiased genetic distance ($\hat{D}$) for all pairwise comparisons of six *A. herodias* groups

Population^a	B-PNW	B-N	B-FP	B-FB	W-FB
B-N	0.134				
B-FP	0.177	0.038			
B-FB	0.230	0.096	0.060		
W-FB	0.219	0.115	0.084	0.010	
W-OK	0.331	0.184	0.138	0.032	0.036

^a W - Great White Heron, B - Great Blue Heron, PNW - Pacific Northwest, N - north-central United States, FP - Florida peninsula, FB - Florida Bay, OK - outer Keys

Table 4.5 presents a matrix of unbiased genetic distances (Nei 1978) for all pairwise comparisons of the six *A. herodias* groups. A few interesting points emerge from this distance data. First, the divergence between Great Blue Herons from the Pacific Northwest (B-PNW) and the other five groups is relatively large, genetic

distances ($\hat{D}$) range from 0.134 to 0.331. This divergence is not surprising because B-PNW is allopatric with all five groups and separated from them by the continental divide. Although Great Blue Herons are capable of long distance dispersal, opportunities for gene flow between B-PNW and the other groups are probably limited.

Second, the smallest genetic distance in Table 4.5 is for the comparison between Great White Herons and Great Blue Herons in Florida Bay ($\hat{D}_{W-FB \text{ vs. } B-FB} = 0.010$). The exact test for population differentiation showed a significant difference in allele frequencies between these two groups (Table 4.4), but this differentiation is relatively weak, as are all three comparisons within the Florida Keys breeding population ($\hat{D}_{W-FB \text{ vs. } B-FB} = 0.010$, $\hat{D}_{W-FB \text{ vs. } W-OK} = 0.036$, and $\hat{D}_{B-FB \text{ vs. } W-OK} = 0.032$). Great Blue Herons breeding in Florida Bay are more similar to Great White Herons ($\hat{D}_{B-FB \text{ vs. } W-FB} = 0.010$, $\hat{D}_{B-FB \text{ vs. } W-OK} = 0.032$) than they are to Great Blue Herons breeding on the nearby Florida peninsula ($\hat{D}_{B-FB \text{ vs. } B-FP} = 0.60$).

Third, the genetic distance between northern Great Blue Herons and Great Blue Herons from the Florida peninsula ($\hat{D}_{B-N \text{ vs. } B-FP} = 0.038$) is comparable to the distances seen among the Florida Keys groups. Even though sampling locations for B-N and B-FP were over 2,000 kilometers apart, the genetic distance between them is only slightly larger than the genetic distance observed between W-FB and W-OK ($\hat{D} = 0.036$), which are separated by less than 100 kilometers.

Finally, although the B-FP sample was collected less than 80 kilometers from Florida Bay, the genetic distance between Great Blue Herons on the Florida peninsula

and Great White Herons in Florida Bay ($\hat{D}_{\text{B-FP vs. W-FB}} = 0.084$) is greater than the B-N vs. B-FP comparison ($\hat{D} = 0.038$). In other words, Great Blue Herons separated by thousands of kilometers (B-N and B-FP) are more alike than Great White Herons and Great Blue Herons separated by less than 80 kilometers (B-FP and W-FB).

These microsatellite data suggest that there are two substantial barriers to gene flow among the six *A. herodias* groups studied. The first lies between the three *A. herodias* subspecies found in eastern North America and *A. h. fannini* in the Pacific Northwest. This divergence is not surprising, given the allopatric distribution of *A. h. fannini* (Figure 4.1) and the formidable terrain that must be negotiated before gene flow can occur between *A. h. fannini* and any of the eastern subspecies. The second occurs between the Florida Keys population and Great Blue Herons on the southern Florida peninsula. This discontinuity in microsatellite allele frequencies is striking because it suggests that barriers to gene flow among Great Blue Heron populations throughout the eastern United States (from south Florida to Minnesota, Wisconsin, and Illinois) are weaker than barriers between the Florida Bay population and Great Blue Herons breeding on the Florida peninsula less than 80 kilometers from Florida Bay.

The patterns of genetic differentiation among the Florida groups (B-FP, B-FB, W-FB, and W-OK) provide evidence that even short distance dispersal within the Great White Heron population is limited. Meyerriecks (1957) proposed that the gap between Florida Bay and the outer Keys might split south Florida's Great White Herons into two distinct breeding populations, but Robertson (1978) doubted the existence of this gap. Significant differences in allele frequencies between W-FB and W-OK support Meyerriecks' hypothesis. Patterns of genetic differentiation in

south Florida also suggest that most Great Blue Herons breeding in Florida Bay are permanent members of this population rather than immigrants from the Florida peninsula. Differences in allele frequencies between Great White and Great Blue herons breeding in Florida Bay are small. It is likely that the plumage polymorphisms found in the Florida Keys are the result of hybridization between Great White Herons and Great Blue Herons from the Florida peninsula. These microsatellite data suggest that hybridization events between Florida Bay and Florida peninsula populations may be relatively rare. Although mate choice within Florida Bay is assortative, the number of mixed pairs appears to be enough to prevent W-FB and B-FB from differentiating to the extent observed between the Florida Bay and Florida peninsula populations.

These microsatellite data provide convincing evidence that barriers to gene flow exist between Great White and Great Blue herons. Two prezygotic isolating mechanisms may be responsible for limiting gene flow between Florida Bay and the Florida peninsula. The first is ecological isolation, where taxa occur in the same geographic area but occupy different habitats. Marked habitat differences between marine environments in the Florida Keys and freshwater wetlands on the southern Florida peninsula may inhibit recruitment in either direction. The second mechanism that may limit gene flow between Florida Bay and the Florida peninsula is temporal isolation. The Florida Keys population breeds throughout the year, but peak breeding activity coincides with south Florida's dry season (October through April). Great Blue Herons on the Florida peninsula generally begin breeding later, in February or March. This difference in the timing of breeding may limit opportunities for hybridization between Florida Bay and Florida peninsula populations.

SUMMARY AND CONCLUSIONS

Resolving the debate surrounding the Great White Heron's taxonomic status depends on whether reproductive barriers exist between Great White Heron and Great Blue Heron populations. My data from Florida Bay do not support the hypothesis that sympatric white and blue herons pair randomly with respect to plumage color. The positive assortative pattern suggests that prezygotic reproductive barriers exist between Great White and Great Blue herons within the Florida Keys breeding population. However, reproductive isolation appears to be incomplete. The putative hybrid, Würdemann's Heron, produces viable offspring and although the pattern of mate choice is assortative, my data suggest that mixed pairs occur at about half the rate expected from a randomly mating population rather than the 10-fold reduction estimated by Robertson (1978).

Microsatellite data provide evidence that gene flow between Florida Bay and the Florida peninsula is limited. There are significant differences in allele frequencies among groups in the Florida Keys, but these differences are small compared to the differences observed between the Florida Keys population and Great Blue Herons breeding on the nearby Florida peninsula. The observed patterns of genetic differentiation suggest that most Great Blue Herons in Florida Bay are permanent residents rather than migrants from the Florida peninsula, and that immigration into the Florida Bay population may be a relatively rare event.

Although Great White Herons are larger than Great Blue Herons on the Florida peninsula (Mayr 1956, Zachow 1983), I found no size differences between Great White Herons and intermediate Würdemann's Herons. This is consistent with the

microsatellite data, which show that Great Blue Herons in Florida Bay are more similar to Great White Herons than to Great Blue Herons from the nearby Florida peninsula. Although Great White and Great Blue Herons from the Florida Keys (W-FK and B-FK respectively) did not differ significantly in size there was a pattern in mean values. $\bar{X}_{B-FK}$ was always intermediate between $\bar{X}_{W-FK}$ and $\bar{X}_{B-N}$ (northern Great Blue Herons) regardless of whether a variable was larger in northern Great Blue Herons (longest occipital plume) or in Great White Herons (culmen, depth of bill, tarsus, and middle toe). This is consistent with the hypothesis of a hybrid origin for Great Blue Herons in the Florida Keys breeding population.

The absence of white individuals in other North American Great Blue Heron populations and the wide range of blue phenotypes in the Florida Keys population are more consistent with a hypothesis that south Florida is a contact zone between two previously isolated taxa rather than a hypothesis that south Florida contains a truly dichromatic subspecies of the Great Blue Heron (Mayr 1956, Mock 1978, Lazell 1989). The Great White Heron probably diverged from the Great Blue Heron in isolation during a Pleistocene interglacial. Given the Great White Heron's present day distribution, it is likely that this isolation occurred somewhere in the Caribbean. About 100,000 years ago, sea level in Florida was approximately eight meters higher than today (Shinn 1988). The Florida Keys were under water and the tip of the submerged Florida peninsula was about 200 kilometers north of its current position. Cuba's present day shoreline is another 150 kilometers from Key West. Approximately 350-450 kilometers of open water is a significant barrier to dispersal, especially given the contemporary low levels of genetic differentiation between Great

White Herons in Florida Bay and those along the outer Keys. With the advent of the Wisconsin Glaciation sea level subsided and fell to its lowest level in recent geological history (approximately 100 meters lower than it is today) by about 15-20,000 years ago. As sea level subsided, movement of North American Great Blue Herons onto the emerging Florida peninsula and westward expansion of the Great White Heron into the Florida Keys could have produced a contact zone where these two taxa currently interbreed.

I believe most Great Blue Herons breeding in Florida Bay today are the result of hybridization between Great White Herons and Great Blue Herons from the Florida peninsula, and that these hybridizations are relatively rare. Once in the Florida Keys population, these blue herons and their offspring can (and do) interbreed with Great White Herons, but even then mate choice is assortative within the Florida Keys population. My morphological, behavioral and genetic data show that the Florida Keys population is distinct from the Florida peninsula Great Blue Heron population, even though their ranges overlap during at least part of the year (some Great White Herons migrate to the southern Florida peninsula during summer). Although reproductive barriers are not complete (hybridization between taxa does occur), I believe the Great White Heron is a good biological species and that a review of its taxonomic status is merited. Regardless of what we ultimately call the Great White Heron, species or subspecies, it should be viewed (and managed) as a small isolated population.

Small populations are more likely to go extinct than large populations (Goodman 1987). Wading bird populations in the Everglades watershed are estimated

to be less than one fifth of their abundance in the 1930's (when populations were still recovering after cessation of the plume trade), and conservative figures estimate the decline in the total number of nesting attempts by all species of wading birds combined to be greater than 90% since the 1940's (Frederick and Spalding 1994, Ogden 1994). These declines have been linked to habitat loss and deterioration resulting from human activities. Given the continued threats to south Florida's ecosystems from human population growth and expanding agriculture, the Great White Heron population will almost certainly face serious challenges to its survival in the future. The scientific community continues to debate the relative importance of genetic and demographic approaches to conservation (Caro and Laurenson 1994, Merola 1994, Schemske et al. 1994); both are critical concerns for small isolated populations. Although microsatellite data show gradual declines in heterozygosity as you move from the Florida peninsula to the outer Keys (Table 4.2), heterozygosity at microsatellite loci is still high within the Great White Heron population, and loss of genetic variation does not appear to be an immediate concern.

However, demographic issues may need to become a focus of future research. Estimates of juvenile survival and nest success for Great White Herons in Florida Bay (Powell 1983, Powell and Powell 1986, Powell and Bjork 1990) are lower than those reported for other Great Blue Heron populations (Henny and Bethers 1971, Forbes et al. 1985). Although the Great White Heron population appears to be stable (Powell et al. 1989, Powell and Bjork 1996), it is not immediately apparent, given Powell's demographic estimates, how it replaces itself. Recruitment from other great blue heron populations seems unlikely and my genetic data suggest that this is not a factor

in maintaining the great white heron population. Whatever the explanation, (published estimates of survival and nest success may be low, birds may rear more than one clutch per year, some portions of the great white heron breeding population may be more productive than others, etc.), effective conservation planning will require a better understanding of this population's demographics.

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APPENDIX A: Morphological variables reported in previous studies of Great White and Great Blue herons

The following table contains a list of morphological variables for which measurements from museum skins^a of adult Great White Herons and intermediate Würdemann's Herons of known sex have been published.

Group	ID Number	Sex	Wing chord	Tail	Culmen	Depth of Bill	Tibia	Tarsus	Middle toe	Occipital plume	Citation ^b
Intermediate	USNM 8690	M	x	x	x	x	x	x	x	x	R1-2, H
	USNM 110210	M	x	x	x	x		x	x	x	R2, H
	CBC 8009	M	x	x	x	x		x	x		R2
	CBC 8011	M	x	x	x	x		x	x		R2
	CMNH 1767	M	x	x	x	x		x		x	H
	CMNH 1405	M	x	x	x	x		x		x	H
	CMNH 1408	M	x	x	x	x		x		x	H
	USNM 110211	F	x	x	x	x		x	x	x	R2, H
	CBC 8010	F	x	x	x	x		x	x		R2
	CBC 8012	F	x	x	x	x		x	x		R2
	CMNH 1765	F	x	x	x	x		x		x	H
	CMNH 1409	F	x	x	x	x		x		x	H
	CMNH 1410	F	x	x	x	x		x		x	H
	CMNH 1407	F	x	x	x	x		x		x	H
White	MCZ 219809	M								x	H
	MCZ 246697	M								x	H
	MCZ 246698	M								x	H
	MCZ 246699	M								x	H
	MCZ 207887	M								x	H
	MCZ 229215	M								x	H
	MCZ 246715	M								x	H
	USNM 110675	M								x	H
	USNM 6540	M								x	H
	USNM 110695	M								x	H
	CMNH 1395	M								x	H
	MCZ 246700	F								x	H
	MCZ 219810	F								x	H
	MCZ 42534	F								x	H
	Sanford Coll.	F								x	H
	USNM 269254	F								x	H
	USNM 110696	F								x	H
	CM 94832	F								x	H
	CM 94889	F								x	H
	CMNH 1397	F								x	H
	CMNH 1393	F								x	H
	CMNH 1394	F								x	H

^a Zacow (1983) measured Great White Heron skeletons

^b H = Holt 1928, R1 = Ridgway 1878, R2 = Ridgway 1887

APPENDIX B: Raw data (in millimeters) for seven morphological variables measured from museum skins of Great White and Great Blue herons.

Museum ID # ^a	Sex	Age ^b	Group ^c	Culmen	Depth of Bill	Tarsus	Middle Toe	Wing Chord	Tail	Longest Occipital Plume
FMNH 356847	M	A	N	144.8	31.5	188.0	113.8	485.5	184.5	218
FMNH 33765	M	A	N	153.5	30.5	177.0	111.3	476.0	185.0	178
FMNH 128623	M	A	N	143.8	30.5	186.8	104.0	489.0	175.3	187
CMNH 26339	M	A	N	141.0	28.8	182.5	114.5	500.0	175.0	198
CMNH 26340	M	A	N	148.5	31.3	186.8	115.5	488.5	179.5	188
CMNH 26341	M	A	N	138.8	30.8	177.8	103.3	486.0	184.5	200
CMNH 26342	M	A	N	146.0	31.3	190.5	112.3	504.0	182.8	204
USNM 422596	M	A	N	145.8	28.5	175.5	102.0	496.5	181.0	168
CM F265	F	A	P			197.8	99.3	483.5		94
MCZ 82593	F	A	P	140.5	29.3	189.0	101.0	473.5	176.0	63
USNM 298360	F	A	P	133.5	31.3	191.0	104.8	489.5	179.3	115
CM F287	M	A	P			212.8	125.8	518.0	202.3	102
USNM 260213	M	A	P	159.3	29.5	189.0	114.0	491.5	179.5	
USNM 287203	M	A	P	151.0	33.0	205.5	118.8	503.0	180.0	61
USNM 78430	U	A	P	156.8	31.5	203.3	116.0	500.5	194.0	68
CM F283	M	J	P			203.3	115.8	483.5	162.8	57
MCZ 229222	M	J	P	154.3	29.5	191.5	107.5	483.5	185.5	94
USNM 255802	U	J	P	138.0		180.8	101.3	489.0	181.0	
FMNH 370621	F	A	I	142.5	30.0	184.3	106.5	465.0	173.8	57
CMNH 11138	F	A	I	141.3	31.0	186.3	100.5	474.5	173.3	108
CMNH 11132	F	A	I	147.5	28.5	192.8	103.0	475.0	168.5	143

APPENDIX B continued

Museum ID # ^a	Sex	Age ^b	Group ^c	Culmen	Depth of Bill	Tarsus	Middle Toe	Wing Chord	Tail	Longest Occipital Plume
CMNH 11139	F	A	I	143.3	29.0	183.0	106.8	451.0	162.8	119
MCZ 246710	F	A	I	148.0	29.3	176.8	101.5	447.5	172.0	151
MCZ 246712	F	A	I	147.5	28.8	182.0	105.8	460.0	177.3	143
MCZ 48302	F	A	I	146.8	28.8	185.5	110.0	470.0	169.0	51
MCZ 246708	F	A	I	153.3	30.8	189.8	110.0	469.5	176.8	147
MCZ 246709	F	A	I	145.0	29.8	187.8	98.0	461.5	167.0	138
MCZ 251272	F	A	I	147.3	31.8	188.8	104.8	467.5	173.0	46
USNM 110211	F	A	I	163.8	35.8	198.0	106.0	484.0	170.0	142
LSUMZ 155814	F	A	I	154.5	29.3	198.2	113.5		184.3	
MCZ 246713	F	J	I	150.0	29.3	191.8	107.8	460.0	171.3	59
MCZ 246711	F	J	I	145.3	31.3	193.3	100.5	459.0	164.0	38
MCZ 246707	F	J	I	163.5	31.5	208.0	114.8	473.5	170.0	74
MCZ 246706	M	A	I	153.0	32.5	189.0	111.0	494.5	181.3	170
MCZ 246705	M	A	I	153.3	31.5	186.5	113.5	473.0	172.3	148
MCZ 246704	M	A	I	157.0	31.0	203.8	113.3	474.0	160.5	161
MCZ 246702	M	A	I	158.0	33.0	200.3	116.0	495.5	177.0	98
MCZ 246701	M	A	I	158.8	33.8	210.3	118.5	474.5	169.3	78
MCZ 101086	M	A	I	160.0	33.8	211.8	116.8	505.5	188.0	69
MCZ 246703	M	A	I	167.8	32.0	209.0	113.0	497.0	183.0	179
FMNH 370620	M	A	I	167.5	32.7	208.8	115.5	495.0	184.8	128
FMNH 385671	M	A	I	156.8	31.5	205.5	116.5	481.0	173.3	86
FMNH 360099	M	A	I	161.0	31.3	188.0	114.5	494.0	187.0	101
FMNH 33767	M	A	I	166.5	32.8	220.3	125.3	500.5	187.3	75
USNM 332552	M	A	I	139.8	29.0	198.5	111.8	486.0	181.5	90

APPENDIX B continued

Museum ID # ^a	Sex	Age ^b	Group ^c	Culmen	Depth of Bill	Tarsus	Middle Toe	Wing Chord	Tail	Longest Occipital Plume
USNM 110210	M	A	I	153.0	34.0	193.5	107.5	472.5	174.3	118
USNM 8690	M	A	I	162.3	32.5	201.3	112.8	520.0	177.3	86
FMNH 385901	F	A	W	134.0	28.0	179.8	105.8	466.0	184.3	73
FMNH 385899	F	A	W	134.0	28.0	188.8	98.8	444.5	169.8	56
FMNH 128640	F	A	W	167.3	34.0	222.0	117.8	501.5	176.8	102
FMNH 128639	F	A	W	147.5	29.5	176.5	106.8	457.5	172.0	72
CM 94832	F	A	W	148.8	29.0	190.5	104.0	465.5	170.5	59
CM 111471	F	A	W	148.0	29.8	188.8	105.8	452.0	164.8	63
CMNH 11147	F	A	W	145.8	30.0	191.0	110.3	460.5	179.0	99
CMNH 11145	F	A	W	150.0	30.0	200.3	110.8	463.5	164.0	73
MCZ 246700	F	A	W	152.8	30.0	184.8	102.3	461.0	178.0	136
MCZ 219810	F	A	W	166.5	33.3	209.5	115.5	484.0	174.0	125
MCZ 42534	F	A	W	152.3	29.3	189.5	101.5	462.0	172.5	60
ANS 160272	F	A	W	145.0	29.5	184.0	99.3	444.5	159.8	68
USNM 400034	F	A	W	128.3	29.0	160.3	106.3	466.5	180.3	86
USNM 110696	F	A	W	171.5	34.8	229.3	119.5	519.5	200.0	67
USNM 302036	F	A	W	153.3	29.0	204.5	110.5	495.5	167.8	
USNM 527757	F	A	W	141.3	30.0	167.8	103.8	497.0	174.3	84
LSUMZ 68303	F	A	W	148.5	32.0	188.7	111.5		175.7	
FMNH 370624	F	J	W	146.3	29.3	194.5	102.0	461.0	169.3	53
FMNH 385900	F	J	W	145.5	28.8	195.5	109.5	458.5	164.8	63
MCZ 327571	F	J	W	149.0	34.3	192.3	105.8	476.0	175.5	60
LSUMZ 155817	F	J	W	145.3	32.0	224.5	121.8		162.5	
LSUMZ 155815	F	J	W	144.2	27.7	196.2	103.8		169.5	

APPENDIX B continued

Museum ID # ^a	Sex	Age ^b	Group ^c	Culmen	Depth of Bill	Tarsus	Middle Toe	Wing Chord	Tail	Longest Occipital Plume
MCZ 246698	M	A	W	162.8	33.0	215.0	108.8	474.0	172.8	153
MCZ 229215	M	A	W	160.0	35.3	199.5	113.8	503.5	187.0	76
MCZ 246697	M	A	W	157.8	32.5	195.3	112.5	488.5	172.0	73
MCZ 219809	M	A	W	161.8	33.5	190.5	115.0	493.5	182.5	171
MCZ 246715	M	A	W	167.3	33.3	191.5	117.5	480.5	186.3	184
MCZ 101085	M	A	W	162.8	32.3	226.3	121.3	511.0	179.8	38
MCZ 207887	M	A	W	160.5	32.3	199.5	103.0	481.5	173.0	146
MCZ 301205	M	A	W	150.8	32.0	191.3	105.5	482.0	188.5	89
MCZ 301207	M	A	W	160.3	31.8	202.5	111.0	498.0	178.0	115
MCZ 186366	M	A	W	160.8	33.0	206.8	122.5	502.5	184.5	55
MCZ 251273	M	A	W	162.5	34.0	224.5	124.3	485.5	173.3	75
FMNH 385669	M	A	W	160.5	31.0	205.3	115.0	504.5	184.0	170
CM F262	M	A	W			219.0	122.3	482.0	193.0	64
CM F263	M	A	W			214.3	118.8	492.0	183.0	146
FMNH 111914	M	A	W	152.0	29.5	191.8	115.8	469.0	173.0	53
FMNH 128638	M	A	W	161.5	32.0	199.0	121.3	492.5	192.0	94
CMNH 11148	M	A	W	159.3	32.3	198.0	116.5	484.0	181.3	37
USNM 110695	M	A	W	162.5	32.5	226.0	126.5	513.0	187.7	75
USNM 110675	M	A	W	171.5	32.8	218.3	120.8	504.0	179.8	81
USNM 89896	M	A	W	162.3	34.0	199.0	122.5	465.0	186.8	69
LSUMZ 136102	M	A	W	152.8	31.3	204.5	118.3		174.3	
LSUMZ 136101	M	A	W	158.3	31.0	223.0	123.5		184.3	
LSUMZ 155818	M	A	W	156.0	34.0	209.3	115.0		178.7	
LSUMZ 155816	M	A	W	161.3	33.0	212.6	115.8		183.7	

APPENDIX B continued

Museum ID # ^a	Sex	Age ^b	Group ^c	Culmen	Depth of Bill	Tarsus	Middle Toe	Wing Chord	Tail	Longest Occipital Plume
FMNH 370867	M	J	W	146.8	30.3	195.0	109.5	475.0	169.8	51
FMNH 370622	M	J	W	158.0	31.8	211.5	112.0	475.0	176.8	48
CM F260	M	J	W			226.5	122.8	499.0	185.8	62
MCZ 246699	M	J	W	160.5	32.3	219.0	123.5	472.5	172.5	59
MCZ 251274	M	J	W	144.0	33.5	208.0	118.0	486.0	173.3	59
FMNH 33769	U	A	W	162.3	31.0	190.5	112.8	477.5	175.8	122
MCZ 46874	U	A	W	160.8	32.5	199.0	111.2	480.0	182.8	69
ANS 48018	U	J	W	164.5	32.3	214.3	114.8	497.5	181.5	52

^a ANS = Academy of Natural Sciences (Philadelphia), CM = Carnegie Museum (Pittsburgh), CMNH = Cleveland Museum of Natural History, FMNH = Field Museum of Natural History (Chicago), LSUMZ = Louisiana State University Museum of Natural Science (Baton Rouge), MCZ = Museum of Comparative Zoology (Harvard University), USNM = United State National Museum (Washington, DC)

^b A = adult, J = juvenile

^c N = Great Blue Heron collected from northeastern and north-central United States, I = intermediate Würdemann's Heron, W = Great White Heron

APPENDIX C: Flow charts of statistical procedures used for morphometric analyses of adult male and female Great White Herons and Great Blue Herons

Statistical procedures used for morphometric analysis of adult male Great White and Great Blue herons

^a W-test for normality (Shapiro and Wilk 1965)

^b Bartlett's test for homogeneity of variances (Sokal and Rohlf 1995)

^c Kruskal Wallis H test (Sokal and Rohlf 1995)

^d One-way ANOVA (Sokal and Rohlf 1995)

B-N = Great Blue Herons from northeast and north-central United States

B-FK = Great Blue Herons from the Florida Keys breeding population

W-FK = Great White Herons from the Florida Keys breeding population

APPENDIX C continued

Statistical procedures used for morphometric analysis of adult female Great White and Great Blue herons

^a W-test for normality (Shapiro and Wilk 1965)

^b F-test (Sokal and Rohlf 1995)

^c Mann-Whitney U-test (Sokal and Rohlf 1995)

^d t-test assuming equal variance (Ott 1993)

^e t'-test assuming unequal variances (Ott 1993)

B-FK = Great Blue Herons from the Florida Keys breeding population

W-FK = Great White Herons from the Florida Keys breeding population

APPENDIX D: Tests of ANOVA assumptions (normality and homogeneity of variance) for seven morphological variables measured from museum skins of adult male herons.

Variable	Group ^a	Normal distribution ^b			Homogeneity of σ^2 ^c		
		W	n	p < W	X ²	df	p > X ²
Culmen	B-N	0.9697	08	0.8927	3.99	2	0.1362
	B-FK	0.9118	14	0.1652			
	W-FK	0.9226	22	0.0840			
Depth of Bill	B-N	0.8384	08	0.0745	0.23	2	0.8922
	B-FK	0.9300	14	0.2963			
	W-FK	0.9785	22	0.8760			
Tarsus	B-N	0.9018	08	0.3044	4.12	2	0.1273
	B-FK	0.9626	14	0.7227			
	W-FK	0.9286	24	0.0925			
Middle Toe	B-N	0.8472	08	0.0914	1.96	2	0.3759
	B-FK	0.9232	14	0.2385			
	W-FK	0.9573	24	0.3940			
Wing	B-N	0.9604	08	0.8137	1.64	2	0.4395
	B-FK	0.9233	14	0.2397			
	W-FK	0.9690	20	0.7255			
Tail	B-N	0.8637	08	0.1332	3.40	2	0.1827
	B-FK	0.9399	14	0.3996			
	W-FK	0.9452	24	0.2190			
Occipital plume	B-N	0.9869	08	0.9857	7.93	2	0.0190*
	B-FK	0.8987	14	0.1073			
	W-FK	0.8957	20	0.0348*			

^a B-N = Great Blue Herons collected in northeastern and north-central United States, B-FK = Würdemann's Herons collected in the Florida Keys (Monroe County, FL), W-FK = Great White Herons collected in the Florida Keys (Monroe County, FL).

^b Test statistic (W), n, and p-value of the W test for normality (Shapiro and Wilk 1965). H_0 : observations within a group are normally distributed.

^c Test statistic (X^2), degrees of freedom (df), and p-value of Bartlett's test for homogeneity of variances (Sokal and Rohlf 1995). $H_0: \sigma^2_{B-N} = \sigma^2_{B-FK} = \sigma^2_{W-FK}$.

APPENDIX E: Tests of t-test assumptions (normality and homogeneity of variance) for seven morphological variables measured from museum skins of adult female herons.

Variable	Group ^a	Normal distribution ^b			Homogeneity of s^2 ^c		
		W	n	p < W	F	df	p > F
Culmen	B-FK	0.8629	12	0.0502	3.49	16, 11	0.0409 [*]
	W-FK	0.9423	17	0.3467			
Depth of Bill	B-FK	0.7618	12	0.0027 ^{**}	1.04	11, 16	0.9194
	W-FK	0.8310	17	0.0049 ^{**}			
Tarsus	B-FK	0.9614	12	0.7479	7.73	16, 11	0.0015 ^{**}
	W-FK	0.9498	17	0.4482			
Middle Toe	B-FK	0.9816	12	0.9677	1.95	16, 11	0.2659
	W-FK	0.9549	16	0.5284			
Wing	B-FK	0.9741	11	0.9135	4.18	15, 10	0.0275 [*]
	W-FK	0.8935	16	0.0641			
Tail	B-FK	0.9700	12	0.8607	2.68	16, 11	0.1021
	W-FK	0.9216	17	0.1601			
Occipital plume	B-FK	0.7871	11	0.0074 ^{**}	2097	10, 14	0.0524
	W-FK	0.8664	15	0.0298 [*]			

^a B-FK = Würdemann's Herons collected in the Florida Keys (Monroe County, FL), W-FK = Great White Herons collected in the Florida Keys (Monroe County, FL).

^b Test statistic (W), n, and p-value of the W test for normality (Shapiro and Wilk 1965). H_0 : observations within a group are normally distributed.

^c Test statistic (F), degrees of freedom (df), and p-value for test of homogeneity of variances (Sokal and Rohlf 1995). H_0 : variances (s^2) among groups are equal.

APPENDIX F: *A. herodias* microsatellite sequences

The following tables contain clone sequences of 60 *A. herodias* microsatellites and their flanking regions. Sequences are reported 5' to 3'. Microsatellite sequences are underlined and, where appropriate, primer sequences are indicated with bold face type.

01. Ah 004: (CA)₁₃TTA(CA)₂AG(CA)₇

GAACCACAGAATGGCTGAGATTGGAAGGGACCTGTCCGGCCTCCCGTGCT
CAAGCAGGGCCACCCACAGCTGCTTGTCCAGGACCAAGTCCAGTTGGTTT
TTGAATATCTCCAAGGAGGGAGACTCCACAGCCTCTGTGGGCAACCTGTA
CCTGTGCTCAGGAACCCTTGCAGTAACAAAGTGTTTCCTGATGTTTCAGAG
GGAACCTCCTGTCTTTCAGT**TTGTGCCCATTCGCTCTGGT**CCTGTCACTG
GGCACCCTGGAAAGAGCCTGGCTCCATCCGCTTTGCAACCTCCCTTCAT
GTGTATGTACACACACACACACACACACACACATTACACAAGCACACA
CACACACATAAGATGCCCTCTGAGGCTTCTCTTCTCTCATCTGAACAGTC
CCAGCTCTCTCAGCCTCTCCTCATAGGAGAGATGCTCCAGTCCCTTAATC
ATCTTTATGTCCCTTTGCTGGACTCTCTCCAGATTGC**CTGCATCTCCCTT**
GTACTGAGGAGCCCAGAACTGGACACAGTACTCCAGGTGTGGCTTCACCA
GTGTATAATAGAGGGGAAG

02. Ah 104: (TC)₉

GATCAGTAGCATGTTTTAAGATATGCTCTACTTTTTTTTTTTATTTTATTG
CTGTGGGACATCTCTCTCTCTCTCTCAATATTTCTTAAATGACTGTGG
TTGCTACAGCAACCTTAGGCCCTGCAGGATGTTGCTGCAGGAGGTATCT
GATACGGCAGAGGCTCCATGCAGCTCAGCTCACTCTTGTGCACAGAACTC
ANGGTCATCCTCGGCACCTCACCTCCTCTGTTGGGGCCAACTCCTTCAGC
AGCCACGCTCTGCCCCCTCCTA

03. Ah 201: (CA)₄CCCCACTA(CA)₃(AC)₂G(CA)₅

ACATACATGTAAGTCATCTCATCAAACCTCAGATACACAATCCCATTGTGG
CTGCTTTTGTCTCTATATGCCATTTAAGTGGCCAAAGGAACGTGCATCTG
TGGCCAGGGATGTTCTTGGTGTTTATGACTTAGATTTCAATAGTGCTACC
CAACATTCACAAGAAAATGTAACGAAAAAAGTTGGAGCAATCACTTGTTA
GATGAGAGCATGTTATCGTTTCCTACACAAAGTAGCAGATTTCTACAGTG
AGCCGTATCTTTTAAAAAACACACACACCCCCCACTACACACAACACGCA
CACACACAAGCAAAAGCCTCTTTTCTATTTCTGGGAGGTTTCTCTGTGGC
TTCAAGACACTGCCACAAAGGTAAAGGAGGTTATGAAAAAGATAAAAGAA
AAGAAAATGGATTTCTTTTTTGCAAGTATCTCATCCTTCTACTACTCATA
ATAGTATTCATGCCCGAGTCCCTGAACAGGGAAA

APPENDIX F continued

04. Ah 202: (CA)₁₀

AAAAGAGAAGCTGCGGTGAGAAAATTGGTCAGCAGTGGGTGCAGAGTTCT
GAGAGGAGCCTCATA**CGTGACCCTGTCTCTTTCCCT**CTTTCTCACACACA
CACACACACACAAAACCTGCGTCATCTAACAGAGGCATGTGGTGGAGAATT
TCTTGAAG**GGGGTAACACAGTGTTTTTGCC**CAGAATAATTTAAAGCATAACT
GAAAA

05. Ah 203: (CA)₁₁

GTGCGTAGGAGTGGGAATGGGAATGGCTTAG**GGATTGGGAGTAGGAGCGG**
GAGCAGGAAGAGCCTGGTCTTGAATTGGAGTAGGGATAGGAACTTCTAGG
GGAAGATGAAAAAGACATAAACACACACACACACACACAAGTTAAC
TCAAAACAGCCACTCTCCCCAGCTTTTCCCCCAAATTTGATTGGTTTTTT
CCTCTCCAAATATGCTAATGTCAAAGCTTCTCTTAGCTGCTGAGATAACG
CTACTGCACAC**GGGAGAACCTGATAACGTGGAAGA**

06. Ah 204: (CA)₉

TGCAGGGGTTTAGTTTCAGGCTCTAG**CCAGCAGCAGAAAAACAAATCTCC**
AGAGTTTTTAATTCTCTCATGAGCCAACAGTCCTCTATATTGATGCGCACA
CACACACACACACAGAGGGAGCATAAAAATAGCCTCAAGCACTTCTGAAC
CTGTGACTCTTGCCAGCTTGACCACACTCTCT**GGGTAGTCCAGGAATTCA**
GACAAAACATCAATCAGAGGACCCAGCTGTACTGGAAT

07. Ah 205: (AC)₁₂

TCCTGTCCAAATAGATGCTTTCTCAATTAGGCGTATCACCTGATATTGCT
GCACATCTTTGAAGCTGACTCATGTTGAGATACCTTGAAGTGAAAGTTTT
AGGGCAGGTAT**GTCCTCATTGTAGGCTGATTCTTGGT**GATTTTGGCTACT
ACTGACTGGTTGGTAGTTGAGTACGAAAAATAATGGAATTTCAAACCTTA
CTACTGTACCATGCTGCTGTCTCCTTAATTCAAGGCATCCTGGCTGACAG
AATATGTAGCGGGTACACACACACACACACACACACATTACAAATATA
TGATTCTTAAGATATGTAAGTATATGTAG**GGGTAGCCTTGCTTCCACAGCA**
GGAACCTTGCTACAGAAGC

08. Ah 206: (CA)₆

TCATCCTTAGTACAAACACCTTTAAAGCAAAATAAAAAACACCCCTGTGCA
ACTCTCCTTTTCCATTACCCTGACATTTTGCAGAAAAAAGAATGCAGCA
GTTAGAAGTATCACACACACACATCCCATGCTCTGGACATCTAGCATGTA
TCCAGATGTTTGCCAAGAGATTATTTCAAATCAGGCTTGTGGAAACTAT
GTGGCCAGGGTGCTGTGACTGTTTATACACACCCAGCTACGTGCACAAGT
TTCCTCCTGGGTTTGTGTGCCTGTAAAGGACTCCTGGAACAGGCTATTT
GTATCCTTTGGCCGCAGGGAACAAGTAACTGGAAAGGGTGG

APPENDIX F continued

09. Ah 207: (AC)₁₀

AAGAGTACACACACACACACACACGACACAGAGTGACAATGACTCAGCT
GAGAGAGCAATCTAATTTTCCTTATTTAAAAAGTCATTTATTTTGCATTA
GGTTCAACTTTCCCCTTATCATAAATGGAAAAAATCAGTTTAAGCCAG
ATGATACCCGTTTAGTTACTTTGGTCACTAAATTTATCCCTGAAGCTTCT
TTATTTATAATCTTAAATNNGGGTAGCNCAGCATTCTCTTGAGAACAAAA
TGGTAGTCAAGGAGAGAAT

10. Ah 208: (CA)₁₀

TATTGAGAAATTCCTTTTCCTTGCAATACATTTCTTCTTCCCTTACTCTT
GACCCTGTACATACACTTCTAAAACCCTTTAACCATATAACTTTTTTTCA
CACACACACACACACACTTACACATATATGTGTGCACATGTATATATA
CATACAGGTATATATTTCAAGGGTCTTTAAACAGCAGTAATATGCAATTT
GTTTAGTCCTCATGT**GGTCCACACTGGGTGTTATTAGCC**CTGGCATGTA
CTGAAATCAGCATTCGTGATTGAGGAACACCTGATATTTGTGAAACTCCC
CAAACAACTTTGCATCTCTGATATGTTTATTCTGTACTTCCTAATTCAG
GGAAGG

11. Ah 209: (AC)₁₆

ACCTTAAGAAAGAAAGATGAATAAAAAATAAACATAATTCTTATAAATTAT
TCCCTTCTTCCACACAGATTTATTACATGTAGTAGCATAGCTTTGAAG**AG**
TTAAGGAACAAATGTTTGGAGGAATGTTTGTA AAAAGGCCGCTAAGGAA
AACAATACCTACACACACACACACACACACACACACACACACAAAATCCT
GCTGTCAGTTAAACCTGTACAAATAGAGCTGAGCACGCAACAAAAGAAA
CTGAATTCAGGCAAAGATG**ACTGCTCTTGCACTGATGTGTTTC**GGCTTAG
GCTATTTCTTGAAATTTCTACTGTTTTAATGAGCTTAAGCTAATCTTTT
AGCTTTTCCTGGTCATGAAGC

12. Ah 210: (CA)₁₁

GATT**CACGGGAACGTTTCAAAAATTTAAGATGTG**AAAGGGAATTCTCAAT
TATTTGCACACACACACACACACACACAAAATCACAAATGAAGGAAAGAA
GAAAGATACATTGAATAAATAGCTCATTGTAATTTATTTTT**CCAGTTTCT**
GAGCCATAGAAACGTGCTTGTCATTAAAGTGACTTTTGGAGATACTTGTAT
TTTCTTACCTTGGATACAGCTTAGATGGATGAGCTACCATTGCTCTCCAT
GCTTATCAAATTAGGAGAATGCATTTCTAAATTCAATTACATCCTCAGTG
GAAATGGTTTCTCTGACTCTGGTAATCTTTAGAGAGGAATTATACACAGT
CTATAAAGCTTCACATTTCAAACCCAATTTTGAATGTTATTCAAGAGGT
TAATTTAAGATTCATCTTCCTAAAGAGGAGTTAAATAAAACGGATAGAAG
GCTGGAACCTGTGCGAGTGGTTGAGAGTTTTATCAGCATGTGAGAAT

APPENDIX F continued

13. Ah 211: (CA)₁₃

AGGAGAGCAGGTTGATTTTCAATGTTTACCTCCTCCCAGCTCAAGAATAG
CCAATCCAAAAGAGTAGGAAGTGAAACAGAAGTGGCAGGAAGCTTGCACA
GGTGAACAAGCAGTTCCTGACTAAATGAGAACGTAAAAATAAAGTGTGTA
AGAGGTGAAAGAAGGGACAGGTGACATGGAAGGAATATAGAACTGTATG
TCACCACATAGGAATGTGGTGAGGAAGCCAAA**GCTCATCAGGAGTTGAAT**
CTGGCAAGACAAAGGTCAATAAGAACAGCGCGCACACACACACACACA
CACACACACCCCTCAACAGTGATGGGAAGACCAAAGGAAATACAG**GTCCA**
TTGCTGAATGACAGAGAACACAGAAAAGTCCAAAGTACTGAAT

14. Ah 212: (CA)₉TA(CA)₃

TGGATGGCTATTCCTATGAATTAGCTCAGTATCCTGTGTGGGCATTACTC
AGAAACATCGATGTATACAACGTGCATACAGACATGATTTGGGTGAAGAA
AAAGAGAACACTTAAAACACATGTGGTGTGATACTTGGAAATCTCATTCT
GGTAGCATTTTAAAAAGAGATAAATATTTATTTAAAAAAACGATAAAAA
GAAAATTAAAATACCCCCACTCTTCTCTTACTGTAGTGCCTTAAATGATA
GTTTTAGCATTCACGT**TCAGGCTAACTTTGGGCAAAGCTTT**ATCTAATTG
TGTTCTAGGCTGTGAAGCATCCATGGCATGCACATTAAAGGGTAAGCAAT
TTCAGTTCTGGGTTTCCCAACACACACACACACACATACACACAA**ACT**
GCAAGTCATGAAAGTGGGCTGATATTGAAA

15. Ah 213: (CA)₁₂

TCAGTGCAAGCCACACACGTGCACACGTGCACACACACACACACACAC
ACACGCATGCACAGAGGGTT**CAGCCTAGCACCAGAATGCTGTTAGTGTA**
ATGAAC**ACTGTACGGCTAAAGTGGC**TGGGGTTTTTGTGTGGTTTTTTA
TTTCCCCC**ACTTTCTGAAACAACCAGCCAATTTAAAGCACAGTTCAGG**
GGGAAG

16. Ah 217: (CA)₁₀

ATTTCTTTAAGACAGAAGTGCTAA**AACTGCTTTTTT**ACCACTGCCAGCAG
AGAATTGGGTAACTCAGGAAGAGTATCCTAGGCTGTGCTCCTGTCCGTAA
GTCTGACATCACTTCTTCACTTGTTAAAGATGCTGCTGTAGTGCCAGCTA
TGACTGAACTGCTGATAACTTCAGTCAGGGAAGAAATCACCTGCATCTCT
GTAACAGACCTTTCACCTATAATGTCAGTTTTCTTTGTA**CTTAGGTAAAA**
TGGTCAGATGAC**CACAGATTCAAAACAAGCACCATGCA**AGTGACACACACA
CACACACACACAGAGAGCCCTACTTGCTTTTGCCACAACCTGCCTACAT
TATCAAGACAGCACAGAGGGTTCGGATGAGCTCATGGATGAAAACCAG**GT**
AGACAAAGCAGAGCCTGAGCAAGAAAGTGGTTATAAAGCTAGGTAAATCA
AAGTATTTTGGAAAA

APPENDIX F continued

17. Ah 220 (AC)₁₁

AGATAGCCTACTTACTGTAATTAGATTGGAACACACACACACACACAC
ACTTTTTCTGCTCTTTACAAGGCATTGTTTCTGGAACGATGAACTGG
TCTCCCTGGAAAGGTTATGACCTTCTATAAAGGACAAGANCCCAATAAAT
ATAGAAGAAAAATGTCATTTAGGACTCAGCAAAAGCAGAACTGCTGATGA
ACTATTTTTAAAGAAGTGTCTGTTCTCAATCCAAATCTTAGAAGTCAATG
TATCATTGTTATTGCTACGCTAAGTGCTTACTAGCACACATTTTATTCAA
AAAAAATCTATACAGAATAAACTAAATTACAGGTGTGCAGCATAGCCAA
TAGTCTAAGAGAGTGATATATCTTGCTGGTCTCCAAATGGGTATACCAG
CATTTTTCATAAAAGAAGT

18. Ah 261: (AC)₁₅

TAGCCTGAGAGCTGGTATTGAGAAGAGAGAATTAATCTCTGCTCAAATCA
CTGGAAATTTTGTCTTATTAAAAGCAGGAAGGACATTTTAATGTTAAGTC
AGTAAAGAACACACACACACACACACACACACACACACCCCGCCCAAGCT
CCAGAGGTGGAATTATTTGCAGAAAATTACTAGCAAGTGGTTTTGAATAA
TGAAC

19. Ah 262: (CATA)₅

TCTCTTCTTTCTGAAAGCATCTAAACACACAAAGTTATATTACATCTTAT
ACGTTTTTTATTTGTATACAAAATGTCATAATTTATCAATATTGTGCAGCA
AAGTATAGATACACGCATACATACATACATACATGCACCACCCACC
CCCCTCGACACACACACATACACAACACAAACACAACCTTTCTTTCTCTCT
GCCCTACAGAAAGGCAACACTGTTTTGGCAGGTGAGCAGCTAGAAATAAG
GTGCTAAAATTAAGCAGTATCGGAAT

20. Ah 265: (AC)₉

AGCAAGCGGGATGGTTTGCACCAGCCCTGACCATAAAACACCTCTGTTTT
AAAAATTAGGCTGAGTCTAGTCCTTCCTCTAAGGCCAGCAATGGCTTGTT
GAAGCATCCATGTTCTGGTCATACAGACTAAGATGTGAAAACCTGCTTAG
ATGTAAAATAAACAGCACTATGCACAGGCCTGGGGAAGGCAGAGGCAGGG
ATGACTTTAGTGCTGTATGTGCACGGTGTATTACACACACACACACACAC
AGAGGAAGC

21. Ah 283: (CA)₁₁

AGTGCAAGCCACACACGTGCACACGTGCACACACACACACACACACAC
GCATGCACAGAGGGTTCAGCCTAGCACCAGAATGCTGTTAGTGTAATGA
ACACTGTCACGGCTAAAGTGGCTGGGGTTTTTGTGTGGTTTTTTATTTT
CCCCCACTTTCTGAAACAACCAGCCAATTTAAAAGCACAGTTCAGAGGGA
AG

APPENDIX F continued

22. Ah 284: (AC)₁₀

TACCTTTTCTCTTCTTATGGCCTTTTCTCCTTATCAGTGAGAGAATTG
CACACAGTTGTTTCTTTCAAAACAGACTAGGGGGAAAAAGGAGGAGAAA
ACCCAACACACACACACACACACCCCAAACCCCAAAAATAAGAGGCTA
AATCAAAGATGAACACATCCACAACCTGAACATGCACATCTTGTACCCAAT
TTGAAAT

23. Ah 291: (CA)₇CG(CA)₆CG(CA)₂

TACAACCTCCCCATTTGTTTGTCTTATTATATTTAAAGTAACACATCTTA
TTTAAGAGTTTCAATGATGGCATGGAGCAGAACTCTGGAAAATAGACAAC
CTGACTTCATAAGTTGTCTGTAGTCTAGAATAATAACTAAACATTTTACA
ACACACAGTATAGATGAACTGTATGCTATTATCAGTATCTGGAATAAAG
GATGCTGTTTGTCTACTGCCCTACAACAGATATTCACATCTGCACATTCA
CACACACACACACGCACACACACACACGCACACTCTTCCAGCTACAGAAC
CGAAGAGATACATTATTTAAAACCACTTCTGAACGAGGGATGTATTTTTT
TATCACCTCCCATCTATACAATATGCTTTATGCACAGAACTATATTGAG
TAGGGAACCTCAGCCTAAAATAAATTGTGTTGTTTTTCTTTTCTTGAGTGT
TAATTTACTTTTAAATATTCTAGACATACCTCTTTAAATGGTACACTC
TGTTTCTATTTTACTGTATTCAGCAATCAGTGTGATTACTCTTGGTAAAC
CATGGCAACAAGCCAGCTAACTGTTCTTAAATCTTCATTTGTCCACTACT
TTTACAAAATACTGTACAGTTAAGAAT

24. Ah 292: (CA)₃TGCATA(CA)₄ and (TA)₄

ATTGACTAAATTAACATTCACAGAATTAGTTATTAAATCTCACATCTACA
GATTACTTAAATTGATTCACACATGCATACACACACAAACATCATTATAT
CCATACAGTATATATACACGCAAGGGTATACCTGCCAAAAATTCCCCTCG
AGTTCAGTGAAAATACTCGATGTGCAATGTCTTAGCGTTTCTCAATGGCC
GAGGGCTGAACCTCAAGGAGTGAAAG

25. Ah 318: (CA)₁₀

AAAAAAAAAAGTGAGGGACAGTACAGGACCAGAACAACACACACACACA
CACACACAAAAAGTGAGTGAAATAAAATAGTAACAAAAATGAAAAAGCA
GGAGCGATGTAAATCCAAATCACACTAAGACATTAAGGTCTCTGATCGT
AAAAACCAATGAAATCAGAAAAATTTAAATTGTCTGCATCAAATTGCCTC
GAGGAGGTAGCAGTAGTCAAATTACATGAACATTATGTTACAATACAA
CATTAATCCTAAATTAACCCCTTTTGGGAGCACATTTGTTTGTCTAA
TACTTGTCAAATTAATAAGAGGCAACTTTAGCCTGGCCTCAACAGGA
ACAGCAAGACCAGGAGATATCAACCAGAAACACTGTGTGAAGCCTGACTC
CAAGAAGTGAAGAATTTAGCAGAAGC

APPENDIX F continued

26. Ah 320: (AC)₁₃

CTTATTGACATAGTTAAATATGGGT**TTAGGAGCAAGATTTTAAAGAAGGT**
GCTACTGCTTTGCTCTCTTTCTTACCTTCTTCCTTTCCTTCTCTCCA
TTTCTTCTTTTCCTACACACACACACACACACACACACGCGTATGTTT
ATGCAAGGTTTTAAACCTAA**CTATTCCAGTATGACCCAGCACTT**GTCCA
ATACTGAAAATTACATCTAGGATAAGTAGACCTATCGCTAGAATGAGATT
CATTTTGGCAAAAGATAGAATAAATATTACACTAGATGGACAACAGAATC
AAGTTTAATTAACACTGATATCAATTTCTAACACTCCGGTTCTTTTCCTG
TTTAATTATCCTACCTAATGAATGATGAAGTTTTTAACAATGCCAGCCTG
CTCACTCTCCAAATGAGGCAGAGGAAGTCATGGATAATCAGCAAGAGAGA
ATTTCTTGATAAAACAGTGCAGTTTTTCTACTGCAGTTTGCAACCTTG
GGCTGTCATTTTATGCCTGCTGTCTGCCTGACAATTAGCAACACCAGAGA
ATAAAAAAAGTCTGAAAGTCTAGTTTCTTTTCAATAGTGGGAGTTTAAAG
TATAA

27. Ah 325: (AC)₁₂

TATAAGAAGATTAGGCATCCT**TCGTGCTGTAGTTTGTAGAGACC**ATGGAT
GCACGTGTACACACACACACACACACACACAGAGTCACACGCTCAAAC
CTCTCAACACTAAGAAAACCCAGGAAAAAAAATCCTGCACTGACTATTG
CACAAAATTTTCATTTTTTAAACTCCCATCTGGATGGGAACCTCACAGCTT
AAATATCT**GCAGAGTTAGCACATTTTACGGC**TATCCTAAGTCTCTGCAA
AATTGATTTAATTGGAAACACCTACAGACACTTAAAGGTAAAAAATGAA
AATTGCNTCATAGGAAACAACGTCAGGCTGTGTTTTTGGAGGGTTTATA
TAGGTAGCTAAACTCCCGGGTCTGCCATGTCGTCTCTCTGCCAGCCAAGC
CCATGCAGCGCGGTGGAGGATGTTACCATGAATACAGCTCTGAAAC

28. Ah 331: (CA)₅TGCAA(AC)₆GTGAA(AC)₈

AAACATGTTTGTACTAAGTCTTATCAGCCTCGCCCTTGGGGCCATTTTCGC
ACATTTACTGTGTTTCTGCAACAGAGGAAGCAGCTCTTGGAATGACCTT
TTTGAATCTGTGGCATATCTGAAATGTGCAGCAGAGCATAGCATTTCTCG
CCAGTAACTTTTTGTCTACTCTCAAATGGTTCCTTTCAGTCTTTCATTC
CTTCTGCATATACCTGGCACTTTCACACACACATGCAAACACACACACAC
GTGCAAACACACACACACACACGACATACGCACGCACACTCTGTAAGAA
TTATAAACAAGCTTCTGCAAACCTCAGAGGCTATTTTCTAACTTGGAATTC
ACAAGTAAATAGTGGAATATTATTGCCTAACAAGCTCTCAGCACCTTT
TGCACCTCTATGTATCTCTGGTGGGTTAGGACTCTGTGAGAAGC

APPENDIX F continued

29. Ah 341: (AC)₁₂

TTTAGTTACCAAGAAT**GGTAATGATTCTGATT**TACC**ACTGAGGG**TTTTAT
CTTAGTGTCACACACACACACACACACTGGGTCACACAAATTTT
ATCAAGTGTCTACTGCGAGTCTTCCATT**GCTGTGAAGACCAGATATGAT**
AACACATCAGGATGATAATATATTTCCATTTCAGTAGTTGAGTTCTCCAT
GCAAATAACTCCTTCAAGGACTATCTTTGGGAAAATAAAAATGCAGTTCC
TTTTGAATTAAAATAGGACAAACTCTTGTTTTAGTGGACCCTTACCTAA
CTAAACGAGTGTGTGCTTTATATTCTACTGTGTTGCTTTTCAAGCTTTC
AAGTTCTTGTAAGCAACTTCTATTATCAGGTTGTTTCTGCTTATATAAATG
TACTACAATGGTCTTCTGCTACAAATT

30. Ah 342: (CA)₈

ACATTTGTTGTATATTAAGTTTGGGAACTGCCATAATTTTTATTTTCAT
CATGCAAACATCATATCATAGCTGGACAGCTGTTATTGTCCCTGCTGGCCT
GAGGGTTATATCACACACTATTANTTTAAAGAATGTGACTTAAACCCACC
TAAAATGGGAAAGGGTAACTNTGTAATTAATCCACTACATTGCACAGGT
TACACCACACACACACACACACGTCCACTTGCTACTCTGAAGTTCTTTCT
CTTTTTTTTCCACCCTCTCTGAAATTGCCATGATGGAGCCCTGTTTCATGTT
AAAAGCACTGGTTTTTCTTTTGCTAACCCCAAGCCTTTTTCTTTTTTTT
TTCTTTT

31. Ah 343: (AC)₁₇

TCAACGGGTCTACCGGAACTAGATGAGATG**CATTGCTTAACTTCTGAAG**
AAACCAGCTTACACGTACACACACACACACACACACACACACACT
GAAGAGGACCCATAAGACTAAATAAGACATGGAAAAATGAGGACAAAAGA
GTTAATTTCAAATTTTTTCGTTTTATGAGTTTGAAAGATAATAAATTA
CTGCACTGAAAA**CAGTTTTATTACAAATGCTGGGTCAAG**TGTTATTTTT
CTTAACCTCTGAAGAGTAGCTCCAGGAAATTATACTGGGACAGAGATAAA
CCAGGTATAACAACCATAATGAAAA

32. Ah 344: (AC)₆

CAGGGTCAGCACAAGGTCATGACTTGTGACATGAGGTTAATGTAGTTGTT
AAGTAACAACATGAGTCTTTTCAATGGCATGAATATTCCACCCCACCTC
GCCCAGACACACACACACGCTTTCTGTCTCCTGGCTGAAGCCTCAGTTGA
ATACTTGTTTAAATGTAAGGGCAACCATGCGAGGCTCATGGACAATATGCA
ATGCAGTTATACACATTTCAATTACCACATACCCAGAAAAATAAGCTATT
TAATACATAGCATGGAAAGGCTACANAAGGCTTNTGCTAGCTANAGTCGA
CCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTG
TGAAATTGTTATCCCGCTA

APPENDIX F continued

33. Ah 346: (AT)₄(AC)₁₀(AT)₃

TAGTTAGCAGTGTCCCTGCTCACTGCAGGGGGGTGGACTAGATGACCAC
TAGTGGTCCCTTCCAACCCTAAACATTCTATGATTCTATGATTCTATAAC
AGAGGTGACTAGTACCTTAGGTAACACCTGAGTGCAAAATAGTTGCTATG
TGGCCACCACCATGGACAAGAGGATAGGGCCAAGACTGATTCTCTGGGAA
GACAACTTCTATGGATATCTACTGTTTTTCATGGACTTTAATTCTTCCAG
ACCCATTTGTACAAATGTATATATATACACACACACACACACACATAT
ATAAAAATCTTTACAATATCAAACCTGCATGGAATGAGCACCAGAAAACAT
TATTAATATTGTGTCAAGCATCCCAAAATGACAAAATTAAGGCTGTCTGT
GCAATATAATTCAATTCCTTGTATGGCTGCATTTAGGAAAAGTTTCTGC
CTTTCAACAGGGGGTGTAACTCAGAAATCTTGTGACTAGTTGGGCTGAA
GC

34. Ah 357: (AC)₁₀

AGCAGGGAATGCTCTCCTTTGAAAAATCCAATACTGTTATGTTTCGTATGA
TGGTTTTTCATTAAACACACACACACACACACCCCCCATAAGAGGAGGCT
GAGGGGAGACCTTATCGCCCTCTACAACTACCTGAAAGAAGGCTGTAGCG
AGGTGGCGGTACCTCTTCTCCCTGGTAATGGGTGATAGAACAAGGGGAA
ATGGTCTGAAGTTGCGCCAGGGCGGGTTTAGGTTGGACATTGAGAAC

35. Ah 381: (AC)₅CCCCACT(AC)₃A(AC)₂G(CA)₅

ACATACATGTAAGTCATCTCATCAAACCTCAGATACACAATCCCATTGTGG
CTGCTTTTGTCTCTATATGCCATTTAAGTGGCCAAAGGAACGTGCATCTG
TGGCCAGGGATGTTCTTGGTGTTTATGACTTAGATTTC AATAGTGCTACC
CAACATTCACAAGAAAATGTAACGAAAAAAGTTGGAGCAATCACTTGTTA
GATGAGAGCATGTTATCGTTTCCTACACAAAGTAGCAGATTTCTACAGTG
AACCGTATCTTTTAAAAAACACACACACCCCCCACTACACACAACACGCA
CACACACAAGCAAAAGCCTCTTTTCTATTTCTGGGAGGTTTCTCTGTGGC
TTCAAGACACTGCCACAAAGGTAAAGGAGGTTATGAAAAAGATAAAAGAA
AAGAAAATGGATTTCTTTTTTGCAAGTATCTCATCCTTCTACTACTCATA
ATAGTATTCATGCCCGAATCCCTGAACAGGGAAA

APPENDIX F continued

36. Ah 414: (AC)₂₂

CTTCCATAGACCTACCCTACAAGAACAAGGTCATTCTTTTTTAATGAGC
AAGGCCTTTTATACTGACAAGGTTTATT**CATTCCAGCTGCTCTTCATTCT**
TGTAACAGAGCCTTGAGGGTTACACACACACACACACACACACACA
CACACACACACACACTTACCTGCAAAGTACAATTTCTGAGCTATTTCTGC
AGAATATTTGCATCCAACTTTAGCAGGGAATATTTATTTCTCAGCCTTG
GCGTGGAACAAATCCGGCGATTGTCTTCTGCCCTAGTTGCTTTTGCCCT
CGCAAATAATTGCTNAGCCATTTCTGTGGAGCTGCTCCCTCTCCCCCA
ACCCCTTTCCCCACTTCTATTTAACATCTGCCCTGCTAATGTGATTATAT
GAACTCTGACAGATAAACTTTACTGGAGCAGAAATGCTTGACAAATAGC
CAAAAAGCACCAGGGATACCCAAATGTGGAGACGAACAAACCACATTGGC
GTACTTGCTAGATTGTGTAGAAAGC

37. Ah 417: (AC)₁₀(AT)₆

TAAGACTGTTCTGGTTCTTATATCCATCTTATATC**CAGATACTTCTTCGC**
TGCATGCACACATGCATAGATGCATACAAGTATTTATTCAGTGACACAC
ACACACACACACACATATATATATATAAAAATATTTACCTTTTACAATTA
ATTTCTTAATTTCTGGNAGGATTGAAA

38. Ah 421: (CA)₁₅

CT**CCCGTTCCACGCTGCTCCT**GTGCGCACGCGGCAGCACAACACACCTGGA
CGCCAATGAGAAAGTACGTTTAATTAATCAGAACTGCAGCCACACACACA
CACACACACACACACACACAAGTTTAATTTTCCCCGAGAGAGTTTT**GGC**
ACTCGGTGAGCAGGCTGTA AAAAGGCCTGTCTCATAGTTGTTGTGCAGAG
GAATCCTGGACCAGGATTGAAGGTAACAGAGCAGGCAGCCCGTGCGGTGCG
CTGGCCCCGCTTGTTGCGGNTTTGGTGAAGCCCCGTGCTCCCGTCTCCCC
CGCTGAGGACCCCTACCAAGCGGTGAAGC

39. Ah 422: (AC)₁₄

ACATGATGGAGTTACACACACACACACACACACACACACACAAATTTAAA
AATAATGATAAGCAAAAGAACAGCNTGAGATGGAACAGGTTGCCAGAGA
GGTTGTGGATGCCCCATGCCTGAAAA

40. Ah 424: (AC)₁₅

CTTATAGAATGTCTAGTTAAGGAGATACACACACACACACACACACAC
ACACACATATGTACATAATATGGCTTTGTAAGACTGAACTGTAAACAAC
ATTTGCAAAATATTACTTCACCAGGGGCATGAAAATCTAGAAAACAGATC
AGCCCCACTATCTTCCCTAAAATAAACACAGACTTCCTCAGAAATTTAAA
CTCTGCAGCATTACTTGCATTTACTAATAACAATTCCTTAACCTTTCCAGA
ATGGAAA

APPENDIX F continued

41. Ah 429: (CA)₆ CCCCCAC(CA)₄

TTACCTTAAAGCTACCTCTACTATCTTCAGGCTTGAAAAAACACACACA
CACACCCCCACCACACACACCCACTGCCATTTTCTTCACTGAAGCACTA
TGTATTTCTGCAGGTAGAACACATACAGGTCTTTAAATTTTGGCCTGAGA
AGTTTAGGTTCAAGCATATTAAGCAACGTATATAACACGCTAATCTCCAT
AATACACCTCATCTATAAAAAGTTACGTGGCAATTTGCAGAGCTCAGAGC
ACTGAAAAACCTCGGAGCTCCTTATTAAGTAAGATATTACAAATGTCTAG
CAAATTAAGCAACAAACCAAGTTGTTGCTTCACTCCATGTTCTGAATAAA
GTTTGCCTTCCCCACCACCAGTAGCAGACATTTGTGAGTGGCCCCAGGG
GCTTGTATCTAACAAGACACATGTGCAAGAAGAAAC

42. Ah 442: (CA)₁₂ and (TG)₅

CCACACACGCACAGGCACACCCGCACGCACACACACACACACACACAC
ACCCCCCCCCAGACATCTCCCGNNNNNTTATTGGATTGACTTTGAAGGTT
GTGTGTGTGATTCAAGGAGGCTGCATGTTGAACCAGNGGTGAATATTTA
AGGCTGGAANAAGTAAAGGGATTTTTCTTTTCTGTGAAGGGAAAATA
TCTCANGGACTAGTCTGACCATCCAAACAGCTAAATGTTTATTATGTAAG
TACATGTGAAATTCAGCATCGGAATAAAATAGAGCGGGCATGGTGCGAAA
GCCCTATGTATGTTTCTGTGCGTTNGGAGAATGTTATCGGTCATTGTA
TGGGGACTGGCATTGAGGTGCTCGCANACTTTTTAAGCCTACGATCGCA
CCGTCAGTTGCTTAAAGTAAGTTTATAGACGAGTAACATAGTTAAATCA
CTGTCTAG

43. Ah 456: (TGC)₆

TTCTTGTTGCTATCCCGCAGAGATTCCTGGCTCAGGGACACTCTCCTCTC
CTGTCCTCCTACCGGTGTTTTTCCAGAAAAGTCAGTTTAANAATTGATT
TTTTTATTTGCCACAGGAATATCTTGCCACTCTTCCCCGAGTTCCTCAT
TATTTTATTACAAGTGGCAGAGGTGCCCTGCAGAGCCTGAAGAGCTACAG
AAGGTGAGACCACAGAACTCATAGTGGTGCTGCTGCTGCTGCTGCAAGGA
AGCAGAGAAAA

44. Ah 475: (CTG)₅, (GCT)₆, and (TGG)₄

GTTCATCTGCTGCTGCTGCTGTTGCTGTTGCTGTGCCTTATTTTCGAGTT
CTCGCTGCTGCTGCTGCTGCTGTTGATTGTGGTGCTGATGGTGGTGGTGG
TCCTTGAAGAGGTCACTCAGCTGACTGGACAGCATCTGATTGCTGCCGCC
ACCACCGCCACCGCCGCCACCTCATCCTCACTGTCGCCCATTTGTTTGG
AGAG

APPENDIX F continued

45. Ah 486: (TGC)₆

AGGGGCCAGGGAGATGCTGCTGCTGCTGCTGCCAAAATTTGGCTTTGCTG
GAGGGATGCACGGCTGGGCGAGGGCAATGCCTGAGCGTGGGTGGCTTTAA
CTTTCCCTGTTTCCTTTCTGCTGTGGCAGCGGGCTGGGGCGCTTCTGCTA
GACTAGCAGAAGCAACATATAGCGATCAGCGTAATGGAGGAGACTGAAGT
CTTCTCAGACGCGACAATCCCAGCCCAGCTCAGGCTGCACCTCTGCCCTT
GTTGCCCCCGTACCTGTCCAGGTTCGTAGAGTGTGGGAGTTCTCAATCACC
ACCTCCACGCCAGCCTCCTTGGCCAGTTTGACGATAGCTGCATCCCTCTC
CTTACCAAATGGCTCCGAGTCGTATTCGAAGGTGAGACGAGTGACTCCCC
ATTCTGCAAGAAACACAATACACACAGCTGTCAAAGAGAAGACTCTTTC
TAACCAGCACAATTATGAATGAGGATGTCAGAATTCCTGGCCAGCGTTAA
GTCCTGGAAGCTTAGAA

46. Ah 517: (TC)₁₅

CTGCAGAAGAGTGTGAAATTAAAAGTATCAGACTAACACTTTAATTTCTG
CCATCTTTCTCTCCCAAAGTCACAAACCCCGGG**TTTTCCATCATGCTTC**
CATCAATACGTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCAGTCTCTTA
GACATGCACATATTAGAAAAACCTTATTTTCTAGACTTCATTTATAAGA
AACAAGGGAAGGGAG**GATTATATTGCTCTACTGGTTTGTGCC**TTTTAATAA
TGTGGGAGGTGCTCAGGTAATAGCATAGTAAGGGACACGTAAGTGGTTC
GTGACATAAATGCAATTCATTCTTGAAC

47. Ah 519: (TC)₁₃

ACTTCTTCACCGAAAGGGTTGTCAGGCTCTGGAATGGGCTGCCCAGGGAG
GTGGTCGAGTCCCCATCCCTGGAGGTATTTAAAAGAAGGATAGATGAGGT
GCTTAGGGACATGGTTTAGTGGTGGGATGGCAGGGTTAGGTAAACGGTTG
GACTTGATGGTCTTAAAGGTCTTTTCCAACCAAGCAATTCTATGATTCT
ACAGTGGAAGTGT**GAATGGCAACGGTTTATGTGGG**TTCAAGAGGGCTTCT
GCTAGCTAGCAGAAGCCCTTTTGCCTCTCTTCCCTTCATCTCTCTCTCT
CTCTCTCTCTCTCTCTGGTTGTACTGCTGTTTTCTCTTTTGGCTGCTTTT
GCTGTGTAAATTCAGGTGAAGACCTGGAAAGAAATGAT**GGATCTACAGCA**
AAACCCTACTTCATGTCA

APPENDIX F continued

48. Ah 522: (TCTTC)₂₅

TCATGCTGAGCCTGCTGTCAACCAGCACCCCCAAATCCCTTTCTGCAGGG
CTGCTGTCCAGCCACTCATCTCCCAATTTATGCTTGTGCCAGCATTACT
CTGTCCTAGGCAGAATCTAGCATTTTACTTGTAAATTTTCATCCCATTA
ATCATAGCCCAATACTCCAGTCTATCTAGAACTCTCTGCAAGGCACCTAG
TCCTTCAAGACATTCAACAGCACCTCCCAGTTTGGTATCACCAGCAAAT
TGCTAATGATGCATTCAACTTCCGCATCCAGAAATAATCTAATAGCTTAT
CTGGCTACAACCAACCCACTCAGTGTCCAGGCACACA**CAAAGCTGATTTAAA**
GATGTTCCATCCCCTCCTCAAGCACAAGGTACAGGTTTCAAACCCACAT
TGCAGTGGGTTTTGCTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCT
TCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCT
TCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCTTCTCT
TTCTGCTTCAGTGT**TTAGTCCCACAAT**CAATCACATCAGCAGAGACCAAG
AAGGGANTGAGACCGAGACACAACAGGAAAAGAGAGAGAAAAAACAGCAC
AGGAAGGGAGTCTGGAAACATATTAG

49. Ah 526: (TC)₂₇

CCAAGGAGCAGTCAT**CACTGCTCAGGGACTGGC**AGGGTGTTGGTTGGTGG
GTGGCGAGCGGTTGCATCAACTTGTTTTCCCTGGGTTCTCTCTCTCTCTC
TCTGTTCCCAA
TCATTACAAATTATTACTATTACAACATTTGAGTTATTAAATGGTTCTTA
TCTCAACCGATGAGTTTTCCCACTTTT**GCTCTTTCAAGTTCTCTTCCCCAT**
TCCACTAGGGGGAGGCGGATAGGGTGTGAGCAACCGGCTGTGTCGTGCTT
AGTTGCCAACTGGAGCCAAACCACAACAGACTCCAATGTCTAGTGTA
GGCTAGATCCCAATGTTTTCCAAGAAAAGTGTATGTTAGCTGGACATTAT
TTTTTTAAAAAAGAAAATAACCAGCCTTTATTCCAATTACTTTATGGAC
TTTTTCTAATTTGGGAAAGGACCAGGTAGTTTTCAGAATCACTAACAAAC
CTCTGTTGTGCATATTACCATTTGCTGGCTGCAGCTTTTAGTATTCCAGC
AGGGATGAATCAAACAATGATAAAAAGTTTTTGTATTATCCTGTCAAGGGG
AAAA

50. Ah 534: (AC)₁₀ and (GT)₈(TA)₅

CCCAGCAGTGCCTCGGATGCTCAGGACAGTAGCCCTTCTTTGTCCATGG
TGTTGCACACTCCTGAGGGGCTGGTACATGGGGAAGCATGTTAGTTGTGG
TGGGGGAATTGTGATTAGCAGACATGCGCGCATGTACACACACACACACA
CACACATAATATAAAAATAGCTTACTTCAGATAATTTCAAGCAACTATTT
TATATGAGTAGTTATTTTAAATAAGCTGCGACTGAATTAGTCTCAGCCAA
AAAGGCTCAGCCTTACATGCAGCAAATTTGGAGAACTGCTTCTGCTAGCA
GAAGCGCTTCATTTTGGTCCCCTCTGGCTGATCAGGGTGAAGGTCTACTA
GTGTGAAGCAGTATACGTGTGTGTGTGTGTGTATATATACGCATGTAT
ATGTGTGATACATACTATCTACGTAAGCAATATACATATGTGTTATATGC
ATAAAGCATGTATACACAACGTGGACCTCTCCAGTAGCTGAGCTCAGTCT
GCCATTAACTGG

51. Ah 536: (AC)₁₄

52. Ah 546: (TC)₂₇

53. Ah 550: (TC)₁₂

54. Ah 556: (TC)₆

55. Ah 573: (TA)₅(AC)₉

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APPENDIX F continued

56. Ah 581: (AC)₉

AAAAATCAGCTCCTTTGCAGGATTCACAACATAGGCTCTTAGTGAGCCT
TTGTGAGAAAGTGCACACAAACACACACACACACACGTCTAAGCAGGA
GGGACCAAAGGAGCAGGTTACCCTGACAATAAAGTGATGTTAGCCAAATG
TGACCTAATTT

57. Ah 590: (CA)₅CT(CA)₁₀

TCACCTACATACACACACACACTCACACACACACACACACAAGTGCAC
TTTGATGGGTGTCACACCTGGGCAACACTCATACTCCTGTTATCTAATGC
AGTGCAATTTGTTAAGTGAATGTGAAAAGGATATGTTTTGNGCATATACC

58. Ah 594: (CA)₁₁

CTTGCTTCCTTCACCATGATGCCTGGCTGTGCACCATGAAAACACTTCTG
CGTGCCCTCACCTGCACACACACACACACACACACTTTATCTCTACA
TTAGTGTTTTCTCACTTGGGTTTCATACCTCCCAAAACATTGAGAACACA
AGGAGAACANACTCTGTANAANACCAA

59. Ah 603: (TCC)₅

TACAGATGTCTACTGGCAGAGCCGCAGGCGTGATCTCCGACCGATGCACC
TGTGTTGGCAAAGCTCTTACGTGGGTGCAATTAAAGCAGCAAACCTGCA
CTTCCACTGAGGCTAATTTTAGTCTTTACTCCTGGGTGCCAGGAATAAA
CTGCACTGGCTCATATAGGACATTGCCGGTGCCAGCTACATCCAACTCA
TGTTTTAGCAACTTCTTTCTGTAGAGCATACCCGTAAGAGTCCCCC
CTTTCCCATAACAACACTGCACACCCCTCCTTCTCTCTCCTCCTCCTCC
TCTTTCTCCTTCTCTTCTCCTCCTTCTCCCCGCTCTTAACTTTTGAGTC
ACAGCATATACAGACAGCAGCAATGACTTTCATGTAAGTGAAGGAA
C

60. Ah 630: (TC)₁₂

GGTGGTGGTGCTGTGAGTTATACAAGACCCAGTAGGAAGCAG**CGGCAGGC**
AGTATTATTTCACTGGTTTGAGCCAAACTTTCTCTCTCTCTCTCTCTCT
CTCTCATTAATAATTACAATAAT**GCAAGTAGCATTGTGAAGGAGGA**ATTTG
GGAAGTGTGTTTCATCATCCAATC

APPENDIX G: *A. herodias* tissue samples used for analysis of genetic differentiation at microsatellite loci

Source^a	Sample ID	Group^b	mm	dd	yyyy	Location State: County
UWBM	EVL 146	B-PNW	08	07	1998	WA: King
UWBM	GKD 01	B-PNW	fall		1990	WA: Mason
UWBM	GKD 133	B-PNW	11	25	2000	WA: Skagit
UWBM	PJG 112	B-PNW	fall		1998	WA: Mason
UWBM	PJG 232	B-PNW				WA: Jefferson
UWBM	SMB 01	B-PNW	07		1998	WA: King
UWBM	SMB 02	B-PNW	11		1990	WA: Mason
UWBM	SMB 129	B-PNW				WA: Island
UWBM	SMB 130	B-PNW				WA: Snohomish
UWBM	SMB 131	B-PNW				WA: Island
UWBM	SMB 132	B-PNW				WA: Island
FMNH	348377	B-N	06	23	1988	WI: Marinette
FMNH	348378	B-N	08	13	1986	WI: Kewaunee
FMNH	348380	B-N	08	17	1986	WI: Door
FMNH	348381	B-N	11	06	1988	WI:
FMNH	348382	B-N	08	29	1985	WI: Kewaunee
FMNH	348383	B-N	09	05	1986	WI: Brown
FMNH	348384	B-N	04	13	1986	WI: Shawano
FMNH	348385	B-N				WI:
FMNH	363357	B-N	08	03	1992	WI: Brown
FMNH	387752	B-N	05	19	1998	WI:
FMNH	395626	B-N	08	22	1998	WI:
FMNH	395888	B-N	09	22	1998	WI:
FMNH	396953	B-N	07	09	1998	MN: Dakota
FMNH	396954	B-N	10	05	1999	MN: Itasca
FMNH	397031	B-N	11	07	1998	WI:
FMNH	397118	B-N	07	17	1999	IL: Aurora
FMNH	397119	B-N	08	03	1999	IL: Batavia
FMNH	429048	B-N	07	21	1995	MN: Hubbard
FMNH	429049	B-N	08	04	1995	MN: Fillmore
FMNH	429086	B-N	06	03	2000	IL: Naperville
BMNH	AF 1010	B-N	08	07	1952	MN:
BMNH	JK 93151	B-N			1990	MN:
BMNH	X 7089	B-N	07	27	1992	MN: Cass
BMNH	X 7090	B-N	04	30	1992	MN: Washington
BMNH	X 7091	B-N	09	28	1991	WI:
BMNH	X 7092	B-N	09	23	1991	MN: Washington
BMNH	X 7093	B-N	07	31	1992	MN: Ramsey
BMNH	X 7094	B-N	08	02	1992	MN: Chisago
BMNH	X 7095	B-N	09	26	1993	MN: Beltrami

APPENDIX G continued

Source^a	Sample ID	Group^b	mm	dd	yyyy	Location State: County
BMNH	X 7096	B-N	08	06	1992	MN: Ramsey
HLM	04 03 00 N01	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N02	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N03	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N04	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N05	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N06	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N07	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N08	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N09	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N10	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N11	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N12	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N13	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N14	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N15	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N16	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N17	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N18	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N19	B-FP	04	03	2000	FL: Dade
HLM	04 03 00 N21	B-FP	04	03	2000	FL: Dade
HLM	04 04 00 N01	B-FP	04	04	2000	FL: Dade
HLM	04 04 00 N02	B-FP	04	04	2000	FL: Dade
HLM	04 04 00 N03	B-FP	04	04	2000	FL: Dade
HLM	12 03 98 N2C1	B-FB	12	03	1998	FL: Monroe
HLM	12 09 98 N1C1	B-FB	12	09	1998	FL: Monroe
HLM	12 15 98 N1C1	B-FB	12	15	1998	FL: Monroe
HLM	12 15 98 N2C1	B-FB	12	15	1998	FL: Monroe
HLM	12 15 98 N3C1	B-FB	12	15	1998	FL: Monroe
HLM	12 16 98 N1C1	B-FB	12	16	1998	FL: Monroe
HLM	12 18 98 N3C1	B-FB	12	18	1998	FL: Monroe
HLM	12 21 98 N1C1	B-FB	12	21	1998	FL: Monroe
HLM	01 10 99 N2C1	B-FB	01	10	1999	FL: Monroe
HLM	01 10 99 N3C1	B-FB	01	10	1999	FL: Monroe
HLM	01 10 99 N4C1	B-FB	01	10	1999	FL: Monroe
HLM	01 12 99 N5C1	B-FB	01	12	1999	FL: Monroe
HLM	01 14 99 N1C1	B-FB	01	14	1999	FL: Monroe
HLM	01 21 99 N1C1	B-FB	01	21	1999	FL: Monroe
HLM	01 21 99 N2C1	B-FB	01	21	1999	FL: Monroe
HLM	01 21 99 N3C2	B-FB	01	21	1999	FL: Monroe
HLM	01 21 99 N4C1	B-FB	01	21	1999	FL: Monroe

APPENDIX G continued

Source^a	Sample ID	Group^b	mm	dd	yyyy	Location State: County
HLM	01 21 99 N5C1	B-FB	01	21	1999	FL: Monroe
HLM	01 27 99 N1C1	B-FB	01	27	1999	FL: Monroe
HLM	01 27 99 N5C1	B-FB	01	27	1999	FL: Monroe
HLM	01 27 99 N6C1	B-FB	01	27	1999	FL: Monroe
HLM	01 27 99 N7C1	B-FB	01	27	1999	FL: Monroe
HLM	02 03 99 N4C1	B-FB	02	03	1999	FL: Monroe
HLM	02 03 99 N5C1	B-FB	02	03	1999	FL: Monroe
HLM	02 03 99 N6C1	B-FB	02	03	1999	FL: Monroe
HLM	02 06 99 N1C1	B-FB	02	06	1999	FL: Monroe
HLM	02 06 99 N2C1	B-FB	02	06	1999	FL: Monroe
HLM	02 06 99 N3C1	B-FB	02	06	1999	FL: Monroe
HLM	02 06 99 N4C1	B-FB	02	06	1999	FL: Monroe
HLM	02 06 99 N6C1	B-FB	02	06	1999	FL: Monroe
HLM	02 06 99 N8C1	B-FB	02	06	1999	FL: Monroe
HLM	02 09 99 N1C2	B-FB	02	09	1999	FL: Monroe
HLM	02 17 99 N1C1	B-FB	02	17	1999	FL: Monroe
HLM	02 17 99 N2C1	B-FB	02	17	1999	FL: Monroe
HLM	02 17 99 N3C1	B-FB	02	17	1999	FL: Monroe
HLM	11 13 98 N1C2	W-FB	11	13	1998	FL: Monroe
HLM	11 13 98 N2C1	W-FB	11	13	1998	FL: Monroe
HLM	11 15 98 N1C1	W-FB	11	15	1998	FL: Monroe
HLM	11 29 98 N1C1	W-FB	11	29	1998	FL: Monroe
HLM	11 30 98 N1C4	W-FB	11	30	1998	FL: Monroe
HLM	11 30 98 N2C1	W-FB	11	30	1998	FL: Monroe
HLM	11 30 98 N3C1	W-FB	11	30	1998	FL: Monroe
HLM	12 03 98 N1C1	W-FB	12	03	1998	FL: Monroe
HLM	12 03 98 N3C1	W-FB	12	03	1998	FL: Monroe
HLM	12 05 98 N1C1	W-FB	12	05	1998	FL: Monroe
HLM	12 06 98 N1C1	W-FB	12	06	1998	FL: Monroe
HLM	12 06 98 N2C1	W-FB	12	06	1998	FL: Monroe
HLM	12 06 98 N3C1	W-FB	12	06	1998	FL: Monroe
HLM	12 09 98 N1C1	W-FB	12	09	1998	FL: Monroe
HLM	12 10 98 N1C1	W-FB	12	10	1998	FL: Monroe
HLM	12 13 98 N1C1	W-FB	12	13	1998	FL: Monroe
HLM	12 13 98 N2C1	W-FB	12	13	1998	FL: Monroe
HLM	12 16 98 N2C1	W-FB	12	16	1998	FL: Monroe
HLM	12 16 98 N3C1	W-FB	12	16	1998	FL: Monroe
HLM	12 16 98 N4C1	W-FB	12	16	1998	FL: Monroe
HLM	12 17 98 N1C1	W-FB	12	17	1998	FL: Monroe
HLM	12 17 98 N2C1	W-FB	12	17	1998	FL: Monroe
HLM	12 17 98 N3C1	W-FB	12	17	1998	FL: Monroe

APPENDIX G continued

Source^a	Sample ID	Group^b	mm	dd	yyyy	Location State: County
HLM	12 18 98 N1C1	W-FB	12	18	1998	FL: Monroe
HLM	12 18 98 N2C1	W-FB	12	18	1998	FL: Monroe
HLM	12 19 98 N1C1	W-FB	12	19	1998	FL: Monroe
HLM	12 20 98 N1C1	W-FB	12	20	1998	FL: Monroe
HLM	12 21 98 N2C1	W-FB	12	21	1998	FL: Monroe
HLM	12 21 98 N3C1	W-FB	12	21	1998	FL: Monroe
HLM	12 21 98 N4C1	W-FB	12	21	1998	FL: Monroe
HLM	12 21 98 N5C1	W-FB	12	21	1998	FL: Monroe
HLM	12 21 98 N6C1	W-FB	12	21	1998	FL: Monroe
HLM	12 22 98 N1C1	W-FB	12	22	1998	FL: Monroe
HLM	12 22 98 N2C1	W-FB	12	22	1998	FL: Monroe
HLM	12 22 98 N3C1	W-FB	12	22	1998	FL: Monroe
HLM	12 23 98 N1C1	W-FB	12	23	1998	FL: Monroe
HLM	12 23 98 N2C1	W-FB	12	23	1998	FL: Monroe
HLM	12 31 98 N1C1	W-FB	12	31	1998	FL: Monroe
HLM	01 06 99 N1C1	W-FB	01	06	1999	FL: Monroe
HLM	01 08 99 N1C1	W-FB	01	08	1999	FL: Monroe
HLM	01 10 99 N1C1	W-FB	01	10	1999	FL: Monroe
HLM	01 12 99 N1C1	W-FB	01	12	1999	FL: Monroe
HLM	01 12 99 N2C1	W-FB	01	12	1999	FL: Monroe
HLM	01 12 99 N3C1	W-FB	01	12	1999	FL: Monroe
HLM	01 12 99 N4C1	W-FB	01	12	1999	FL: Monroe
HLM	01 12 99 N6C1	W-FB	01	12	1999	FL: Monroe
HLM	01 12 99 N7C1	W-FB	01	12	1999	FL: Monroe
HLM	01 13 99 N1C1	W-FB	01	13	1999	FL: Monroe
HLM	01 13 99 N2C1	W-FB	01	13	1999	FL: Monroe
HLM	01 14 99 N2C1	W-FB	01	14	1999	FL: Monroe
HLM	01 17 99 N1C1	W-FB	01	17	1999	FL: Monroe
HLM	01 17 99 N2C1	W-FB	01	17	1999	FL: Monroe
HLM	01 17 99 N3C1	W-FB	01	17	1999	FL: Monroe
HLM	01 18 99 N1C1	W-FB	01	18	1999	FL: Monroe
HLM	01 18 99 N2C1	W-FB	01	18	1999	FL: Monroe
HLM	01 18 99 N3C1	W-FB	01	18	1999	FL: Monroe
HLM	01 19 99 N1C1	W-FB	01	19	1999	FL: Monroe
HLM	01 19 99 N2C1	W-FB	01	19	1999	FL: Monroe
HLM	01 19 99 N3C1	W-FB	01	19	1999	FL: Monroe
HLM	01 19 99 N4C1	W-FB	01	19	1999	FL: Monroe
HLM	01 20 99 N1C1	W-FB	01	20	1999	FL: Monroe
HLM	01 26 99 N1C1	W-FB	01	26	1999	FL: Monroe
HLM	01 26 99 N2C1	W-FB	01	26	1999	FL: Monroe
HLM	01 27 99 N2C1	W-FB	01	27	1999	FL: Monroe

APPENDIX G continued

Source^a	Sample ID	Group^b	mm	dd	yyyy	Location
						State: County
HLM	01 27 99 N3C1	W-FB	01	27	1999	FL: Monroe
HLM	01 27 99 N4C1	W-FB	01	27	1999	FL: Monroe
HLM	01 29 99 N1C1	W-FB	01	29	1999	FL: Monroe
HLM	01 31 99 N1C1	W-FB	01	31	1999	FL: Monroe
HLM	01 31 99 N2C1	W-FB	01	31	1999	FL: Monroe
HLM	02 02 99 N1C1	W-FB	02	02	1999	FL: Monroe
HLM	02 02 99 N2C1	W-FB	02	02	1999	FL: Monroe
HLM	02 03 99 N1C1	W-FB	02	03	1999	FL: Monroe
HLM	02 03 99 N2C1	W-FB	02	03	1999	FL: Monroe
HLM	02 03 99 N3C1	W-FB	02	03	1999	FL: Monroe
HLM	02 06 99 N5C1	W-FB	02	06	1999	FL: Monroe
HLM	02 06 99 N7C1	W-FB	02	06	1999	FL: Monroe
HLM	02 07 99 N1C1	W-FB	02	07	1999	FL: Monroe
HLM	12 26 96 - 1	W-OK	12	26	1996	FL: Monroe
HLM	12 30 96 N1C2	W-OK	12	30	1996	FL: Monroe
HLM	12 30 96 N2C1	W-OK	12	30	1996	FL: Monroe
HLM	01 02 97 N1C1	W-OK	01	02	1997	FL: Monroe
HLM	01 02 97 N2C1	W-OK	01	02	1997	FL: Monroe
HLM	01 02 97 N3C1	W-OK	01	02	1997	FL: Monroe
HLM	01 02 97 N4C1	W-OK	01	02	1997	FL: Monroe
HLM	01 02 97 N5C1	W-OK	01	02	1997	FL: Monroe
HLM	01 04 97 N1C1	W-OK	01	04	1997	FL: Monroe
HLM	01 06 97 N1C1	W-OK	01	06	1997	FL: Monroe
HLM	01 06 97 N2C1	W-OK	01	06	1997	FL: Monroe
HLM	01 07 97 N1C1	W-OK	01	07	1997	FL: Monroe
HLM	01 12 98 N1C1	W-OK	01	12	1998	FL: Monroe
HLM	01 17 98 N1C1	W-OK	01	17	1998	FL: Monroe
HLM	01 17 98 N2C1	W-OK	01	17	1998	FL: Monroe
HLM	01 17 98 N3C1	W-OK	01	17	1998	FL: Monroe
HLM	01 17 98 N5C1	W-OK	01	17	1998	FL: Monroe
HLM	01 17 98 N6C1	W-OK	01	17	1998	FL: Monroe
HLM	01 18 98 N1C1	W-OK	01	18	1998	FL: Monroe
HLM	01 18 98 N2C1	W-OK	01	18	1998	FL: Monroe
HLM	01 20 98 N1C1	W-OK	01	20	1998	FL: Monroe
HLM	01 21 98 N2C1	W-OK	01	21	1998	FL: Monroe
HLM	01 31 98 N1C1	W-OK	01	31	1998	FL: Monroe
HLM	01 31 98 N2C1	W-OK	01	31	1998	FL: Monroe
HLM	01 12 98 N2C1	W-OK	01	12	1998	FL: Monroe
HLM	02 26 99 N5C1	W-OK	02	26	1999	FL: Monroe
HLM	02 08 99 N1C1	W-OK	02	08	1999	FL: Monroe
HLM	02 19 99 N1C1	W-OK	02	19	1999	FL: Monroe

APPENDIX G continued

Source^a	Sample ID	Group^b	mm	dd	yyyy	Location State: County
HLM	02 08 99 N4C1	W-OK	02	08	1999	FL: Monroe
HLM	02 08 99 N5C1	W-OK	02	08	1999	FL: Monroe
HLM	02 08 99 N3C1	W-OK	02	08	1999	FL: Monroe
HLM	02 26 99 N1C1	W-OK	02	26	1999	FL: Monroe
HLM	02 26 99 N2C1	W-OK	02	26	1999	FL: Monroe
HLM	02 26 99 N3C1	W-OK	02	26	1999	FL: Monroe
HLM	02 26 99 N4C1	W-OK	02	26	1999	FL: Monroe
HLM	03 10 99 N4C1	W-OK	03	10	1999	FL: Monroe
HLM	03 10 99 N5C1	W-OK	03	10	1999	FL: Monroe

^a UWBM = University of Washington, Burke Museum, BMNH = Bell Museum of Natural History, HLM = Heather L. McGuire

^b B = great blue heron, W = great white heron, PNW = Pacific Northwest, N = north-central United states, FP = Florida peninsula, FB = Florida Bay, OK = outer Keys

APPENDIX H: Results of tests for independence of loci; Chi-square (X^2), degrees of freedom (df) and p-values for each locus pair across all populations

Locus pair	X^2	df	p-value	Locus pair	X^2	df	p-value
Ah 205 - Ah 209	10.5	10	0.400	Ah 217 - Ah 517	10.7	10	0.378
Ah 205 - Ah 211	8.2	10	0.606	Ah 320 - Ah 517	16.8	10	0.078
Ah 209 - Ah 211	10.9	10	0.366	Ah 341 - Ah 517	4.1	10	0.942
Ah 205 - Ah 217	8.0	10	0.626	Ah 343 - Ah 517	8.4	10	0.593
Ah 209 - Ah 217	15.6	10	0.110	Ah 414 - Ah 517	12.4	10	0.276
Ah 211 - Ah 217	9.4	10	0.498	Ah 421 - Ah 517	2.3	10	0.971
Ah 205 - Ah 320	5.5	10	0.856	Ah 205 - Ah 526	11.0	08	0.202
Ah 209 - Ah 320	10.1	10	0.430	Ah 209 - Ah 526	5.7	08	0.679
Ah 211 - Ah 320	2.2	10	0.995	Ah 211 - Ah 526	16.9	08	0.031
Ah 217 - Ah 302	8.2	10	0.611	Ah 217 - Ah 526	1.8	08	0.988
Ah 205 - Ah 341	16.5	10	0.085	Ah 320 - Ah 526	4.9	08	0.767
Ah 209 - Ah 341	5.3	10	0.870	Ah 341 - Ah 526	1.8	08	0.985
Ah 211 - Ah 341	20.7	10	0.023	Ah 343 - Ah 526	10.6	08	0.226
Ah 217 - Ah 341	13.4	10	0.202	Ah 414 - Ah 526	2.9	08	0.940
Ah 320 - Ah 341	14.0	10	0.173	Ah 421 - Ah 526	0.9	06	0.989
Ah 205 - Ah 343	5.3	10	0.871	Ah 517 - Ah 526	1.0	08	0.998
Ah 209 - Ah 343	4.3	10	0.931	Ah 205 - Ah 536	3.3	10	0.974
Ah 211 - Ah 343	3.0	10	0.982	Ah 209 - Ah 536	14.0	10	0.175
Ah 217 - Ah 343	3.2	10	0.976	Ah 211 - Ah 536	4.6	10	0.915
Ah 320 - Ah 343	5.6	10	0.844	Ah 217 - Ah 536	8.2	10	0.604
Ah 341 - Ah 343	5.5	10	0.853	Ah 320 - Ah 536	10.1	10	0.436
Ah 205 - Ah 414	6.5	10	0.770	Ah 341 - Ah 536	10.5	10	0.394
Ah 209 - Ah 414	5.7	10	0.842	Ah 343 - Ah 536	6.3	10	0.788
Ah 211 - Ah 414	7.2	10	0.711	Ah 414 - Ah 536	16.9	10	0.077
Ah 217 - Ah 414	3.4	10	0.971	Ah 421 - Ah 536	8.3	08	0.403
Ah 320 - Ah 414	6.7	10	0.750	Ah 517 - Ah 536	7.8	10	0.653
Ah 341 - Ah 414	11.8	10	0.300	Ah 526 - Ah 536	7.0	08	0.538
Ah 343 - Ah 414	12.8	10	0.238	Ah 205 - Ah 630	18.0	10	0.055
Ah 205 - Ah 421	1.4	08	0.994	Ah 209 - Ah 630	5.4	10	0.863
Ah 209 - Ah 421	5.4	08	0.714	Ah 211 - Ah 630	24.4	10	0.007
Ah 211 - Ah 421	6.5	08	0.589	Ah 217 - Ah 630	5.4	10	0.866
Ah 217 - Ah 421	8.2	08	0.413	Ah 320 - Ah 630	8.5	10	0.576
Ah 320 - Ah 421	8.2	08	0.412	Ah 341 - Ah 630	6.7	10	0.752
Ah 341 - Ah 421	8.7	08	0.354	Ah 343 - Ah 630	7.2	10	0.706
Ah 343 - Ah 421	6.1	08	0.628	Ah 414 - Ah 630	6.4	10	0.779
Ah 414 - Ah 421	5.5	08	0.708	Ah 421 - Ah 630	5.0	08	0.750
Ah 205 - Ah 517	12.6	10	0.249	Ah 517 - Ah 630	3.1	10	0.978
Ah 209 - Ah 517	7.4	10	0.684	Ah 526 - Ah 630	9.6	08	0.292
Ah 211 - Ah 517	1.6	10	0.998	Ah 536 - Ah 630	6.1	10	0.808

APPENDIX 1: Genotypes of the 213 Great White and Great Blue herons used for tests of population differentiation

The following table contains genotypes of 213 Great White and Great Blue herons at 13 microsatellite loci. Alleles at each locus are numbered consecutively and genotypes are expressed as a four digit number (for example, sample EVL 146 is homozygous for allele number 2 at locus Ah 205 and heterozygous for alleles 3 and 6 at locus Ah 209).

Source ^a	Sample ID	Group ^b	Ah 205	Ah 209	Ah 211	Ah 217	Ah 320	Ah 341	Ah 343	Ah 414	Ah 421	Ah 517	Ah 526	Ah 536	Ah 630
UWBM	EVL 146	B-PNW	0202	0306	0707	0303	0202	0203	0407	0810	0303	0409	0506	0606	0303
UWBM	GKD 01	B-PNW	0303	0507	0207	0303	0203	0203	0707	0808	0304	0409	0614	0506	0203
UWBM	GKD 133	B-PNW	0000	0510	0207	0303	0202	0202	0404	0810	0000	0409	0715	0305	0303
UWBM	PJG 112	B-PNW	0203	0000	0307	0303	0203	0203	0404	0608	0304	0309	0816	0306	0303
UWBM	PJG 232	B-PNW	0303	0310	0707	0304	0203	0202	0404	0810	0303	0309	0606	0303	0303
UWBM	SMB 01	B-PNW	0303	0810	0707	0404	0203	0203	0204	0606	0303	0609	0814	0306	0303
UWBM	SMB 02	B-PNW	0203	0708	0207	0304	0202	0202	0405	0710	0303	0407	0815	0303	0203
UWBM	SMB 129	B-PNW	0303	0606	0707	0303	0202	0202	0207	0708	0404	0509	1516	0606	0203
UWBM	SMB 130	B-PNW	0303	0505	0207	0303	0204	0203	0507	0408	0304	0509	0000	0304	0303
UWBM	SMB 131	B-PNW	0303	0608	0307	0303	0202	0202	0404	0610	0304	0409	1216	0304	0303
UWBM	SMB 132	B-PNW	0202	0606	0202	0304	0202	0202	0204	0408	0303	0607	0000	0406	0303
FMNH	348377	B-N	0203	0508	0507	0304	0303	0202	0102	0910	0000	0404	0516	0505	0304
FMNH	348378	B-N	0303	0808	0507	0304	0202	0203	0103	0000	0000	0306	0916	0505	0203
FMNH	348380	B-N	0203	0508	0707	0102	0203	0202	0102	0710	0000	0308	0716	0303	0303
FMNH	348381	B-N	0203	0508	0707	0304	0202	0202	0107	0808	0000	0404	0712	0606	0303
FMNH	348382	B-N	0203	0508	0507	0304	0304	0102	0101	0608	0000	0404	0106	0505	0303
FMNH	348383	B-N	0203	0510	0205	0103	0203	0103	0407	0000	0000	0608	1217	0303	0203
FMNH	348384	B-N	0203	0000	0305	0304	0202	0303	0407	0707	0000	0306	0102	0305	0303
FMNH	348385	B-N	0303	0608	0305	0303	0204	0303	0204	0310	0000	0107	1515	0405	0303
FMNH	363357	B-N	0203	0808	0303	0303	0303	0202	0105	0308	0000	0609	0708	0505	0303
FMNH	387752	B-N	0303	0000	0307	0304	0203	0203	0107	0609	0000	0707	0000	0506	0204
FMNH	395626	B-N	0203	0303	0303	0304	0203	0203	0203	0608	0000	0404	1216	0303	0303

APPENDIX I continued

Source ^a	Sample ID	Group ^b	Ah	209	Ah	211	Ah	217	Ah	320	Ah	341	Ah	343	Ah	414	Ah	421	Ah	517	Ah	526	Ah	536	Ah	630
FMNH	395888	B-N	0203	0508	0303	0404	0202	0202	0303	0505	0610	0000	0608	0812	0303	0203										
FMNH	396953	B-N	0303	0408	0303	0303	0202	0202	0202	0307	0810	0000	0608	1014	0306	0303										
FMNH	396954	B-N	0303	0308	0103	0404	0303	0303	0203	0101	0608	0000	0406	1016	0506	0203										
FMNH	397031	B-N	0203	0808	0305	0404	0202	0103	0107	0710	0000	0303	0303	0510	0303	0304										
FMNH	397118	B-N	0203	0408	0507	0303	0202	0202	0202	0104	0810	0000	0608	1212	0304	0203										
FMNH	397119	B-N	0303	0308	0303	0404	0202	0202	0202	0407	0811	0000	0407	1010	0505	0303										
FMNH	429048	B-N	0203	0808	0707	0303	0203	0203	0303	0109	0000	0000	0406	1416	0506	0304										
FMNH	429049	B-N	0202	0508	0303	0304	0205	0205	0203	0405	0608	0000	0204	1014	0505	0303										
FMNH	429086	B-N	0202	0508	0305	0203	0203	0203	0203	0306	0608	0000	0608	0911	0505	0304										
BMNH	AF 1010	B-N	0303	0808	0103	0103	0303	0303	0203	0203	0608	0303	0608	0610	0306	0303										
BMNH	JK 93151	B-N	0202	0508	0303	0304	0202	0202	0303	0304	0609	0303	0506	0510	0305	0303										
BMNH	X 7089	B-N	0203	0408	0305	0304	0203	0203	0303	0404	0609	0303	0409	1616	0404	0203										
BMNH	X 7090	B-N	0203	0508	0305	0204	0202	0202	0303	0404	0508	0303	0404	1616	0306	0304										
BMNH	X 7091	B-N	0303	0508	0305	0304	0303	0303	0203	0507	0407	0303	0408	1115	0505	0203										
BMNH	X 7092	B-N	0203	0808	0507	0204	0203	0203	0103	0103	0608	0303	0608	0516	0303	0203										
BMNH	X 7093	B-N	0203	0505	0307	0203	0203	0203	0303	0105	0707	0303	0408	0809	0505	0303										
BMNH	X 7094	B-N	0303	0305	0707	0203	0203	0203	0203	0205	0710	0303	0809	1616	0303	0303										
BMNH	X 7095	B-N	0203	0508	0303	0304	0203	0203	0303	0103	0308	0303	0408	0708	0305	0202										
BMNH	X 7096	B-N	0304	0808	0306	0404	0303	0303	0203	0104	0607	0303	0909	1416	0304	0304										
HLM	04 03 00 N01	B-FP	0303	0408	0205	0304	0202	0202	0203	0102	0709	0303	0406	0405	0000	0102										
HLM	04 03 00 N02	B-FP	0000	0000	0505	0000	0303	0303	0203	0102	0407	0303	0000	0000	0000	0000										
HLM	04 03 00 N03	B-FP	0303	0808	0303	0203	0303	0303	0303	0204	0809	0303	0405	0612	0406	0304										
HLM	04 03 00 N04	B-FP	0203	0808	0307	0304	0203	0304	0203	0204	0608	0303	0309	0815	0404	0303										
HLM	04 03 00 N05	B-FP	0202	0808	0303	0304	0202	0304	0203	0203	1010	0303	0000	0816	0606	0303										
HLM	04 03 00 N06	B-FP	0203	0505	0303	0404	0102	0404	0303	0304	0708	0304	0509	1016	0405	0303										
HLM	04 03 00 N07	B-FP	0303	0508	0307	0204	0303	0204	0202	0102	0307	0303	0406	1216	0306	0203										
HLM	04 03 00 N08	B-FP	0103	0707	0707	0404	0202	0404	0202	0103	0210	0303	0408	0610	0406	0203										

APPENDIX I continued

Source ^a	Sample ID	Group ^b	Ah	205	Ah	209	Ah	211	Ah	217	Ah	320	Ah	341	Ah	343	Ah	414	Ah	421	Ah	517	Ah	526	Ah	536	Ah	630
HLM	04 03 00 N09	B-FP	0202	0410	0707	0204	0102	0203	0205	0709	0304	0406	0913	0105	0304	0203	0105	0709	0304	0304	0406	0406	0913	0105	0304	0105	0304	0304
HLM	04 03 00 N10	B-FP	0203	0808	0707	0102	0102	0203	0105	1010	0104	0209	0808	0305	0104	0203	0105	1010	0104	0104	0209	0808	0305	0404	0305	0404	0305	0404
HLM	04 03 00 N11	B-FP	0000	0000	0303	0000	0203	0303	0107	0707	0303	0000	0000	0303	0303	0107	0707	0707	0303	0303	0000	0000	0000	0405	0303	0405	0303	
HLM	04 03 00 N12	B-FP	0203	0408	0303	0000	0102	0303	0105	0610	0303	0406	0000	0303	0303	0105	0610	0610	0303	0303	0406	0000	0000	0000	0303	0000	0303	
HLM	04 03 00 N13	B-FP	0203	0808	0307	0000	0202	0303	0307	0106	0305	0609	0815	0303	0303	0307	0106	0106	0305	0305	0609	0815	0105	0203	0105	0203	0203	
HLM	04 03 00 N14	B-FP	0203	1010	0307	0303	0202	0202	0505	0707	0303	0609	1016	0202	0202	0505	0707	0707	0303	0303	0609	1016	0303	0303	0303	0303	0303	
HLM	04 03 00 N15	B-FP	0203	0508	0405	0202	0202	0203	0203	0710	0303	0609	0308	0203	0203	0203	0710	0710	0303	0303	0609	0308	0406	0303	0406	0303	0303	
HLM	04 03 00 N16	B-FP	0202	0405	0507	0404	0203	0203	0203	0707	0305	0608	0608	0203	0203	0203	0707	0707	0305	0608	0608	0608	0000	0202	0000	0202	0202	
HLM	04 03 00 N17	B-FP	0102	0305	0707	0404	0203	0303	0203	0707	0303	0409	1015	0303	0303	0203	0707	0707	0303	0409	1015	0606	0304	0606	0304	0606	0304	
HLM	04 03 00 N18	B-FP	0203	0308	0103	0304	0303	0103	0303	0708	0303	0608	0512	0203	0203	0303	0708	0708	0303	0608	0512	0405	0304	0405	0304	0405	0304	
HLM	04 03 00 N19	B-FP	0000	0000	0303	0203	0000	0303	0205	0404	0000	0000	0000	0303	0303	0205	0404	0404	0000	0000	0000	0000	0000	0000	0000	0000	0000	
HLM	04 03 00 N21	B-FP	0000	0910	0808	0000	0000	0808	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	0000	
HLM	04 04 00 N01	B-FP	0203	0808	0303	0203	0203	0303	0103	0707	0303	0709	0412	0203	0203	0103	0707	0707	0303	0303	0709	0412	0000	0000	0000	0000	0000	
HLM	04 04 00 N02	B-FP	0102	0808	0307	0304	0203	0303	0102	0910	0305	0408	0309	0303	0303	0102	0910	0910	0305	0408	0309	0408	0000	0304	0000	0304	0304	
HLM	04 04 00 N03	B-FP	0203	0610	0207	0404	0202	0303	0405	0607	0000	0000	0000	0303	0303	0405	0607	0607	0000	0000	0000	0000	0606	0000	0606	0000	0000	
HLM	12 03 98 N2C1	B-FB	0202	0308	0707	0304	0202	0303	0304	0102	0303	0809	0303	0303	0303	0304	0102	0102	0303	0809	0303	0000	0305	0303	0305	0303	0303	
HLM	12 09 98 N1C1	B-FB	0203	0505	0307	0203	0102	0203	0205	0810	0303	0000	0000	0203	0203	0205	0810	0810	0303	0000	0000	0000	0305	0202	0305	0202	0202	
HLM	12 15 98 N1C1	B-FB	0202	0508	0303	0202	0102	0203	0303	1010	0303	0809	0408	0203	0203	0303	1010	1010	0303	0809	0408	0305	0303	0303	0305	0303	0303	
HLM	12 15 98 N2C1	B-FB	0202	0000	0207	0203	0103	0303	0202	0610	0303	0909	1616	0303	0202	0202	0610	0610	0303	0909	1616	0303	0303	0203	0303	0203	0203	
HLM	12 15 98 N3C1	B-FB	0202	0508	0707	0404	0102	0203	0104	0108	0303	0606	0000	0203	0203	0104	0108	0108	0303	0606	0000	0000	0506	0202	0506	0202	0202	
HLM	12 16 98 N1C1	B-FB	0203	0505	0307	0204	0203	0303	0203	0110	0303	0106	1516	0303	0303	0203	0110	0110	0303	0106	1516	0305	0305	0304	0305	0304	0304	
HLM	12 18 98 N3C1	B-FB	0303	0405	0207	0204	0102	0203	0205	0910	0303	0609	0710	0203	0203	0205	0910	0910	0303	0609	0710	0506	0204	0204	0506	0204	0204	
HLM	12 21 98 N1C1	B-FB	0202	0808	0707	0304	0203	0303	0205	0608	0303	0509	0707	0303	0303	0205	0608	0608	0303	0509	0707	0000	0303	0303	0303	0303	0303	
HLM	01 10 99 N2C1	B-FB	0202	0304	0307	0404	0102	0202	0204	0809	0203	0606	1616	0202	0202	0204	0809	0809	0203	0606	1616	0404	0404	0303	0404	0303	0303	
HLM	01 10 99 N3C1	B-FB	0202	0508	0707	0303	0102	0202	0204	0110	0303	0309	1016	0202	0202	0204	0110	0110	0303	0309	1016	0506	0303	0303	0506	0303	0303	
HLM	01 10 99 N4C1	B-FB	0203	0305	0307	0203	0101	0303	0202	0110	0303	0609	0711	0303	0303	0202	0110	0110	0303	0609	0711	0306	0303	0303	0306	0303	0303	
HLM	01 12 99 N5C1	B-FB	0202	0305	0707	0204	0102	0303	0204	0110	0303	0606	0000	0303	0303	0204	0110	0110	0303	0606	0000	0303	0303	0303	0303	0303	0303	

APPENDIX I continued

Source ^a	Sample ID	Group ^b	Ah 205	Ah 209	Ah 211	Ah 217	Ah 320	Ah 341	Ah 343	Ah 414	Ah 421	Ah 517	Ah 526	Ah 536	Ah 630
HLM	01 14 99 NIC1	B-FB	0202	0308	0305	0204	0202	0203	0102	0107	0303	0104	0406	0305	0303
HLM	01 21 99 NIC1	B-FB	0203	0808	0307	0202	0202	0203	0104	0310	0303	0909	1216	0205	0303
HLM	01 21 99 N2C1	B-FB	0202	0305	0207	0204	0303	0303	0105	0909	0303	0506	0614	0205	0203
HLM	01 21 99 N3C2	B-FB	0202	0303	0707	0303	0204	0303	0405	0110	0303	0609	0516	0306	0303
HLM	01 21 99 N4C1	B-FB	0202	0305	0505	0202	0203	0203	0202	0110	0303	0609	0305	0306	0303
HLM	01 21 99 N5C1	B-FB	0202	0308	0207	0304	0203	0303	0205	0810	0303	0406	0608	0606	0303
HLM	01 27 99 NIC1	B-FB	0202	0408	0707	0304	0303	0303	0305	0108	0303	0609	1216	0404	0303
HLM	01 27 99 N5C1	B-FB	0202	0808	0307	0304	0103	0202	0205	1010	0303	0304	0616	0000	0203
HLM	01 27 99 N6C1	B-FB	0203	0305	0305	0204	0303	0303	0204	0106	0303	0809	1616	0303	0203
HLM	01 27 99 N7C1	B-FB	0203	0808	0707	0303	0303	0203	0304	0810	0303	0609	1216	0406	0304
HLM	02 03 99 N4C1	B-FB	0203	0108	0707	0204	0103	0303	0202	0810	0303	0606	1116	0303	0303
HLM	02 03 99 N5C1	B-FB	0202	0308	0707	0304	0202	0202	0204	1010	0303	0105	0516	0306	0303
HLM	02 03 99 N6C1	B-FB	0202	0308	0707	0202	0204	0303	0205	1010	0303	0406	0814	0306	0303
HLM	02 06 99 NIC1	B-FB	0203	0508	0507	0202	0203	0303	0405	0708	0303	0609	0306	0406	0203
HLM	02 06 99 N2C1	B-FB	0202	0305	0707	0404	0303	0203	0202	0610	0303	0809	1015	0106	0303
HLM	02 06 99 N3C1	B-FB	0202	0608	0207	0404	0203	0303	0104	0810	0303	0406	1016	0406	0203
HLM	02 06 99 N4C1	B-FB	0202	0508	0707	0304	0303	0303	0405	0810	0303	0608	0516	0000	0303
HLM	02 06 99 N6C1	B-FB	0203	0208	0303	0304	0304	0203	0104	0810	0303	0209	1516	0606	0000
HLM	02 06 99 N8C1	B-FB	0203	0608	0207	0202	0203	0203	0202	0810	0303	0409	1216	0206	0203
HLM	02 09 99 NIC2	B-FB	0202	0303	0707	0303	0203	0303	0404	0609	0303	0409	0808	0303	0303
HLM	02 17 99 NIC1	B-FB	0203	0305	0307	0404	0203	0203	0102	0707	0303	0409	0816	0406	0202
HLM	02 17 99 N2C1	B-FB	0202	0508	0307	0203	0303	0203	0407	0108	0303	0608	0000	0505	0202
HLM	02 17 99 N3C1	B-FB	0202	0308	0707	0303	0202	0303	0203	0608	0303	0608	0312	0203	0303
HLM	11 13 98 NIC2	W-FB	0202	0308	0507	0304	0203	0303	0407	0101	0303	0809	0511	0203	0203
HLM	11 13 98 N2C1	W-FB	0202	0208	0707	0303	0103	0303	0407	0101	0303	0609	1516	0506	0303
HLM	11 15 98 NIC1	W-FB	0202	0305	0207	0203	0102	0303	0202	1010	0303	0608	1114	0606	0304
HLM	11 29 98 NIC1	W-FB	0203	0305	0303	0203	0102	0203	0202	1010	0303	0609	1016	0406	0203

APPENDIX I continued

Source ^a	Sample ID	Group ^b	Ah	205	Ah	209	Ah	211	Ah	217	Ah	320	Ah	341	Ah	343	Ah	414	Ah	421	Ah	517	Ah	526	Ah	536	Ah	630
HLM	11 30 98 N1C4	W-FB	0202	0508	0507	0303	0202	0303	0202	0303	0202	0303	0202	0303	0404	0106	0106	0303	0303	0606	0616	0000	0303	0303	0202	0303	0303	0303
HLM	11 30 98 N2C1	W-FB	0202	0305	0307	0303	0203	0303	0203	0303	0203	0203	0203	0203	0205	0110	0110	0303	0303	0609	0813	0306	0202	0303	0306	0202	0303	
HLM	11 30 98 N3C1	W-FB	0202	0203	0707	0304	0102	0304	0102	0304	0102	0203	0203	0203	0205	0108	0108	0303	0303	0909	0810	0306	0303	0303	0306	0303	0303	
HLM	12 03 98 N1C1	W-FB	0202	0305	0707	0304	0303	0304	0303	0304	0303	0303	0303	0303	0202	1010	1010	0303	0303	0409	1516	0404	0303	0303	0404	0303	0303	
HLM	12 03 98 N3C1	W-FB	0203	0808	0707	0303	0103	0303	0103	0303	0103	0303	0303	0303	0407	0609	0609	0303	0303	0609	0814	0202	0303	0303	0202	0303	0303	
HLM	12 05 98 N1C1	W-FB	0202	0208	0207	0203	0102	0203	0102	0203	0102	0203	0203	0203	0105	0609	0609	0303	0303	0106	0404	0506	0304	0304	0506	0304	0304	
HLM	12 06 98 N1C1	W-FB	0202	0505	0307	0303	0204	0303	0204	0303	0204	0303	0204	0303	0207	0110	0110	0303	0303	0608	1415	0303	0202	0303	0303	0202	0303	
HLM	12 06 98 N2C1	W-FB	0202	0000	0707	0204	0103	0204	0103	0204	0103	0203	0203	0303	0204	0202	0202	0303	0303	0409	0615	0303	0303	0303	0303	0303	0303	
HLM	12 06 98 N3C1	W-FB	0202	0303	0707	0304	0203	0304	0203	0304	0203	0203	0203	0000	0107	0710	0710	0303	0303	0406	0816	0303	0303	0303	0303	0303	0303	
HLM	12 09 98 N1C1	W-FB	0203	0505	0307	0203	0102	0203	0102	0203	0102	0203	0102	0203	0205	0810	0810	0303	0303	0606	1616	0305	0202	0303	0305	0202	0303	
HLM	12 10 98 N1C1	W-FB	0303	0308	0307	0204	0202	0204	0202	0204	0202	0202	0202	0103	0205	0808	0808	0303	0303	0608	0000	0405	0303	0303	0405	0303	0303	
HLM	12 13 98 N1C1	W-FB	0202	0308	0307	0304	0303	0307	0304	0304	0303	0303	0303	0303	0202	0910	0910	0303	0303	0106	0816	0606	0303	0303	0606	0303	0303	
HLM	12 13 98 N2C1	W-FB	0202	0304	0307	0404	0202	0404	0202	0404	0202	0202	0202	0303	0304	0110	0110	0303	0303	0608	1016	0306	0303	0303	0306	0303	0303	
HLM	12 16 98 N2C1	W-FB	0202	0000	0303	0304	0304	0303	0304	0304	0304	0304	0304	0203	0104	0109	0109	0303	0303	0606	0000	0506	0203	0303	0506	0203	0303	
HLM	12 16 98 N3C1	W-FB	0203	0000	0307	0202	0203	0307	0202	0202	0203	0203	0203	0203	0205	0110	0110	0303	0303	0609	0000	0105	0303	0303	0105	0303	0303	
HLM	12 16 98 N4C1	W-FB	0203	0000	0707	0303	0102	0303	0102	0303	0102	0203	0102	0203	0207	0110	0110	0303	0303	0306	0611	0406	0303	0303	0406	0303	0303	
HLM	12 17 98 N1C1	W-FB	0203	0808	0507	0404	0303	0507	0404	0404	0303	0203	0303	0203	0102	0409	0409	0303	0303	0609	0816	0505	0303	0303	0505	0303	0303	
HLM	12 17 98 N2C1	W-FB	0203	0305	0307	0304	0202	0307	0304	0304	0202	0202	0202	0202	0207	0101	0101	0303	0303	0308	0816	0303	0303	0303	0303	0303	0303	
HLM	12 17 98 N3C1	W-FB	0202	0000	0707	0304	0202	0707	0304	0304	0202	0202	0202	0303	0202	0409	0409	0303	0303	0404	0000	0406	0203	0303	0406	0203	0303	
HLM	12 18 98 N1C1	W-FB	0000	0508	0303	0404	0102	0303	0404	0404	0102	0303	0303	0303	0305	0101	0101	0303	0303	0809	0811	0303	0203	0303	0303	0303	0303	
HLM	12 18 98 N2C1	W-FB	0203	0305	0707	0304	0202	0707	0304	0304	0202	0202	0202	0303	0307	0110	0110	0303	0303	0608	1616	0306	0303	0303	0306	0303	0303	
HLM	12 19 98 N1C1	W-FB	0203	0304	0707	0304	0101	0707	0304	0304	0101	0303	0303	0303	0204	1010	1010	0303	0303	0606	0815	0306	0203	0303	0306	0203	0303	
HLM	12 20 98 N1C1	W-FB	0202	0808	0707	0304	0102	0707	0304	0304	0102	0102	0102	0202	0202	0609	0609	0303	0303	0709	0000	0303	0203	0303	0303	0203	0303	
HLM	12 21 98 N2C1	W-FB	0203	0205	0707	0203	0102	0707	0203	0203	0102	0102	0102	0303	0205	1010	1010	0303	0303	0909	1616	0506	0303	0303	0506	0303	0303	
HLM	12 21 98 N3C1	W-FB	0202	0305	0303	0303	0202	0303	0303	0303	0202	0202	0202	0202	0102	0609	0609	0303	0303	0304	0616	0306	0303	0303	0306	0303	0303	
HLM	12 21 98 N4C1	W-FB	0202	0308	0707	0404	0202	0707	0404	0404	0202	0202	0202	0303	0202	0410	0410	0303	0303	0608	0909	0405	0303	0303	0405	0303	0303	
HLM	12 21 98 N5C1	W-FB	0203	0305	0303	0203	0103	0303	0203	0203	0103	0103	0103	0203	0307	0107	0107	0303	0303	0308	0000	0606	0303	0303	0606	0303	0303	

APPENDIX I continued

Source ^a	Sample ID	Group ^b	Ah	205	Ah	209	Ah	211	Ah	217	Ah	320	Ah	341	Ah	343	Ah	414	Ah	421	Ah	517	Ah	526	Ah	536	Ah	630
HLM	12 21 98 N6C1	W-FB	0203	0303	0707	0202	0203	0303	0202	0910	0303	0202	0910	0303	0609	1616	0406	0203										
HLM	12 22 98 N1C1	W-FB	0203	0508	0707	0304	0102	0303	0407	0810	0303	0407	0810	0303	0609	1016	0304	0303										
HLM	12 22 98 N2C1	W-FB	0202	0000	0707	0203	0102	0203	0405	0103	0303	0407	0516	0306	0303	0303	0303	0303										
HLM	12 22 98 N3C1	W-FB	0202	0808	0507	0304	0202	0303	0207	0108	0303	0000	0000	0000	0304	0606	0306	0303										
HLM	12 23 98 N1C1	W-FB	0202	0105	0507	0304	0202	0303	0204	0110	0303	0304	0606	0306	0303	0303	0303	0303										
HLM	12 23 98 N2C1	W-FB	0202	0305	0707	0304	0202	0303	0608	0106	0303	0608	1616	0306	0303	0303	0303	0303										
HLM	12 31 98 N1C1	W-FB	0202	0303	0707	0303	0202	0303	0202	1010	0303	0609	0808	0305	0303	0303	0303	0303										
HLM	01 06 99 N1C1	W-FB	0202	0308	0307	0304	0101	0203	0203	0710	0303	0606	0615	0405	0303	0303	0303	0303										
HLM	01 08 99 N1C1	W-FB	0202	0508	0707	0303	0101	0303	0105	0110	0203	0607	1416	0303	0303	0303	0303	0303										
HLM	01 10 99 N1C1	W-FB	0202	0303	0707	0203	0101	0000	0202	0607	0303	0409	0909	0606	0204	0203	0203	0203										
HLM	01 12 99 N1C1	W-FB	0203	0308	0707	0304	0202	0000	0204	0110	0203	0608	1116	0203	0203	0203	0203	0203										
HLM	01 12 99 N2C1	W-FB	0202	0203	0307	0303	0102	0303	0404	0107	0303	0406	0116	0406	0303	0406	0303	0303										
HLM	01 12 99 N3C1	W-FB	0202	0808	0707	0304	0202	0303	0505	0910	0303	0606	0316	0206	0203	0203	0203	0203										
HLM	01 12 99 N4C1	W-FB	0202	0305	0307	0304	0203	0303	0404	1010	0303	0000	0211	0304	0203	0303	0303	0303										
HLM	01 12 99 N6C1	W-FB	0203	0508	0307	0303	0202	0303	0202	0110	0303	0909	1416	0303	0303	0303	0303	0303										
HLM	01 12 99 N7C1	W-FB	0202	0305	0707	0303	0202	0303	0507	0610	0303	0606	0515	0606	0203	0203	0203	0203										
HLM	01 13 99 N1C1	W-FB	0202	0808	0707	0303	0102	0303	0304	0109	0303	0409	0516	0204	0204	0204	0204	0204										
HLM	01 13 99 N2C1	W-FB	0202	0808	0707	0202	0203	0303	0204	1010	0303	0000	0816	0303	0303	0303	0303	0303										
HLM	01 14 99 N2C1	W-FB	0202	0205	0507	0203	0102	0203	0204	0610	0303	0408	1014	0406	0303	0303	0303	0303										
HLM	01 17 99 N1C1	W-FB	0202	0308	0107	0303	0102	0303	0205	0709	0303	0106	0811	0306	0303	0303	0303	0303										
HLM	01 17 99 N2C1	W-FB	0202	0308	0305	0303	0202	0303	0204	0710	0303	0408	0308	0505	0203	0203	0203	0203										
HLM	01 17 99 N3C1	W-FB	0203	0808	0707	0204	0202	0303	0205	0109	0303	0408	1616	0303	0303	0303	0303	0303										
HLM	01 18 99 N1C1	W-FB	0202	0308	0707	0304	0203	0303	0304	1010	0303	0109	1016	0406	0203	0203	0203	0203										
HLM	01 18 99 N2C1	W-FB	0202	0808	0303	0304	0202	0103	0404	0407	0103	0608	0609	0505	0303	0303	0303	0303										
HLM	01 18 99 N3C1	W-FB	0303	0505	0307	0104	0102	0202	0205	0910	0303	0608	1016	0305	0303	0303	0303	0303										
HLM	01 19 99 N1C1	W-FB	0202	0505	0307	0303	0102	0303	0304	0810	0303	0409	1116	0000	0303	0303	0303	0303										
HLM	01 19 99 N2C1	W-FB	0203	0508	0707	0303	0202	0303	0102	0710	0303	0609	0616	0303	0303	0303	0303	0303										

APPENDIX I continued

Source ^a	Sample ID	Group ^b	Ah	205	Ah	209	Ah	211	Ah	217	Ah	320	Ah	341	Ah	343	Ah	414	Ah	421	Ah	517	Ah	526	Ah	536	Ah	630
HLM	01 19 99 N3C1	W-FB	0203	0308	0707	0303	0102	0303	0303	0102	0303	0102	0303	0303	0102	0303	0110	0203	0306	1616	0303	0303	0303	0303	0303	0303	0303	0303
HLM	01 19 99 N4C1	W-FB	0203	0308	0307	0304	0103	0304	0303	0103	0303	0103	0303	0303	0407	0410	0303	0309	1111	0206	0203	0203	0203	0203	0203	0203	0203	
HLM	01 20 99 N1C1	W-FB	0202	0305	0307	0303	0102	0303	0303	0102	0303	0102	0303	0203	0105	0709	0303	0607	0814	0206	0303	0303	0303	0303	0303	0303	0303	
HLM	01 26 99 N1C1	W-FB	0202	0505	0707	0303	0303	0303	0303	0303	0303	0303	0303	0303	0104	0106	0303	0607	0516	0306	0303	0303	0303	0303	0303	0303	0303	
HLM	01 26 99 N2C1	W-FB	0203	0808	0707	0204	0202	0204	0303	0202	0303	0202	0303	0303	0204	0110	0303	0406	0610	0306	0203	0203	0203	0203	0203	0203	0203	
HLM	01 27 99 N2C1	W-FB	0202	0305	0707	0404	0204	0404	0303	0204	0303	0204	0303	0303	0205	0109	0305	0409	1616	0606	0203	0203	0203	0203	0203	0203	0203	
HLM	01 27 99 N3C1	W-FB	0203	0305	0307	0102	0203	0102	0303	0203	0303	0203	0303	0303	0202	0110	0303	0406	1616	0306	0203	0203	0203	0203	0203	0203	0203	
HLM	01 27 99 N4C1	W-FB	0405	0708	0307	0204	0203	0204	0303	0204	0303	0203	0303	0203	0407	0810	0303	0406	0508	0405	0203	0203	0203	0203	0203	0203	0203	
HLM	01 29 99 N1C1	W-FB	0202	0508	0707	0303	0202	0303	0303	0202	0303	0202	0303	0303	0405	0910	0303	0709	0810	0306	0303	0303	0303	0303	0303	0303	0303	
HLM	01 31 99 N1C1	W-FB	0202	0308	0307	0203	0203	0203	0303	0203	0303	0203	0303	0203	0102	0110	0303	0809	0811	0206	0203	0203	0203	0203	0203	0203	0203	
HLM	01 31 99 N2C1	W-FB	0203	0808	0707	0303	0303	0303	0303	0303	0303	0303	0303	0303	0405	0610	0303	0606	0814	0306	0303	0303	0303	0303	0303	0303	0303	
HLM	02 02 99 N1C1	W-FB	0202	0303	0307	0203	0102	0203	0303	0102	0303	0102	0303	0303	0405	0110	0303	0404	0611	0203	0303	0303	0303	0303	0303	0303	0303	
HLM	02 02 99 N2C1	W-FB	0203	0303	0707	0203	0204	0203	0303	0204	0303	0204	0303	0202	0307	0110	0303	0406	1010	0303	0303	0303	0303	0303	0303	0303	0303	
HLM	02 03 99 N1C1	W-FB	0202	0505	0305	0404	0303	0404	0303	0303	0303	0303	0202	0202	0202	0810	0303	0606	0808	0305	0203	0203	0203	0203	0203	0203		
HLM	02 03 99 N2C1	W-FB	0202	0305	0707	0303	0203	0303	0303	0203	0303	0203	0303	0303	0407	0110	0303	0608	0815	0306	0203	0203	0203	0203	0203	0203		
HLM	02 03 99 N3C1	W-FB	0202	0308	0707	0304	0103	0304	0303	0103	0303	0103	0303	0303	0204	0110	0303	0408	0812	0306	0304	0304	0304	0304	0304	0304		
HLM	02 06 99 N5C1	W-FB	0303	0505	0707	0303	0101	0303	0303	0101	0303	0101	0303	0303	0507	0810	0303	0406	0616	0606	0202	0202	0202	0202	0202	0202		
HLM	02 06 99 N7C1	W-FB	0203	0508	0307	0304	0304	0304	0303	0304	0304	0304	0203	0203	0205	0809	0303	0408	1016	0606	0203	0203	0203	0203	0203	0203		
HLM	02 07 99 N1C1	W-FB	0202	0204	0707	0404	0101	0404	0303	0101	0303	0101	0303	0303	0202	0809	0303	0109	0815	0305	0303	0303	0303	0303	0303	0303		
HLM	12 26 96 - 1	W-OK	0203	0810	0307	0304	0303	0304	0303	0304	0303	0303	0303	0303	0000	1010	0303	0109	1217	0305	0304	0304	0304	0304	0304	0304		
HLM	12 30 96 N1C2	W-OK	0202	0508	0707	0304	0202	0304	0303	0202	0103	0202	0103	0202	0202	0109	0304	0404	1616	0306	0303	0303	0303	0303	0303	0303		
HLM	12 30 96 N2C1	W-OK	0202	0308	0707	0404	0102	0404	0303	0102	0303	0102	0303	0303	0202	0108	0303	0406	0608	0606	0303	0303	0303	0303	0303	0303		
HLM	01 02 97 N1C1	W-OK	0202	0000	0707	0303	0103	0303	0303	0103	0203	0103	0203	0203	0205	0110	0000	0000	0000	0303	0303	0303	0303	0303	0303	0303		
HLM	01 02 97 N2C1	W-OK	0202	0305	0707	0304	0202	0304	0303	0202	0203	0202	0203	0203	0202	0101	0304	0606	0816	0103	0303	0303	0303	0303	0303	0303		
HLM	01 02 97 N3C1	W-OK	0202	0303	0707	0304	0103	0304	0303	0304	0103	0103	0303	0303	0404	0612	0304	0609	0505	0606	0303	0303	0303	0303	0303	0303		
HLM	01 02 97 N4C1	W-OK	0203	0308	0707	0304	0202	0304	0303	0202	0303	0202	0303	0303	0202	0810	0303	0406	1616	0101	0303	0303	0303	0303	0303	0303		
HLM	01 02 97 N5C1	W-OK	0202	0303	0303	0000	0203	0000	0303	0000	0203	0203	0303	0303	0205	0612	0303	0606	0816	0606	0203	0203	0203	0203	0203	0203		

APPENDIX I continued

Source ^a	Sample ID	Group ^b	Ah	205	Ah	209	Ah	211	Ah	217	Ah	320	Ah	341	Ah	343	Ah	414	Ah	421	Ah	517	Ah	526	Ah	536	Ah	630
HLM	01 04 97 N1C1	W-OK	0202	0508	0707	0000	0103	0303	0204	1010	0303	0107	0516	0103	0303	0303	0303	0303	0303	0303	0303	0107	0516	0103	0303	0303	0303	0303
HLM	01 06 97 N1C1	W-OK	0202	0508	0207	0203	0203	0203	0205	0109	0303	0607	0506	0303	0303	0303	0303	0303	0303	0303	0303	0607	0506	0303	0303	0303	0303	0303
HLM	01 06 97 N2C1	W-OK	0202	0808	0707	0303	0103	0203	0204	0104	0303	0000	0506	0606	0000	0303	0303	0303	0303	0303	0303	0000	0506	0606	0606	0000	0000	0000
HLM	01 07 97 N1C1	W-OK	0202	0303	0707	0203	0102	0203	0204	0109	0303	0404	0607	0405	0303	0303	0303	0303	0303	0303	0303	0404	0607	0405	0405	0303	0303	0303
HLM	01 12 98 N1C1	W-OK	0202	0303	0707	0404	0102	0203	0205	0910	0303	0406	0206	0204	0303	0303	0303	0303	0303	0303	0303	0406	0206	0204	0204	0303	0303	0303
HLM	01 12 98 N2C1	W-OK	0202	0000	0707	0303	0103	0203	0105	0808	0000	0606	0416	0101	0303	0303	0303	0303	0303	0303	0303	0606	0416	0101	0101	0303	0303	0303
HLM	01 17 98 N1C1	W-OK	0202	0308	0707	0404	0203	0303	0405	0708	0303	0606	1616	0203	0303	0303	0303	0303	0303	0303	0303	0606	1616	0203	0303	0303	0303	0303
HLM	01 17 98 N2C1	W-OK	0203	0305	0307	0404	0204	0303	0202	0106	0303	0406	1516	0505	0303	0303	0303	0303	0303	0303	0303	0406	1516	0505	0303	0303	0303	0303
HLM	01 17 98 N3C1	W-OK	0202	0203	0707	0204	0203	0203	0204	0104	0303	0606	0505	0306	0202	0303	0303	0303	0303	0303	0303	0606	0505	0306	0202	0202	0303	0303
HLM	01 17 98 N5C1	W-OK	0202	0308	0307	0304	0203	0203	0205	1010	0303	0308	0516	0202	0303	0303	0303	0303	0303	0303	0308	0516	0202	0202	0203	0203	0303	0303
HLM	01 17 98 N6C1	W-OK	0202	0505	0707	0404	0103	0303	0205	0610	0303	0406	0310	0203	0203	0303	0303	0303	0303	0303	0406	0310	0203	0203	0203	0203	0203	0203
HLM	01 18 98 N1C1	W-OK	0202	0305	0707	0404	0203	0303	0205	0610	0303	0409	1616	0306	0203	0303	0303	0303	0303	0303	0409	1616	0306	0306	0203	0203	0203	0203
HLM	01 18 98 N2C1	W-OK	0202	0308	0307	0304	0103	0303	0202	0108	0303	0406	0508	0304	0303	0303	0303	0303	0303	0303	0406	0508	0304	0304	0303	0303	0303	0303
HLM	01 20 98 N1C1	W-OK	0202	0308	0707	0404	0404	0303	0204	0609	0303	0406	0508	0306	0303	0303	0303	0303	0303	0303	0406	0508	0306	0306	0303	0303	0303	0303
HLM	01 21 98 N2C1	W-OK	0202	0308	0707	0404	0102	0203	0204	0106	0303	0606	0616	0303	0303	0303	0303	0303	0303	0303	0606	0616	0303	0303	0303	0303	0303	0303
HLM	01 31 98 N1C1	W-OK	0202	0305	0707	0404	0101	0303	0203	0610	0303	0308	0316	0304	0303	0303	0303	0303	0303	0303	0308	0316	0304	0304	0303	0303	0303	0303
HLM	01 31 98 N2C1	W-OK	0202	0305	0707	0304	0101	0203	0505	0410	0303	0309	0000	0206	0304	0303	0303	0303	0303	0303	0309	0000	0206	0206	0304	0304	0304	0304
HLM	02 08 99 N1C1	W-OK	0202	0303	0303	0202	0104	0203	0203	0610	0303	0406	0810	0306	0303	0303	0303	0303	0303	0303	0406	0810	0306	0306	0303	0303	0303	0303
HLM	02 08 99 N3C1	W-OK	0203	0505	0707	0304	0101	0203	0203	0808	0303	0406	0816	0306	0303	0303	0303	0303	0303	0303	0406	0816	0306	0306	0303	0303	0303	0303
HLM	02 08 99 N4C1	W-OK	0202	0308	0707	0304	0202	0203	0204	1010	0303	0406	0516	0206	0303	0303	0303	0303	0303	0303	0406	0516	0206	0206	0303	0303	0303	0303
HLM	02 08 99 N5C1	W-OK	0202	0308	0707	0204	0103	0203	0207	0410	0203	0406	1216	0305	0303	0303	0303	0303	0303	0303	0406	1216	0305	0305	0303	0303	0303	0303
HLM	02 19 99 N1C1	W-OK	0202	0808	0707	0204	0103	0303	0204	0110	0303	0409	1616	0103	0303	0303	0303	0303	0303	0303	0409	1616	0103	0304	0303	0303	0303	0303
HLM	02 26 99 N1C1	W-OK	0202	0308	0707	0404	0303	0203	0204	1010	0303	0409	0506	0304	0303	0303	0303	0303	0303	0303	0409	0506	0304	0304	0303	0303	0303	0303
HLM	02 26 99 N2C1	W-OK	0202	0303	0303	0304	0203	0203	0204	0107	0304	0608	0616	0404	0303	0303	0303	0303	0303	0303	0608	0616	0404	0404	0303	0303	0303	0303
HLM	02 26 99 N3C1	W-OK	0202	0308	0307	0303	0103	0303	0105	0410	0303	0404	1616	0304	0303	0303	0303	0303	0303	0303	0404	1616	0304	0304	0303	0303	0303	0303
HLM	02 26 99 N4C1	W-OK	0202	0303	0307	0303	0101	0303	0202	0810	0103	0406	1616	0306	0303	0303	0303	0303	0303	0303	0406	1616	0306	0306	0303	0303	0303	0303
HLM	02 26 99 N5C1	W-OK	0202	0808	0707	0404	0103	0303	0205	1010	0303	0406	0516	0303	0303	0303	0303	0303	0303	0303	0406	0516	0303	0303	0303	0303	0303	0303

APPENDIX I continued

Source ^a	Sample ID	Group ^b	Ah 205	Ah 209	Ah 211	Ah 217	Ah 320	Ah 341	Ah 343	Ah 414	Ah 421	Ah 517	Ah 526	Ah 536	Ah 630
HLM	03 10 99 N4C1	W-OK	0202	0508	0707	0404	0101	0303	0205	0109	0303	0404	1616	0306	0303
HLM	03 10 99 N5C1	W-OK	0202	0308	0507	0304	0103	0303	0202	0609	0303	0407	1116	0303	0203

^a UWBM = University of Washington, Burke Museum, BMNH = Bell Museum of Natural History, HLM = Heather L. McGuire

^b B = great blue heron, W = great white heron, PNW = Pacific Northwest, N = north-central United states, FP = Florida peninsula, FB = Florida Bay, OK = outer Keys

APPENDIX J: Allele frequencies at 12 microsatellite loci for six *A. herodias* groups

The following tables contain allele frequencies at 12 microsatellite loci for six *A. herodias* groups (number of individuals genotyped in parentheses). Abbreviations: B = Great Blue Heron, W = Great White Heron, PNW = Pacific Northwest, N = north-central United states, FP = Florida peninsula, FB = Florida Bay, OK = outer Keys.

Allele frequencies at locus Ah 205

Allele	(10) B-PNW	(30) B-N	(19) B-FP	(35) B-FB	(76) W-FB	(37) W-OK
01			0.079			
02	0.300	0.383	0.474	0.814	0.789	0.946
03	0.700	0.600	0.447	0.186	0.197	0.054
04		0.017			0.007	
05					0.007	

Allele frequencies at locus Ah 209

Allele	(10) B-PNW	(28) B-N	(20) B-FP	(34) B-FB	(71) W-FB	(35) W-OK
01				0.015	0.007	
02				0.015	0.049	0.014
03	0.100		0.050	0.265	0.310	0.457
04		0.089	0.100	0.044	0.021	
05	0.200	0.054	0.150	0.265	0.282	0.186
06	0.300	0.268	0.025	0.029		
07	0.100	0.018	0.050	0.000	0.007	
08	0.150	0.554	0.475	0.368	0.324	0.329
09			0.025			
10	0.150	0.018	0.125			0.014

Allele frequencies at locus Ah 217

Allele	(11) B-PNW	(30) B-N	(18) B-FP	(35) B-FB	(77) W-FB	(35) W-OK
01		0.050	0.028		0.013	
02		0.100	0.222	0.329	0.156	0.100
03	0.773	0.450	0.278	0.314	0.545	0.343
04	0.277	0.400	0.472	0.357	0.286	0.557

APPENDIX J continued

Allele frequencies at locus Ah 320

Allele	(11) B-PNW	(30) B-N	(21) B-FP	(35) B-FB	(77) W-FB	(37) W-OK
01			0.095	0.171	0.247	0.365
02	0.773	0.550	0.571	0.400	0.513	0.270
03	0.182	0.400	0.333	0.386	0.208	0.311
04	0.045	0.033		0.043	0.032	0.054
05		0.017				

Allele frequencies at locus Ah 341

Allele	(11) B-PNW	(30) B-N	(22) B-FP	(35) B-FB	(74) W-FB	(37) W-OK
01		0.067			0.020	0.014
02	0.773	0.417	0.364	0.300	0.209	0.230
03	0.227	0.517	0.636	0.700	0.770	0.757

Allele frequencies at locus Ah 343

Allele	(11) B-PNW	(30) B-N	(22) B-FP	(35) B-FB	(77) W-FB	(36) W-OK
01		0.283	0.205	0.100	0.071	0.028
02	0.136	0.100	0.273	0.386	0.377	0.542
03		0.133	0.227	0.100	0.058	0.042
04	0.545	0.200	0.091	0.243	0.221	0.181
05	0.091	0.117	0.159	0.157	0.149	0.194
06		0.017			0.006	
07	0.227	0.133	0.045	0.014	0.110	0.014
08					0.006	
09		0.017				

APPENDIX J continued

Allele frequencies at locus Ah 414

Allele	(11) B-PNW	(27) B-N	(22) B-FP	(35) B-FB	(77) W-FB	(37) W-OK
01			0.023	0.171	0.247	0.203
02			0.023	0.014	0.013	
03		0.056	0.023	0.014	0.006	
04	0.091	0.019	0.068		0.032	0.068
05		0.019				
06	0.182	0.222	0.091	0.086	0.071	0.135
07	0.091	0.167	0.409	0.057	0.065	0.027
08	0.409	0.278	0.091	0.214	0.078	0.122
09		0.074	0.091	0.071	0.123	0.095
10	0.227	0.148	0.182	0.371	0.364	0.324
11		0.019				
12						0.027

Allele frequencies at locus Ah 421

Allele	(10) B-PNW	(10) B-N	(20) B-FP	(35) B-FB	(77) W-FB	(35) W-OK
01			0.025		0.006	0.014
02				0.014	0.019	0.014
03	0.700	1.000	0.825	0.986	0.968	0.914
04	0.300		0.075			0.057
05			0.075		0.006	

Allele frequencies at locus Ah 517

Allele	(11) B-PNW	(30) B-N	(17) B-FP	(34) B-FB	(74) W-FB	(35) W-OK
01		0.017		0.044	0.034	0.029
02		0.017	0.029	0.015		
03	0.091	0.083	0.029	0.029	0.047	0.043
04	0.227	0.300	0.235	0.118	0.169	0.357
05	0.091	0.017	0.059	0.044		
06	0.091	0.217	0.265	0.338	0.365	0.400
07	0.091	0.067	0.029		0.041	0.043
08		0.200	0.118	0.103	0.142	0.043
09	0.409	0.083	0.235	0.309	0.203	0.086

APPENDIX J continued

Allele frequencies at locus Ah 526

Allele	(9) B-PNW	(29) B-N	(17) B-FP	(31) B-FB	(70) W-FB	(35) W-OK
01		0.034			0.007	
02		0.017			0.007	0.014
03			0.059	0.081	0.014	0.029
04			0.059	0.032	0.014	0.014
05	0.056	0.069	0.059	0.065	0.043	0.186
06	0.222	0.034	0.088	0.081	0.086	0.114
07	0.056	0.069		0.065		0.014
08	0.167	0.069	0.206	0.097	0.171	0.100
09		0.052	0.059		0.036	
10		0.138	0.118	0.065	0.086	0.029
11		0.034		0.032	0.086	0.014
12	0.056	0.103	0.118	0.081	0.007	0.029
13			0.029		0.007	
14	0.111	0.069		0.032	0.057	
15	0.167	0.052	0.088	0.048	0.064	0.014
16	0.167	0.241	0.118	0.323	0.314	0.429
17		0.017				0.014

Allele frequencies at locus Ah 536

Allele	(11) B-PNW	(30) B-N	(15) B-FP	(32) B-FB	(74) W-FB	(37) W-OK
01			0.067	0.016	0.007	0.095
02				0.063	0.068	0.095
03	0.409	0.367	0.133	0.328	0.358	0.392
04	0.136	0.083	0.267	0.125	0.108	0.108
05	0.091	0.417	0.200	0.188	0.135	0.068
06	0.364	0.133	0.333	0.281	0.324	0.243

Allele frequencies at locus Ah 630

Allele	(11) B-PNW	(30) B-N	(19) B-FP	(34) B-FB	(77) W-FB	(36) W-OK
01			0.026			
02	0.136	0.183	0.158	0.235	0.214	0.083
03	0.864	0.700	0.632	0.721	0.747	0.889
04		0.117	0.184	0.044	0.039	0.028

VITA

Heather L. McGuire was born in Randolph, Vermont, 15 April 1961. She graduated from Queensbury High School, Queensbury, New York, in 1979 and earned a Bachelor of Arts in Biology from the University of Rochester, Rochester, New York, in 1983. Heather was employed as a research technician in the Department of Surgery, Strong Memorial Hospital, University of Rochester School of Medicine and Dentistry, Rochester, New York, until 1987. She entered the graduate program of the College of William and Mary, Virginia Institute of Marine Science (VIMS), Gloucester Point, Virginia, in the fall of 1987. She was employed as a graduate research assistant for the Department of Ocean and Coastal Law, VIMS (1987-1990), served as a graduate intern with the King William County Planning Department, King William, Virginia (1989), and earned a Master of Arts in Marine Science from VIMS in 1990. Heather was employed as a marine scientist, Department of Biological Sciences, VIMS (1990), a field assistant for the United States Department of the Interior, Bureau of Land Management, Northern Field Office, Fairbanks, Alaska (1991), an instructor for the Department of Natural Resources, Planning, and Interpretation, Humboldt State University, Arcata, California (1992), and a biologist for the United States Department of the Interior, National Park Service, Redwood National Park, Orick, California (1993-1994). In 1995, Heather entered the Ph.D. program in Wildlife and Fisheries Science, School of Forestry, Wildlife, and Fisheries, Louisiana State University and Agricultural and Mechanical College, Baton Rouge, Louisiana.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Heather L. McGuire

Major Field: Wildlife & Fisheries Science

Title of Dissertation: Evaluating the Taxonomic Status of the Great White Heron (*Ardea herodias occidentalis*) Using Morphological, Behavioral and Genetic Evidence.

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

24 October 2001