Taxonomic Status of the Great White Heron (*Ardea herodias occidentalis*): An Analysis of Behavioral, Genetic, and Morphometric Evidence

FINAL REPORT

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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*. Support for this classification stems largely from limited observations of interbreeding between white and blue herons. Breeding ranges overlap in the Florida Keys (USA) providing an opportunity to study the degree to which these 2 taxa are reproductively isolated. The current classification leads to predictions of random mate choice ($H_0$1), no genetic divergence ($H_0$2), and no significant size differences ($H_0$3) between sympatric white and blue herons. I tested $H_0$1 by monitoring breeding herons in Florida Bay (Monroe County, Florida). I observed more white/white and blue/blue pairs and fewer mixed pairs than expected in a randomly mating population, suggesting that premating isolating mechanisms exist within the Florida Bay breeding population. Although the pattern of mate choice was assortative, the number of mixed pairs (17 of 114 pairs) suggested that significant gene flow may occur between white and blue herons in Florida Bay or between the Florida Bay breeding population and other great blue heron populations. I compared allele frequencies at 12 microsatellite loci from 6 *A. herodias* groups: great blue herons from the Pacific Northwest (B-PNW), great blue herons from the north-central United States (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). I found significant differences in allele frequencies among all groups compared. Differences among the Florida Keys groups (W-FB, B-FB, and W-OK) were small compared to differences between these Florida Keys groups and the B-FP group, even though the B-FP sample was collected <80 km from Florida Bay. I found no significant size differences between sympatric great white herons and intermediate Würdemann's herons at any of 6 morphometric variables; however, Würdemann's herons averaged slightly smaller than great white herons at all 6 variables. This observation is consistent with the hypothesis that these herons are the result of hybridization between great white herons and great blue herons, which are significantly smaller than great white herons. Together, these data show that the Florida Keys population is distinct and, although reproductive isolation does not appear to be complete, suggest that migration into the Florida Keys population from other great blue heron populations is limited. The great white heron appears to be a good biological species, and a review of its taxonomic status is merited. Because recruitment from other great blue heron populations does not appear to be a factor in maintaining the great white heron population, conservation will require understanding and managing this population as an isolate.

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INTRODUCTION

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* (American Ornithologists' Union [AOU] 1973). Seven great blue heron subspecies are recognized in North America and are distinguished by differences in size, plumage, and geographic distribution (AOU 1957, 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). 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). This population's vulnerability to natural catastrophic events and to habitat loss and deterioration resulting from human activities motivated my research, which seeks to understand the relationships among North American great white and great blue heron populations.

Despite a lively historical debate over 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 2 taxa are reproductively isolated (if at all). I collected behavioral, genetic, and morphometric data to test hypotheses regarding mate choice, genetic differences, and size differences between white and blue herons. My objectives are to clarify the great white heron's taxonomic status and provide information that may inform conservation efforts.

Background and Project Objectives

Support for the current classification stems largely from 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). There are also "blue" herons (Würdemann's herons) in south Florida with plumage that is clearly intermediate between a great white heron and a great blue heron. The Würdemann's heron is widely believed to be a hybrid, but it has also been regarded as a distinct species (*A. würdemannii*), a light color phase of the great

blue heron, or a dark color phase of the great white heron (Holt 1928). Not only is the status of the Würdemann's heron unclear, but there are conflicting accounts of which blue form predominates in the Florida Keys. Holt (1928) and Mayr (1956) independently examined museum skins of herons in breeding condition collected 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, 59) state that there are "apparently no published reports of the nesting of 2 Würdemann's herons," casting doubt on the fertility of these intermediates and implying that great blue herons breeding in the Florida Keys may be something other than Würdemann's herons (presumably *A. h. wardi*).

A leading hypothesis for the situation, as it is understood today, is that the great white heron diverged from the great blue heron in isolation (Mayr 1956, Lazell 1989), perhaps in the Caribbean during a Pleistocene interglacial when much of the Florida peninsula was submerged (Shinn 1988). 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 2 taxa currently interbreed. The importance of observations of mixed pairs and the existence of putative hybrids may be overemphasized. A key question is whether white and blue individuals interbreed freely or only rarely (Mayr 1956). The current classification implies that isolating mechanisms have not accrued between great white and great blue herons, but there are no published field studies that support or refute this assumption. 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. In contrast, Robertson (1978) suggested that mixed pairs occur "about" one order of magnitude lower than expected from a randomly mating population (no details of methodology are given) and Powell's unpublished surveys reportedly support Robertson's hypothesis of positive assortative mating (Powell and Bjork 1996).

Morphological data are also equivocal. Size differences between great white herons and great blue herons have been documented, but comparable measurements from sympatric blue and white herons have not been published. According to Holt (1928), an index of proportion (culmen length divided by tarsus length) "sharply separates" white *occidentalis* collected in Monroe County (extreme south Florida including Florida Bay and the Florida Keys) from blues (*A. h. wardi*) collected on the Florida peninsula. Holt does not provide his supporting data. Mayr (1956) presents another index (bill length divided by wing length) for 11 white *occidentalis* and 14 *wardi*. While

acknowledging that *occidentalis* differs from *wardi* by an average longer bill, Mayr notes much overlap in the values of this index. Zachow (1983) showed significant size differences among "northern" great blue, "southern" great blue, and great white herons. However, the southern blue specimens were collected from the Florida peninsula outside the area where white and blue herons are sympatric. Because these analyses compare allopatric populations, they fail to directly address the species/subspecies debate surrounding south Florida's "polymorphic population."

As a first step toward answering the question of whether reproductive isolating mechanisms exist between these 2 taxa, I collected behavioral, genetic, and morphometric data to evaluate the relationships between great white and great blue heron populations. The current classification leads to predictions of random mate choice between sympatric white and blue herons ($H_0$1), no genetic divergence between sympatric white and blue herons ($H_0$2), and no significant size differences between sympatric white and blue herons ($H_0$3). First, I tested the hypothesis that sympatric great white and great blue herons pair randomly with respect to plumage color. Rejection of this hypothesis suggested that premating isolating mechanisms currently exist within this population. Second, I compared the distribution of mitochondrial haplotypes (inferred from cytochrome *b* sequence data) among phenotypically distinct great white and great blue heron populations. I also isolated nuclear microsatellite markers and tested for genetic differentiation among these populations (H_0: no difference in allele frequencies among populations). The patterns of genetic differentiation provide insight into the amount of gene flow between Florida's great white heron population and other great blue heron populations. Finally, I collected morphometric data from museum specimens to test for size differences between sympatric white and blue herons.

METHODS

Mate Choice

Natural History and Study Area.—Approximately 850 pairs of great white herons breed in the shallow marine and coastal mangrove environments of Florida Bay and the Florida Keys (Powell and Bjork 1996). Florida Bay is a large shallow estuary which 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. The outer Keys refer to the portion of the Florida Keys that extend beyond Florida Bay's western boundary, which lies approximately along a line drawn between Cape Sable on the southwestern Florida peninsula and Long Key in the Florida Keys. Although some nonbreeding birds do move seasonally to freshwater wetlands on the southern Florida peninsula, the great white heron population is essentially nonmigratory and many birds spend the entire year within the Florida Keys ecosystem (Powell and Bjork 1990).

Great white herons build nests on small mangrove islands within Florida Bay and 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, where they are considered *A. h. occidentalis*, but they also breed in a variety of environments on the Florida peninsula, where they are considered *A. h. wardi.* 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 and occurs from 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 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 (Leica 8x44 binoculars or Swarovski 60x spotting scope) to observe nests from a distance and monitored each nest until I observed adults engaged in activities that positively identified them as a pair (e.g., switching incubation duties). I attempted to determine whether the plumage of each blue adult was characteristic of a "typical" great blue heron or of an "intermediate"

Würdemann's heron. However, for reasons discussed below (see Results: Mate Choice), adult phenotype is reported here as either white or blue.

I determined mate choice at 114 nests and used the number of white and blue adults from this sample 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 3 pairing categories (white/white, white/blue, blue/blue). I used a χ^2 goodness of fit test for the difference between observed and expected values (Sokal and Rohlf 1995), subtracting 1 degree of freedom for the total sample size and 1 degree of freedom because sample frequencies were used to generate expected values (df = 1).

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.

Mitochondrial DNA: Cytochrome *b* Sequence Data

Mitochondrial DNA (mtDNA) sequence data has proven to be a valuable tool for resolving phylogenetic relationships among avian taxa that have recently diverged (Moore and DeFilippis 1997). In herons, cytochrome *b* has evolved at 5–10 times the rate of single-copy nuclear DNA (Sheldon et al. 2000). In general, cytochrome *b* works well for resolving relatively recent evolutionary history and has been used to examine genetic variation at the population level in a number of taxa (Randi et al. 1994, Apostolidis et al. 1997, Moore and DeFilippis 1997, Rassmann et al. 1997, Ruedi et al. 1997, Brown and Pestano 1998). I compared mtDNA cytochrome *b* sequences from great white herons and great blue herons representing 4 of the 7 recognized great blue heron subspecies (*occidentalis, wardi, herodias,* and *fannini*). I chose these groups because I wanted to look at large-scale geographic patterns of genetic divergence among phenotypically distinct allopatric and parapatric populations. 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). *A. h. wardi* is distributed throughout the southeastern and south-central United States, including the southern Florida peninsula. *A. h. occidentalis* is restricted to extreme south Florida (Florida Bay and the Florida Keys). *A. h. fannini* is narrowly distributed along the Pacific coasts of Washington (USA), British Columbia (Canada), and southeastern Alaska (USA). *A. h. fannini* is allopatric with the other 3

subspecies and probably rarely interbreeds with any of these. *A. h. herodias* and *A. h. wardi* are parapatric. *A. h. herodias* is migratory, especially in the northern portion of its range, and many individuals have winter ranges that overlap with *A. h. wardi* (Eckert 1981). The degree to which these 2 subspecies interbreed has not been investigated. *A. h. wardi* and *A. h. occidentalis* are also parapatric. Although Würdemann's heron is commonly believed to be a hybrid between these 2 subspecies, the degree to which *wardi* and *occindentalis* interbreed is also unknown.

Tissue Samples.—*A. h. herodias* (n = 5) and *A. h. fannini* (n = 5) tissues were provided by the Bell Museum of Natural History (University of Minnesota) and the Burke Museum (University of Washington), respectively (Table 1). Although the *A. h. fannini* tissues are not identified to subspecies, it is unlikely that they contain individuals from other subspecies. Some, especially those collected in the fall, could be migrants from more northern populations, but these migrants, although not local breeders, would be members of the same subspecies. I collected *A. h. wardi* tissues (n = 5) in the southern portion this subspecies' range (Water Conservation Area 3A, Miami-

Table 1. *A. herodias* tissue samples used for mtDNA cytochrome *b* sequencing.

Subspecies	Specimen ID[a]	Type[b]	Date Collected mm	dd	yyyy	Location State: County
fannini[c]	UWBM SMB 01	m	07		1998	Wash.: King
	UWBM EVL 146	m	08	07	1998	Wash.: King
	UWBM GKD 01	m	fall		1990	Wash.: Mason
	UWBM PJG 112	m	fall		1998	Wash.: Mason
	UWBM PJG 232b	m				Wash.: Jefferson
herodias	BMNH AF1010	m	08	07	1952	Minn.
	BMNH JK93151	m			1990	Minn.
	BMNH X7089	m	07	27	1992	Minn.: Cass
	BMNH X7090	m	04	30	1992	Minn.: Washington
	BMNH X7091	m	09	28	1991	Wis.
wardi	HLM 04 03 00 N1	f	04	03	2000	Fla.: Miami-Dade
	HLM 04 03 00 N2	f	04	03	2000	Fla.: Miami-Dade
	HLM 04 03 00 N3	f	04	03	2000	Fla.: Miami-Dade
	HLM 04 03 00 N4	f	04	03	2000	Fla.: Miami-Dade
	HLM 04 03 00 N5	f	04	03	2000	Fla.: Miami-Dade
occidentalis	LSUMZ B-29641	m				Fla.: Monroe
	LSUMZ B-29642	m	12	22	1996	Fla.: Monroe
	LSUMZ B-29643	m				Fla.: Monroe
	LSUMZ B-29644	m	03	30	1997	Fla.: Monroe
	LSUMZ B-29645	m	01	30	1997	Fla.: Monroe

[a]UWBM = University of Washington, Burke Museum; BMNH = Bell Museum of Natural History; HLM = Heather L. McGuire; LSUMZ = Louisiana State University, Museum of Natural Science.

[b]m = skeletal muscle, f = feather.

[c]Putative subspecies based on locality data.

Dade County, Florida) where individuals are unlikely to interbreed directly with *A. h. herodias*. To minimize the possibility of including migrants from northern subspecies, I collected feathers from nestlings (Marsden and May 1984). These samples, therefore, are representative of the local breeding population. Feathers were kept on ice for 3–4 days and then stored at -80°C. I collected *A. h. occidentalis* (n = 5) tissues from fatally injured great white herons donated to the Museum of Natural Science at Louisiana State University (LSUMNS) by the Florida Keys Wild Bird Center (Key Largo, Florida). Carcasses were packed in dry ice and shipped to LSUMNS where samples of heart, liver, and skeletal muscle were collected and stored at -80°C.

DNA Extraction and Cytochrome* b *Sequencing.—I isolated genomic DNA from muscle and feather samples with a DNeasy Tissue Kit (QIAGEN, Valencia, California). 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 (microliter) of 10 mM (millimolar) 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).

I modified existing PCR (polymerase chain reaction) primers (Helm-Bychowski and Cracraft 1993, Sheldon et al. 2000) to create 3 primer pairs with similar melting temperatures (Tm) and to maximize sequence similarity between the new primers and great blue heron mtDNA cytochrome *b* sequence (Sheldon et al. 2000). The 3 primer pairs (Table 2) amplify overlapping fragments, which cover 1,048 base pairs (bp) of the cytochrome *b* gene and 28 bp of the adjacent $tRNA^{Thr}$ gene. Standard PCR reagents and thermal profiles

Table 2. PCR primers used for amplification of *A. herodias* mtDNA cytochrome *b*.

Primer ID[a]	Primer Sequence (5' to 3')	Tm (°C)
L 14990[b]	CCA TCC AAC ATC TCT GCT TGA TGA AA	60.00
H 15424[c]	GGA AGT GAA GGG CGA AGA ATC G	59.23
L 15320[c]	TCC ATG AGG ACA AAT ATC CTT CTG AGG	59.71
H 15710[b]	GAA TGG CGT AGG CAA ATA GGA AGT ATC	59.22
L 15660[b]	CAT ACC TCT TAG GAG ACC CAG AAA AC	58.17
H 16067[b]	GGA GTC TTC AGT CTC TGG TTT ACA AG	58.41

[a]L and H denote primers located on the light and heavy strand of the mtDNA genome. The 5-digit number refers to the base pair location at the 3' end of the primer, referenced to the complete chicken mtDNA sequence (Desjardins and Morais 1990).

[b]Modified from Helm-Bychowski and Cracraft (1993).

[c]Modified from Sheldon et al. (2000).

were used to amplify these 1,076 bp of the mtDNA genome. Each 50 µl reaction volume contained the following: 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 1.5 mM $MgCl_2$, 200 µM (micromolar) dNTPs, 0.05 µM L-strand primer, 0.05 µM H-strand primer, 1.25 units *Taq* polymerase (Applied Biosystems, Foster City, California), and approximately 20–200 ng DNA template. I overlaid reaction mixtures with light mineral oil. I used a Hybaid Omn-E thermal cycler and the following touchdown PCR thermal profile for all reactions: an initial denaturing step at 95°C for 5 minutes; then X cycles of 94°C for 60 seconds, Y°C for 30 seconds, 72°C for 30 seconds; followed by a final rapid thermal ramp to 40°C; X and Y equal 3 and 61, then 3 and 58, then 3 and 55, and finally 24 and 52. A 5 µl aliquot of the reaction mixture was visualized under ultraviolet illumination after electrophoresis through a 2% agarose gel stained with ethidium bromide. For successful reactions (a single distinct band of the appropriate length), I cleaned the remaining PCR product with a QIAquick PCR Purification Kit (QIAGEN) and eluted clean product from the QIAGEN mini column with 30 µl sterile distilled deionized water (ddH_20).

I used an ABI PRISM® Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) to sequence PCR products. Each 10 µl reaction volume contained 2 µl PCR product (clean), 2 µl BigDye ready reaction mix, and 3.2 picomolar (final concentration) H-strand or L-strand primer. 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 seconds, 50°C for 15 seconds, and 60°C for 4 minutes. 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 LSUMNS where they were resuspended 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. I repeated PCR amplification and sequencing reactions for any PCR products that produced ambiguous electropherograms. I sequenced both strands (heavy and light) and aligned sequences by eye.

Nuclear DNA: Microsatellite Allele Frequency Data

Given the lack of resolution afforded by the mtDNA cytochrome *b* sequence data (see Results: Mitochondrial DNA), I chose to isolate microsatellite markers, which are highly variable nuclear genetic markers. Because they evolve so rapidly, microsatellites are particularly well suited for

genetic analyses at the level of populations or individuals and 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), and provide a resolution not possible with many other more slowly evolving co-dominant nuclear markers (e.g., allozymes).

To evaluate large- and small-scale geographic patterns of genetic differentiation among great white and great blue heron populations, I analyzed microsatellite allele frequency data for 6 *A. herodias* groups: great blue herons from the Pacific Northwest (B-PNW), great blue herons from the north-central United States (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) (Table 3). My primary objectives were to determine if allele frequencies were significantly different between great white herons and great blue herons breeding in Florida Bay and whether either (or both) were different from great blue herons breeding on the nearby Florida peninsula. I also wanted to compare these differences (if any) with those found among geographically distant populations of allopatric and parapatric great blue heron subspecies (see Mitochondrial DNA: Cytochrome *b* Sequence Data, above, for rational regarding sample selection). Finally, I looked for evidence of genetic structure within the great white heron population by comparing samples collected along the outer Keys with those collected in Florida Bay. Meyerriecks (1957) proposed that the gap between Florida Bay and the outer Keys might

Table 3. *A. herodias* groups used to examine large scale geographic patterns of genetic differentiation and number of individuals genotyped in each group.

Group[a]	Subspecies	*n*	Comments
B-PNW	*fannini*[b]	11	Tissues collected in Washington
B-N	*herodias*	30	Tissues collected in Minnesota and Wisconsin
B-FP	*wardi*	23	Tissues collected in Miami-Dade County, Florida
B-FB	*occidentalis*	35	Tissues collected in Monroe County, Florida
W-FB	*occidentalis*	77	Tissues collected in Monroe County, Florida
W-OK	*occidentalis*	37	Tissues collected in Monroe County, Florida

[a]B-PNW = great blue herons from the Pacific Northwest, B-N = great blue herons from the north-central United States, B-FP = great blue herons from the southern Florida peninsula, B-FB = great blue herons from Florida Bay, W-FB = great white herons from Florida Bay, and W-OK = great white herons from the outer Keys.

[b]Putative subspecies based on locality data.

effectively split south Florida's great white herons into 2 distinct breeding populations, but Robertson (1978) doubted the existence of this gap.

Sample Collection and Preparation.—*A. h. fannini* (B-PNW) tissues from adult great blue herons collected in Washington were provided by the Burke Museum. *A. 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 (Chicago). 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. Many adult great blue herons in south Florida during winter and early spring are nonbreeding winter migrants. By collecting tissues from nestlings, I ensured that the B-FB and B-FP samples were representative of local breeding populations. Sample details can be found in Appendix A.

I collected blood samples from the tibio-tarsal vein using a sterile 1-ml syringe and 25-gauge needle (Gaunt and Oring 1997). Approximately 0.1 ml of blood was mixed with 1.0 ml 10% EDTA (ethylenediaminetetraacetic acid) anticoagulant/preservative buffer. I allowed red blood cells (RBCs) to settle overnight, removed and discarded the plasma/EDTA supernatant, resuspended the RBCs in 1 ml 10% EDTA, and refrigerated the samples at 4°C. I isolated genomic DNA from each tissue (blood, feather, or muscle) as described above (see DNA Extraction and Cytochrome *b* Sequencing). 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).

Microsatellite Isolation and Selection of Loci.—I isolated microsatellite markers using an enrichment technique developed by Hamilton 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). The enrichment protocol used here employs a subtractive hybridization to increase the proportion of microsatellite repeats in the genomic DNA insert library prior to cloning. 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 priming site (to recover double-stranded DNA) and contain restriction sites to create compatible ends for cloning. Details of the enrichment protocol are provided in Hamilton et al. (1999). Except where indicated, I used reagent concentrations and reaction conditions suggested by the authors.

I used *Sau*3AI, *Nhe*I and *Hha*I to digest *A. herodias* genomic DNA and conducted separate hybridization reactions for 4 different biotinylated oligonucleotide repeat motifs: $(CA)_{15}$, $(TC)_{15}$, $(AGC)_{10}$, and $(CATA)_7CA$. I used *Nhe*I to digest linker sequences and ligated the repeat enriched library into pUC19 plasmid DNA that had been digested with *Xba*I. I transformed plasmids into *Escherichia coli* (Life Technologies, Library Efficiency® DH5α™) 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 µl reaction volume contained 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 µM 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 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 minutes; 30 cycles of 94°C for 60 seconds, 55°C for 30 seconds, 72°C for 30 seconds; rapid thermal ramp to 40°C. A 5 µl aliquot of the reaction mixture was visualized under ultraviolet light after electrophoresis through a 2% agarose gel stained with ethidium bromide. For successful reactions (i.e., those with a distinct band 300–1,000 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 µl sterile ddH20. I used 2 µl of clean product in subsequent sequencing reactions, which were performed as above (see DNA Extraction and Cytochrome *b* Sequencing), except pUC19 primers (forward or reverse) were used in the reaction mix.

I used Oligo Analyzer 2.0 (Integrated DNA Technologies, <http://www.idtdna.com>) to design PCR primers for sequences that contained a microsatellite with 9 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 3 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 µl 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 µM forward and reverse primers, 0.04 µM IRD labeled M13 primer (LI-COR, Lincoln,

Nebraska), 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 minutes; X cycles of 94°C for 60 seconds, Y°C for 30 seconds, 72°C for 30 seconds; rapid thermal ramp to 40°C; X and Y equal 3 and 61, then 3 and 58, and finally 27 and Tm -5°C (see Table 6 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). Microsatellites with 2 or more alleles were used to genotype the remainder of the *A. herodias* DNA samples.

I used a number of criteria to select loci for genetic analyses. First, I omitted those 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 (1,000 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, 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 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, 1998) to amplify homologous regions of 2 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 2 bands and males have 1 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 2 CHD genes. Each 10 µl PCR volume contained 50 mM Tris-HCl (pH 8.3), 20 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 1 µM 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 5 units *Hae*III (New England Biolabs, Beverly, Massachusetts) and a 1x final concentration of the restriction buffer supplied with the enzyme (37°C, 1 hour). Restriction digests were visualized under ultraviolet illumination after electrophoresis through a 2% agarose gel stained with ethidium bromide. Females had 2 bands (approximately 400 bp and 335 bp) and males had 1 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 < 1, 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 1995, 2000) and TFPGA (Miller 1997), respectively. I used FSTAT to calculate pairwise F_{ST} and R_{ST} (an F_{ST} analog often calculated for microsatellite data) values; F_{ST} and R_{ST} 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 $\alpha = 0.0033$).

Morphometrics

Although Zachow (1983) found significant size differences between great white herons in the Florida Keys and great blue herons from the Florida peninsula, no study has published comparable measurements from sympatric great white and great blue herons. To determine whether size differences exist between sympatric white and blue herons, I examined museums skins of adult great white herons and intermediate Würdemann's herons and measured 6 variables (length of exposed culmen, depth of bill at base, length of tarsus, wing chord, length of tail, length of middle toe) commonly reported for herons (Appendix B). My selection criteria included great blue herons collected in Monroe County, Florida, during summer (to exclude possible winter migrants) and great white and intermediate Würdemann's herons regardless of collection date. Because size differences between males and females require that they be analyzed separately, I omitted birds whose sex was unknown. I did not find any herons with "typical" great blue heron plumage that met my selection criteria. My data set, therefore, contains great white herons (17 females, 24 males) and intermediate Würdemann's herons (12 females, 14 males). I used calipers to measure depth of bill at base to the nearest 0.1 mm (Baldwin et al. 1931, Proctor and Lynch 1993). I used a ruler with an upright stop at the zero point to measure the wing chord (leading edge of the wrist joint to the tip of the longest primary) to the nearest 0.5 mm (Proctor and Lynch 1993). I used dividers and a ruler to measure the remaining variables to the nearest 0.5 mm (Baldwin et al. 1931, Proctor and Lynch 1993). To guard against errors, I measured each variable at least twice. I calculated a mean and 95% confidence interval (CI) for each variable in each group (intermediate or white).

RESULTS

Mate Choice

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. However, among blue adults there appeared to be a continuum of phenotypes ranging 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, white breast and belly streaked with gray or black, and pale bluish-gray wings and back). If I define 3 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 4). I rejected the random mating hypothesis using a χ^2 goodness of fit test for the difference between observed and expected values ($\chi^2 = 31.32$, df = 1, $p < 0.001$).

Table 5 contains nestling and adult phenotypes for 2 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

Table 4. Observed (O) and expected (E) values for each pairing category expressed as number of mated pairs and percentage of total.

Adult Phenotypes		Number of Mated Pairs		Percentage of Total	
		O	E[a]	O	E[a]
White	White	83	72.96	73	64
White	Blue	17	36.48	15	32
Blue	Blue	14	4.56	12	4

[a]Expected values were calculated assuming p(white) = 0.8, q(blue) = 0.2, and adults pair randomly with respect to plumage color.

Table 5. 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

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 2 blue parents could produce a brood of all white offspring.

Mitochondrial DNA: Cytochrome *b* Sequence Data

I sequenced 1,076 bp of the mtDNA genome in great white herons and great blue herons collected from 4 *A. herodias* subspecies. Three PCR primer pairs amplified overlapping fragments, which covered 1,048 bp of the cytochrome *b* gene and 28 bp of the adjacent $tRNA^{Thr}$ gene. The primers were highly specific and produced unambiguous sequence data. I found 2 haplotypes among the 20 individuals sampled (complete sequences provided in Appendix C). Nineteen sequences shared Haplotype 1. Haplotype 2, which differed from Haplotype 1 by a single base pair, was found in a great blue heron sample from Miami-Dade County, Florida (sample ID: HLM 04 03 00 N2). This single base pair difference, a third position transition, is a synonymous substitution.

Nuclear DNA: Microsatellite Allele Frequency Data

Microsatellite Isolation and Selection of Loci.—Sixty sequences contained microsatellites with 4 or more repeat units (GenBank accession numbers AF447926–AF447985). I designed PCR primers for 28 loci that had 9 or more repeats. Twenty-six primer pairs amplified products of the appropriate length (2 failed to amplify any product). Seventeen produced PCR products that could be reliably scored (Table 6). 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 9 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. Attempts to isolate CATA and ACG microsatellites did not produce many clones containing these repeat

units. Since ACG was reported to be the most abundant microsatellite repeat in the brown-headed cowbird (*Molothrus ater*) (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*.

I used 12 of the 17 loci, presented in Table 6, 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 contained too little variation across the groups being compared. 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 (H_0: genotypes at 1 locus are independent from genotypes at another locus) in 3 of 78 pairwise tests. All 3 involved the same microsatellite locus—Ah 211 vs. Ah 341 (p = 0.0235, df = 10), Ah 211 vs. Ah 526 (p = 0.0312, df = 8), and Ah 211 vs. Ah 630 (p = 0.0067, df = 10). Although I would expect, by chance, to reject approximately 4 of 78 tests at the 0.05 significance level and only 1 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 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 6 *A. herodias* groups at the remaining 12 microsatellite loci. I rejected the null hypothesis (H_0: observed genotype frequencies do not deviate from HW expectations, α = 0.05) in 8 of 72 tests. One locus in W-FB (Ah 205, p = 0.0111), 1 locus in B-FP (Ah 209, p = 0.0097), 2 loci in W-OK (Ah 517, p = 0.0166; Ah 536, p = 0.0375), and 4 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 2 loci (Ah 517 and Ah 536) deviated from HW expectations within more than 1 *A. herodias* group, suggesting that null alleles are not an issue for any of these loci. I would expect to reject, by chance, only about 4 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.

Table 6. Microsatellite loci for *Ardea herodias* and cross-species amplification in closely related taxa.

Locus	Repeat[a]	Size (bp)[b]	# of alleles[c]	n[d]	Amplified in[e]: al	ci	co	Primer sequences (5' to 3')	Tm (°C)
Ah 205	$(AC)_{12}$	256	05	282	x[f]	x	x	F: CTGTGGAAGCAAGGCTACCC	58.9
								R: GTCCTCATTGTAGGCTGATTCTTGG	59.7
Ah 208	$(CA)_{10}$	207	01	40	x[f]	x[f]	x[f]	F: GCTAATAACACCCAGTGTGGACC	59.0
								R: GACCCTGTACATACACTTCTAAAACCC	58.7
Ah 209	$(AC)_{16}$	214	10	270	np	np	x[f]	F: GAAACACATCAGTGCAAGAGCAG	58.1
								R: AGTTAAGGAACAAATGTTTGGAAGGAATG	59.3
Ah 210	$(CA)_{11}$	179	02	40	x[f]	x	x	F: ACGGGAACGTTTCAAAAATTTAAGATGTG	59.0
								R: ACGTTTCTATGGCTCAGAAACTGG	58.7
Ah 211	$(CA)_{13}$	152	10	291	x[f]	x	x	F: GCTCATCAGGAGTTGAATCTGGC	59.2
								R: TCTGTCATTCAGCAATGGACC	56.3
Ah 212	$(CA)_{9}$	173	01	247	x[f]	x[f]	x	F: TCAGGCTAACTTTGGGCAAAGC	59.7
								R: AGCCCACTTTCATGACTTGCAG	59.4
Ah 217	$(CA)_{10}$	178	04	286	x	np	x[f]	F: GCTCAGGCTCTGCTTTGTCTAC	59.0
								R: CACAGATTCAAAACAAGCACCATGC	59.3
Ah 320	$(AC)_{13}$	188	05	289	x	x	x	F: TTAGGAGCAAGATTTTAAAGAAGGTGC	57.7
								R: AAGTGCTGGGTCATACTGGAATAG	58.0
Ah 341	$(AC)_{12}$	160	03	287	x[f]	x[f]	x[f]	F: GGTAATGATTCTGATTTACCACTGAGGG	58.9
								R: ATGTGTTATCATATCTGGTCTTCACAGC	58.7
Ah 343	$(AC)_{17}$	228	09	288	x	x[f]	np	F: CATTGCTTAACTTCTGAAGAAAC	58.6
								R: CTTGACCCAGCATTTGTGAATAAAACTG	59.0
Ah 414	$(AC)_{22}$	240	12	287	x	np	np	F: CATTCCAGCTGCTCTTCATTCTTG	57.9
								R: GGCAAAAGCAACTAGGGGC	57.7
Ah 421	$(CA)_{15}$	182	05	263	x[f]	x[f]	x[f]	F: CCCGTTCCACGCTGCTC	59.2
								R: GCCTGCTCACCGAGTGC	59.2
Ah 517	$(TC)_{15}$	176	09	277	np	np	x	F: TTTTCCATCATGCTTCCATCAATACG	57.8
								R: GGCACAAACCAGTAGAGCAATATAATC	57.8
Ah 522	$(TCTTC)_{25}$	260	18	37	x	x	nd	F: TTGTGGGACTAAACAGTGAAGCAG	58.9
								R: CAAAGCTGATTTAAAGATGTTCCATCCC	58.5

Table 6. Continued.

Locus	Repeat[a]	Size (bp)[b]	# of alleles[c]	n[d]	Amplified in[e]: al	ci	co	Primer sequences (5' to 3')	Tm (°C)
Ah 526	$(TC)_{27}$	256	18	267	x	np	x	F: GAATGGGGAAGAGAACTGAAAGAGC	59.2
								R: CACTGCTCAGGGACTGGC	58.5
Ah 536	$(AC)_{14}$	130	07	273	x[f]	x[f]	x[f]	F: CCCTGGTTTAGATCACATGATGGAG	58.6
								R: CTGGGCAACCTGTTCCATCT	58.4
Ah 630	$(TC)_{12}$	122	04	286	x	np	x	F: TCCTCCTTCACAATGCTACTTGC	58.7
								R: CGGCAGGCAGTATTATTTCAGTGG	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*.

Descriptive Statistics and Tests of Population Differentiation.—I determined the genotype of 213 *A. herodias* individuals at 12 microsatellite loci. Allele frequencies and sample sizes for each locus in each of the *A. herodias* groups are provided in Appendix D. In general, heterozygosity estimates (Table 7) were high in all 6 *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). Values for F_{ST} and R_{ST} (Table 8) reveal genetic structure among the 6 *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 9). Allele frequencies of great white herons breeding in Florida Bay (W-FB) were significantly different from those of great blue herons breeding in Florida Bay (B-FB). Both W-FB and B-FB were significantly different from great white herons breeding along the outer Keys (W-OK). All 3 of these groups were significantly different 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 7. Nei's (1978) unbiased heterozygosity (H) and number of individuals genotyped (*n*) for each *A. herodias* group at each microsatellite locus.

	B-PNW[a]		B-N		B-FP		B-FB		W-FB		W-OK	
Locus	**H**	***n***	**H**	***n***	**H**	***n***	**H**	***n***	**H**	***n***	**H**	***n***
Ah 205	0.442	10	0.501	30	0.585	19	0.307	35	0.340	76	0.104	37
Ah 209	0.847	10	0.621	28	0.738	20	0.732	34	0.722	71	0.658	35
Ah 217	0.368	11	0.636	30	0.668	18	0.675	35	0.600	77	0.570	35
Ah 320	0.385	11	0.545	30	0.567	21	0.670	35	0.636	77	0.704	37
Ah 341	0.368	11	0.564	30	0.474	22	0.426	35	0.365	74	0.380	37
Ah 343	0.654	11	0.834	30	0.815	22	0.758	35	0.771	77	0.642	36
Ah 414	0.766	11	0.830	27	0.786	22	0.782	35	0.780	77	0.817	37
Ah 421	0.442	10	0.000	10	0.315	20	0.029	35	0.064	77	0.163	35
Ah 517	0.784	11	0.818	30	0.824	17	0.772	34	0.777	74	0.709	35
Ah 526	0.895	09	0.899	29	0.913	17	0.863	31	0.845	70	0.766	35
Ah 536	0.706	11	0.678	30	0.782	15	0.770	32	0.737	74	0.763	37
Ah 630	0.247	11	0.471	30	0.556	19	0.430	34	0.398	77	0.205	36
All	0.572		0.622		0.671		0.597		0.576		0.540	

[a]B-PNW = great blue herons from the Pacific Northwest, B-N = great blue herons from the north-central United States, B-FP = great blue herons from the southern Florida peninsula, B-FB = great blue herons from Florida Bay, W-FB = great white herons from Florida Bay, and W-OK = great white herons from the outer Keys.

Table 8. Pairwise F_{ST} values across all loci (above diagonal) and 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		0.074[b]	0.088[b]	0.123[b]	0.122[b]	0.186[b]
B-N	0.022		0.020[b]	0.055[b]	0.068[b]	0.111[b]
B-FP	0.039	0.016		0.035[b]	0.051[b]	0.087[b]
B-FB	0.120	0.045	0.023		0.007	0.025[b]
W-FB	0.102	0.058	0.043	0.001		0.026[b]
W-OK	0.161	0.083	0.059	0.030	0.027	

[a]B-PNW = great blue herons from the Pacific Northwest, B-N = great blue herons from the north-central United States, B-FP = great blue herons from the southern Florida peninsula, B-FB = great blue herons from Florida Bay, W-FB = great white herons from Florida Bay, and W-OK = great white herons from the outer Keys.

[b]99% CI does not include zero (confidence intervals were not calculated for R_{ST} estimates).

Table 9. 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	0.0001				
B-FP	0.0001	0.0002			
B-FB	0.0001	0.0001	0.0001		
W-FB	0.0001	0.0001	0.0001	0.0029	
W-OK	0.0001	0.0001	0.0001	0.0001	0.0001

[a]B-PNW = great blue herons from the Pacific Northwest, B-N = great blue herons from the north-central United States, B-FP = great blue herons from the southern Florida peninsula, B-FB = great blue herons from Florida Bay, W-FB = great white herons from Florida Bay, and W-OK = great white herons from the outer Keys.

Morphometrics

I found no significant size differences between great white herons and intermediate Würdemann's herons for any of the variables measured (Table 10). Although differences were not significant, mean values for great white herons were larger than those for intermediates for every variable. With 2 exceptions, males were significantly larger than females. Length of tail was not significantly different (95% confidence intervals overlapped) between male and female intermediate Würdemann's herons or between male and female white herons.

Table 10. Mean (mm), 95% confidence interval (CI), and sample size (*n*) for 6 morphometric variables measured from museum skins of great white herons and intermediate Würdemann's herons collected in the Florida Keys (Monroe County, Florida).

		Females			Males		
	Group	**Mean**	**± 95% CI**	***n***	**Mean**	**± 95% CI**	***n***
Culmen	Intermediate	148.4	4.0	12	158.2	4.2	14
	White	149.1	5.9	17	160.2	2.1	22
Depth of Bill	Intermediate	30.2	1.3	12	32.2	0.8	14
	White	30.3	1.0	17	32.5	0.6	22
Tarsus	Intermediate	189.0	5.9	12	201.9	5.8	14
	White	191.5	9.0	17	207.4	5.3	24
Middle Toe	Intermediate	105.2	2.8	11	114.7	2.4	14
	White	107.6	3.1	16	116.9	2.5	24
Wing	Intermediate	466.0	7.1	11	490.2	8.1	14
	White	471.3	11.6	16	490.3	6.3	20
Tail	Intermediate	172.3	3.5	12	178.3	4.6	14
	White	174.3	4.7	17	181.6	2.6	24

DISCUSSION

Mate Choice

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, thus allowing for a statistical test of the random mate choice hypothesis and inferences regarding the genetic basis of the plumage polymorphism found in the Florida Keys population. My data from Florida Bay do not support the hypothesis that sympatric great white herons and great blue herons pair randomly with respect to plumage color ($\chi^2 = 31.32$, df = 1, $p < 0$ .001). I observed more white/white and blue/blue pairs and fewer mixed pairs than expected from a randomly mating population. This suggests that premating isolating mechanisms exist within this population. Although the pattern of mate choice is assortative, the mechanism producing this pattern is unknown. The nonrandom pattern does not necessarily imply that these herons use plumage color as a criterion for mate choice. Other factors (e.g., habitat preference, timing of breeding, sex ratios, geographic distribution) may also influence mate choice. These factors may function at several spatial and/or temporal scales and could either inhibit or promote mixed pairs.

My observations also provide some insight into the genetic basis of the plumage polymorphism observed in the Florida Bay population. Some ornithologists have suspected that 2 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 5). If plumage color is determined primarily at a single locus and white is dominant, the probability of observing this sample is extremely low unless most pairs (> 88%) have at least 1 member that is homozygous for the dominant white allele (Table 11). Given the assortative pattern of mate choice and the ratio of white: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, 2 blue parents can produce white offspring only if white plumage is a recessive trait and both parents are heterozygotes. Furthermore, as Table 12 illustrates, the proportion of white offspring found in nests of blue/blue pairs (19.4 ± 13.9%) is within the range expected under the hypothesis that white plumage is recessive (0–25%) and is significantly different from the expected value for the hypothesis that white plumage is dominant (0%).

Table 11. Probability of producing 113 white offspring (Table 5) 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	3.04×10^{-3}
0.12	0.88	0.96	0.01
0.08	0.92	0.98	0.10
0.00	1.00	1.00	1.00

[a]WW and Ww = white, ww = blue.

Table 12. Possible genotype crosses and percent white offspring expected from these crosses for 2 hypotheses of color dominance (assuming color is controlled by a single locus) compared with the percent of white offspring (±95% CI) observed in nests with known parental phenotypes.

	White Dominant		White Recessive		
Parental phenotypes	Possible genotype crosses[a]	% white offspring expected	Possible genotype crosses[b]	% white offspring expected	% white offspring observed
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 (±18.5)
Blue/Blue	ww x ww	0	Bb x Bb BB x Bb BB x BB	0–25	19.4 (±13.9)

[a]WW and Ww = white, ww = blue.
[b]BB and Bb = blue, bb = white.

Although nestling and adult phenotype data indicate that white plumage behaves as a recessive trait, these single locus hypotheses for the inheritance of plumage color presented above 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 1 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 would 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 1 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 migration of either white or blue individuals out of the predominantly white Florida Bay population into other great blue heron populations.

Inferences regarding migration from other great blue heron populations into 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 migration from other great blue heron populations into Florida Bay.

Mitochondrial DNA: Cytochrome *b* Sequence Data

Contemporary distributions of mtDNA haplotypes among populations have been used to infer phylogeny, patterns of historical fragmentation, dispersal, and changes in population size and distribution in a variety of taxa (Avise et al. 1987). Given the utility of mtDNA cytochrome *b* sequence data in phylogeographic studies of birds and the levels of intraspecific variation reported for other avian taxa (Wenink et al. 1993, Wood and Krajewski 1996, Mundy et al. 1997, Questiau et al. 1998, Kirchman et al. 2000), the absence of haplotype diversity observed among great blue heron subspecies is remarkable. Although past studies provide mixed results with respect to concordance between distribution of mtDNA haplotypes and subspecies boundaries (Zink 1997), none have reported such low haplotype diversity within such a widespread species like the great blue heron. The absence of genetic variation at cytochrome *b* could suggest a rapid post-Pleistocene range expansion or a recent adaptive mutation followed by a selective sweep (Rand 1996). It is also possible that a more slowly evolving nuclear pseudogene (Quinn 1997) was amplified rather than mtDNA cytochrome *b*. However, 15 of the 20 samples were obtained from muscle tissue, which is a mtDNA-rich tissue source, and the sequences code for amino acids (pseudogenes often contain stop codons). A curious aspect of these sequences is that they differ

from the great blue heron mtDNA cytochrome *b* sequence reported by Sheldon et al. (2000) (GenBank accession number AF193821). AF193821 was collected in Louisiana (within the range for *A. h. wardi*) and, given the lack of variation exhibited among any of my sequences, would not be expected to differ substantially from the *A. h wardi* sequences reported here. However, AF193821 differs from Haplotype 1 by 9 bp. These base pair differences include non-synonymous substitutions. Non-synonymous substitutions should be rare because changing the amino acid composition often radically alters protein structure (and, thus, function). The number of non-synonymous substitutions (4) makes these *A. herodias* sequences suspect. The 3 sequences (AF193821, Haplotype 1, and Haplotype 2) do not conain "stop" condons and, therefore, none can be easily dismissed as a pseudogene. However, because no other study reports such low levels of haplotype diversity in any widespread bird species, suspicion must fall on the sequences reported here, and further work is required to either confirm or refute them (e.g., re-extract DNA and order new primers, enzymes, buffers, etc. to rule out contamination of PCR reagents; amplify my tissue samples with primers used by Sheldon et al. [2000] to see if the same sequences were obtained; conduct partition analysis to see if the sequences contain pseudogene properties). Until such work is completed, these sequences should be viewed as preliminary and interpreted with caution.

Nuclear DNA: Microsatellite Allele Frequency Data

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 *A. herodias* DNA fragments enriched for microsatellites. 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.

My analyses of allele frequencies at microsatellite loci in *A. herodias* revealed large- and small-scale patterns of genetic differentiation (Tables 8–9). Values for F_{ST} and R_{ST}, which are measures of the consequence of population subdivision, are comparable to those found in isolated populations or allopatric subspecies of other birds (Goostrey et al. 1998, Tarr et al. 1998, von Segesser et al. 1999). I found significant differences in allele frequencies among all 4 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 great white heron population itself. Great white herons and great blue herons breeding in Florida Bay (W-FB and B-FB) were significantly different from each other and both were significantly different from great white herons breeding along the outer Keys (W-OK). Although the W-FB vs. B-FB comparison was significant (Table 9), *F*-statistics indicate that the difference between these 2 groups is very small relative to the other comparisons (Table 8).

Unweighted pair-group method using arithmetic averages (UPGMA) is a phenetic clustering algorithm which uses genetic distances to group taxa according to their overall similarity but does not provide information on evolutionary relationships (Quicke 1993). An UPGMA analysis of the microsatellite data using Nei's (1978) unbiased genetic distance defined 3 clusters (Fig. 1). The first cluster contains the 3 Florida Keys groups (W-FB, B-FB, and W-OK), the second cluster contains B-FP and B-N, and the B-PNW group forms the third cluster. It is not surprising that B-PNW is distinct. It is allopatric with the other groups and, although great blue herons are capable of

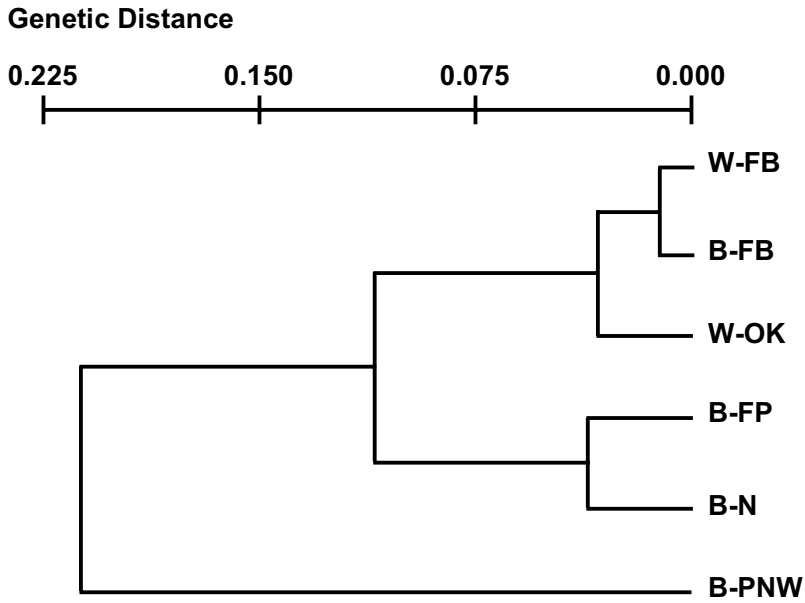

Fig. 1. UPGMA cluster phenogram using Nei's (1978) unbiased genetic distance. B-PNW = great blue herons from the Pacific Northwest, B-N = great blue herons from the north-central United States, B-FP = great blue herons from the southern Florida peninsula, B-FB = great blue herons from Florida Bay, W-FB = great white herons from Florida Bay, and W-OK = great white herons from the outer Keys.

long distance travel, opportunities for gene flow between B-PNW and the other groups are probably limited because it is separated from them by the continental divide. A few interesting points emerge from the patterns of genetic differentiation among the remaining groups. First, B-FP and B-N are very similar to each other. Even though sampling locations for these groups were over 2,000 km apart, the genetic distance between them is only slightly larger than the genetic distance observed between W-FB and W-OK, which are separated by <100 km. Second, the Florida Keys population is distinct from the B-FP/B-N cluster despite its proximity to the B-FP group (B-FP samples were collected <80 km from Florida Bay). Third, great blue herons breeding in Florida Bay are more similar to great white herons than they are to great blue herons breeding on the nearby Florida peninsula.

These patterns of genetic differentiation suggest that barriers to gene flow among great blue heron populations from south Florida to the north-central U.S. are far less than those between Florida Bay and the adjacent southern Florida peninsula. They also suggest that most great blue herons breeding in Florida Bay are permanent members of this population rather than migrants from the Florida peninsula. It is likely that plumage polymorphisms found in the Florida Bay population are the result of hybridization between great white and great blue herons, but these genetic 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 Florida Bay and Florida peninsula populations.

Two prezygotic isolating mechanisms may limit 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 shallow marine environments of the Florida Keys and freshwater wetlands of the southern Florida peninsula may inhibit recruitment in either direction. Furthermore, unlike most great blue heron populations, the Florida Keys great white heron population is nonmigratory. My microsatellite data show significant differences in allele frequencies between W-FB and W-OK and provide evidence that even short-distance dispersal within the great white heron population is limited. The second isolating mechanism that may limit gene flow between Florida Bay and the Florida peninsula is temporal. Peak great white heron breeding activity coincides with south Florida's dry season (October through April). This is typical of birds nesting in tropical or sub-tropical regions and is consistent with a hypothesis of a Caribbean origin for the great white heron. Great blue herons on the Florida peninsula generally begin breeding in February or March, which is typical of birds nesting in southern temperate regions. This

difference in the timing of breeding may limit opportunities for hybridization between Florida Bay and Florida peninsula populations.

Morphometrics

Previous investigators have made morphometric comparisons of great white and great blue herons (Holt 1928, Mayr 1956, Zachow 1983). In one of the most thorough treatments, Zachow (1983) found significant size differences between great white herons, great blue herons from the Florida peninsula, and northern great blue herons. Great white herons were the largest, northern great blue herons were smallest, and great blue herons from the Florida peninsula were intermediate in size. To date, no one has made the direct comparison between sympatric white and blue herons. Attempts to extract information for this comparison from existing literature (Ridgway 1887, Oberholser 1912, Holt 1928, Zachow 1983) were fruitless because different investigators measured different variables. This is the first study to collect comparable measurements from sympatric great white and great blue herons.

The plumage of blue herons in my sample varied, but all appeared to be intermediate Würdemann's herons. None had plumage characteristic of typical great blue herons. This may 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 if they were collected during winter), or the phenotypic composition of this sample may accurately reflect the resident great blue heron population in the Florida Keys. I found no significant size differences between great white herons and intermediate Würdemann's herons at any of the 6 morphometric variables (Table 10). Mean values of all variables were smaller in intermediate herons (females and males). This pattern is consistent with the hypothesis that these intermediates are the result of hybridization between great white herons and the smaller great blue herons from the Florida peninsula. These findings are also consistent with my genetic data set, which show small differences in allele frequencies between great white and great blue herons breeding in Florida Bay.

CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

Resolving the debate surrounding the great white heron's taxonomic status depends on determining 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 ($p < 0.001$). The positive assortative pattern suggests that premating isolating mechanisms exist within this 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 (W-FB, B-FB, and W-OK), but these differences are small compared to the differences observed between the Florida Keys population and B-FP. 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 migration 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 (Table 10). This finding 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 (Fig. 1). Together, these data show that the Florida Keys great white heron 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 do migrate to the southern Florida peninsula during summer). Although barriers are not complete, the Florida Keys population does appear to be reproductively isolated from the Florida peninsula population. The great white heron appears to be a good biological species (Dobzhensky 1937, Mayr 1942), and a review of its taxonomic status is merited. Regardless of whether we ultimately call the great white heron a species or subspecies, it should be viewed and managed as a small, isolated population.

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); however, genetic issues can be a critical concern for small isolated populations. Although the microsatellite data show a gradual decline in heterozygosity as you move from the Florida

peninsula to the outer Keys (Table 7), heterozygosity is still high within the great white heron population, and loss of genetic variation does not appear to be an immediate concern for this population. Demographic issues, however, 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 (e.g., Powell's estimates may be low, birds may rear more than 1 clutch per year, some portions of the great white heron breeding population may be more productive than others), effective conservation planning will require a better understanding of this population's demographics.

A final issue concerns a statement in one of the species accounts for the great white heron and my observations in the field. Adult great white herons are believed to be immune from many predators (Stevenson and Anderson 1994) and, aside from bald eagles (*Haliaeetus leucocephalus*), they have evolved in a system that is relatively free of nest predators. Stevenson and Anderson (1994, 58) state, "The Fish Crow, which may steal eggs from Great Blue Herons, is chiefly out of the Great White's range." American crows (*Corvus brachyrhynchos*) are fixtures at picnic areas along the road that leads from the eastern edge of Everglades National Park to Flamingo, which lies on the northwestern edge of Florida Bay. My observations indicate that they are largely absent from Florida Bay, except near Flamingo where I found evidence of crow depredation (eggs with holes punched in their center and emptied of their contents) on Clive Key and Sandy Key. I also observed crows on Oyster Keys, Catfish Key, and Frank Key. Although great white herons rarely leave eggs unattended, they will flush when disturbed (e.g., by recreational fishermen fishing the mangrove roots or by scientific researchers accessing study sites). If crows are novel predators in this system, great white herons and other wading birds may have few defenses against them. Combined with disturbance from recreational users, expansion of crows into Florida Bay could pose a threat to wading birds and may be worth monitoring.

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Appendix A. Tissue samples used for microsatellite analyses of population differentiation.

Source[a]	Sample ID	Group[b]	Collection Date mm	dd	yyyy	Location State: County
UWBM	EVL 146	B-PNW	08	07	1998	Wash.: King
UWBM	GKD 01	B-PNW	fall		1990	Wash.: Mason
UWBM	GKD 133	B-PNW	11	25	2000	Wash.: Skagit
UWBM	PJG 112	B-PNW	fall		1998	Wash.: Mason
UWBM	PJG 232	B-PNW				Wash.: Jefferson
UWBM	SMB 01	B-PNW	07		1998	Wash.: King
UWBM	SMB 02	B-PNW	11		1990	Wash.: Mason
UWBM	SMB 129	B-PNW				Wash.: Island
UWBM	SMB 130	B-PNW				Wash.: Snohomish
UWBM	SMB 131	B-PNW				Wash.: Island
UWBM	SMB 132	B-PNW				Wash.: Island
FMNH	348377	B-N	06	23	1988	Wis.: Marinette
FMNH	348378	B-N	08	13	1986	Wis.: Kewaunee
FMNH	348380	B-N	08	17	1986	Wis.: Door
FMNH	348381	B-N	11	06	1988	Wis.
FMNH	348382	B-N	08	29	1985	Wis.: Kewaunee
FMNH	348383	B-N	09	05	1986	Wis.: Brown
FMNH	348384	B-N	04	13	1986	Wis.: Shawano
FMNH	348385	B-N				Wis.
FMNH	363357	B-N	08	03	1992	Wis.: Brown
FMNH	387752	B-N	05	19	1998	Wis.
FMNH	395626	B-N	08	22	1998	Wis.
FMNH	395888	B-N	09	22	1998	Wis.
FMNH	396953	B-N	07	09	1998	Minn.: Dakota
FMNH	396954	B-N	10	05	1999	Minn.: Itasca
FMNH	397031	B-N	11	07	1998	Wis.
FMNH	397118	B-N	07	17	1999	Ill.: Aurora
FMNH	397119	B-N	08	03	1999	Ill.: Batavia
FMNH	429048	B-N	07	21	1995	Minn.: Hubbard
FMNH	429049	B-N	08	04	1995	Minn.: Fillmore
FMNH	429086	B-N	06	03	2000	Ill.: Naperville
BMNH	AF 1010	B-N	08	07	1952	Minn.
BMNH	JK 93151	B-N			1990	Minn.
BMNH	X 7089	B-N	07	27	1992	Minn.: Cass
BMNH	X 7090	B-N	04	30	1992	Minn.: Washington
BMNH	X 7091	B-N	09	28	1991	Wis.
BMNH	X 7092	B-N	09	23	1991	Minn.: Washington
BMNH	X 7093	B-N	07	31	1992	Minn.: Ramsey
BMNH	X 7094	B-N	08	02	1992	Minn.: Chisago
BMNH	X 7095	B-N	09	26	1993	Minn.: Beltrami
BMNH	X 7096	B-N	08	06	1992	Minn.: Ramsey
HLM	04 03 00 N01	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N02	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N03	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N04	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N05	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N06	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N07	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N08	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N09	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N10	B-FP	04	03	2000	Fla.: Miami-Dade

Appendix A. Continued.

Source[a]	Sample ID	Group[b]	Collection Date mm	dd	yyyy	Location State: County
HLM	04 03 00 N11	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N12	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N13	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N14	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N15	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N16	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N17	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N18	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N19	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N20	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 03 00 N21	B-FP	04	03	2000	Fla.: Miami-Dade
HLM	04 04 00 N01	B-FP	04	04	2000	Fla.: Miami-Dade
HLM	04 04 00 N02	B-FP	04	04	2000	Fla.: Miami-Dade
HLM	04 04 00 N03	B-FP	04	04	2000	Fla.: Miami-Dade
HLM	12 03 98 N2C1	B-FB	12	03	1998	Fla.: Monroe
HLM	12 09 98 N1C1	B-FB	12	09	1998	Fla.: Monroe
HLM	12 15 98 N1C1	B-FB	12	15	1998	Fla.: Monroe
HLM	12 15 98 N2C1	B-FB	12	15	1998	Fla.: Monroe
HLM	12 15 98 N3C1	B-FB	12	15	1998	Fla.: Monroe
HLM	12 16 98 N1C1	B-FB	12	16	1998	Fla.: Monroe
HLM	12 18 98 N3C1	B-FB	12	18	1998	Fla.: Monroe
HLM	12 21 98 N1C1	B-FB	12	21	1998	Fla.: Monroe
HLM	01 10 99 N2C1	B-FB	01	10	1999	Fla.: Monroe
HLM	01 10 99 N3C1	B-FB	01	10	1999	Fla.: Monroe
HLM	01 10 99 N4C1	B-FB	01	10	1999	Fla.: Monroe
HLM	01 12 99 N5C1	B-FB	01	12	1999	Fla.: Monroe
HLM	01 14 99 N1C1	B-FB	01	14	1999	Fla.: Monroe
HLM	01 21 99 N1C1	B-FB	01	21	1999	Fla.: Monroe
HLM	01 21 99 N2C1	B-FB	01	21	1999	Fla.: Monroe
HLM	01 21 99 N3C2	B-FB	01	21	1999	Fla.: Monroe
HLM	01 21 99 N4C1	B-FB	01	21	1999	Fla.: Monroe
HLM	01 21 99 N5C1	B-FB	01	21	1999	Fla.: Monroe
HLM	01 27 99 N1C1	B-FB	01	27	1999	Fla.: Monroe
HLM	01 27 99 N5C1	B-FB	01	27	1999	Fla.: Monroe
HLM	01 27 99 N6C1	B-FB	01	27	1999	Fla.: Monroe
HLM	01 27 99 N7C1	B-FB	01	27	1999	Fla.: Monroe
HLM	02 03 99 N4C1	B-FB	02	03	1999	Fla.: Monroe
HLM	02 03 99 N5C1	B-FB	02	03	1999	Fla.: Monroe
HLM	02 03 99 N6C1	B-FB	02	03	1999	Fla.: Monroe
HLM	02 06 99 N1C1	B-FB	02	06	1999	Fla.: Monroe
HLM	02 06 99 N2C1	B-FB	02	06	1999	Fla.: Monroe
HLM	02 06 99 N3C1	B-FB	02	06	1999	Fla.: Monroe
HLM	02 06 99 N4C1	B-FB	02	06	1999	Fla.: Monroe
HLM	02 06 99 N6C1	B-FB	02	06	1999	Fla.: Monroe
HLM	02 06 99 N8C1	B-FB	02	06	1999	Fla.: Monroe
HLM	02 09 99 N1C2	B-FB	02	09	1999	Fla.: Monroe
HLM	02 17 99 N1C1	B-FB	02	17	1999	Fla.: Monroe
HLM	02 17 99 N2C1	B-FB	02	17	1999	Fla.: Monroe
HLM	02 17 99 N3C1	B-FB	02	17	1999	Fla.: Monroe
HLM	11 13 98 N1C2	W-FB	11	13	1998	Fla.: Monroe
HLM	11 13 98 N2C1	W-FB	11	13	1998	Fla.: Monroe

Appendix A. Continued.

Source[a]	Sample ID	Group[b]	Collection Date mm	dd	yyyy	Location State: County
HLM	11 15 98 N1C1	W-FB	11	15	1998	Fla.: Monroe
HLM	11 29 98 N1C1	W-FB	11	29	1998	Fla.: Monroe
HLM	11 30 98 N1C4	W-FB	11	30	1998	Fla.: Monroe
HLM	11 30 98 N2C1	W-FB	11	30	1998	Fla.: Monroe
HLM	11 30 98 N3C1	W-FB	11	30	1998	Fla.: Monroe
HLM	12 03 98 N1C1	W-FB	12	03	1998	Fla.: Monroe
HLM	12 03 98 N3C1	W-FB	12	03	1998	Fla.: Monroe
HLM	12 05 98 N1C1	W-FB	12	05	1998	Fla.: Monroe
HLM	12 06 98 N1C1	W-FB	12	06	1998	Fla.: Monroe
HLM	12 06 98 N2C1	W-FB	12	06	1998	Fla.: Monroe
HLM	12 06 98 N3C1	W-FB	12	06	1998	Fla.: Monroe
HLM	12 09 98 N1C1	W-FB	12	09	1998	Fla.: Monroe
HLM	12 10 98 N1C1	W-FB	12	10	1998	Fla.: Monroe
HLM	12 13 98 N1C1	W-FB	12	13	1998	Fla.: Monroe
HLM	12 13 98 N2C1	W-FB	12	13	1998	Fla.: Monroe
HLM	12 16 98 N2C1	W-FB	12	16	1998	Fla.: Monroe
HLM	12 16 98 N3C1	W-FB	12	16	1998	Fla.: Monroe
HLM	12 16 98 N4C1	W-FB	12	16	1998	Fla.: Monroe
HLM	12 17 98 N1C1	W-FB	12	17	1998	Fla.: Monroe
HLM	12 17 98 N2C1	W-FB	12	17	1998	Fla.: Monroe
HLM	12 17 98 N3C1	W-FB	12	17	1998	Fla.: Monroe
HLM	12 18 98 N1C1	W-FB	12	18	1998	Fla.: Monroe
HLM	12 18 98 N2C1	W-FB	12	18	1998	Fla.: Monroe
HLM	12 19 98 N1C1	W-FB	12	19	1998	Fla.: Monroe
HLM	12 20 98 N1C1	W-FB	12	20	1998	Fla.: Monroe
HLM	12 21 98 N2C1	W-FB	12	21	1998	Fla.: Monroe
HLM	12 21 98 N3C1	W-FB	12	21	1998	Fla.: Monroe
HLM	12 21 98 N4C1	W-FB	12	21	1998	Fla.: Monroe
HLM	12 21 98 N5C1	W-FB	12	21	1998	Fla.: Monroe
HLM	12 21 98 N6C1	W-FB	12	21	1998	Fla.: Monroe
HLM	12 22 98 N1C1	W-FB	12	22	1998	Fla.: Monroe
HLM	12 22 98 N2C1	W-FB	12	22	1998	Fla.: Monroe
HLM	12 22 98 N3C1	W-FB	12	22	1998	Fla.: Monroe
HLM	12 23 98 N1C1	W-FB	12	23	1998	Fla.: Monroe
HLM	12 23 98 N2C1	W-FB	12	23	1998	Fla.: Monroe
HLM	12 31 98 N1C1	W-FB	12	31	1998	Fla.: Monroe
HLM	01 06 99 N1C1	W-FB	01	06	1999	Fla.: Monroe
HLM	01 08 99 N1C1	W-FB	01	08	1999	Fla.: Monroe
HLM	01 10 99 N1C1	W-FB	01	10	1999	Fla.: Monroe
HLM	01 12 99 N1C1	W-FB	01	12	1999	Fla.: Monroe
HLM	01 12 99 N2C1	W-FB	01	12	1999	Fla.: Monroe
HLM	01 12 99 N3C1	W-FB	01	12	1999	Fla.: Monroe
HLM	01 12 99 N4C1	W-FB	01	12	1999	Fla.: Monroe
HLM	01 12 99 N6C1	W-FB	01	12	1999	Fla.: Monroe
HLM	01 12 99 N7C1	W-FB	01	12	1999	Fla.: Monroe
HLM	01 13 99 N1C1	W-FB	01	13	1999	Fla.: Monroe
HLM	01 13 99 N2C1	W-FB	01	13	1999	Fla.: Monroe
HLM	01 14 99 N2C1	W-FB	01	14	1999	Fla.: Monroe
HLM	01 17 99 N1C1	W-FB	01	17	1999	Fla.: Monroe
HLM	01 17 99 N2C1	W-FB	01	17	1999	Fla.: Monroe
HLM	01 17 99 N3C1	W-FB	01	17	1999	Fla.: Monroe

Appendix A. Continued.

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

Appendix A. Continued.

Source[a]	Sample ID	Group[b]	Collection Date mm	dd	yyyy	Location State: County
HLM	02 19 99 N1C1	W-OK	02	19	1999	Fla.: Monroe
HLM	02 08 99 N4C1	W-OK	02	08	1999	Fla.: Monroe
HLM	02 08 99 N5C1	W-OK	02	08	1999	Fla.: Monroe
HLM	02 08 99 N3C1	W-OK	02	08	1999	Fla.: Monroe
HLM	02 26 99 N1C1	W-OK	02	26	1999	Fla.: Monroe
HLM	02 26 99 N2C1	W-OK	02	26	1999	Fla.: Monroe
HLM	02 26 99 N3C1	W-OK	02	26	1999	Fla.: Monroe
HLM	02 26 99 N4C1	W-OK	02	26	1999	Fla.: Monroe
HLM	03 10 99 N4C1	W-OK	03	10	1999	Fla.: Monroe
HLM	03 10 99 N5C1	W-OK	03	10	1999	Fla.: Monroe

[a]BMNH = Bell Museum of Natural History, FMNH = Field Museum of Natural History (Chicago), HLM = Heather L. McGuire, UWBM = University of Washington, Burke Museum.

[b]B-PNW = great blue herons from the Pacific Northwest, B-N = great blue herons from the north-central United States, B-FP = great blue herons from the southern Florida peninsula, B-FB = great blue herons from Florida Bay, W-FB = great white herons from Florida Bay, and W-OK = great white herons from the outer Keys.

Appendix B. Morphometric variables (mm) measured from museum specimens of adult great white herons and intermediate Würdemann's herons.

Museum ID[a]	Sex	Group[b]	Culmen	Depth of Bill	Tarsus	Middle Toe	Wing Chord	Tail
FMNH 370621	F	I	142.50	30.00	184.25	106.50	465.00	173.75
CMNH 11138	F	I	141.30	31.00	186.25	100.50	474.50	173.25
CMNH 11132	F	I	147.50	28.50	192.75	103.00	475.00	168.50
CMNH 11139	F	I	143.25	29.00	183.00	106.75	451.00	162.75
MCZ 246710	F	I	148.00	29.25	176.75	101.50	447.50	172.00
MCZ 246712	F	I	147.50	28.75	182.00	105.75	460.00	177.25
MCZ 48302	F	I	146.75	28.75	185.50	110.00	470.00	169.00
MCZ 246708	F	I	153.25	30.75	189.75	110.00	469.50	176.75
MCZ 246709	F	I	145.00	29.75	187.75	98.00	461.50	167.00
MCZ 251272	F	I	147.25	31.75	188.75	104.75	467.50	173.00
USNM 110211	F	I	163.75	35.83	198.00	106.00	484.00	170.00
LSUMZ 155814	F	I	154.50	29.30	212.70	113.50		184.30
FMNH 385901	F	W	134.00	28.00	179.75	105.75	466.00	184.25
FMNH 385899	F	W	134.00	28.00	188.75	98.75	444.50	169.75
FMNH 128640	F	W	167.25	34.00	222.00	117.75	501.50	176.75
FMNH 128639	F	W	147.50	29.50	176.50	106.75	457.50	172.00
CM 94832	F	W	148.75	29.00	190.50	104.00	465.50	170.50
CM 111471	F	W	148.00	29.75	188.75	105.75	452.00	164.75
CMNH 11147	F	W	145.75	30.00	191.00	110.25	460.50	179.00
CMNH 11145	F	W	150.00	30.00	200.25	110.75	463.50	164.00
MCZ 246700	F	W	152.75	30.00	184.75	102.25	461.00	178.00
MCZ 219810	F	W	166.50	33.25	209.50	115.50	484.00	174.00
MCZ 42534	F	W	152.25	29.25	189.50	101.50	462.00	172.30
ANS 160272	F	W	145.00	29.50	184.00	99.25	444.50	159.75
USNM 400034	F	W	128.25	29.00	160.33	106.25	466.50	180.25
USNM 110696	F	W	171.50	34.75	229.25	119.50	519.50	200.00
USNM 302036	F	W	153.33	29.00	204.50	110.50	495.50	167.75
USNM 527757	F	W	141.25	30.00	167.75	103.75	497.00	174.25
LSUMZ 68303	F	W	148.50	32.00	188.70	111.50		175.70
MCZ 246706	M	I	153.00	32.50	189.00	111.00	494.50	181.25
MCZ 246705	M	I	153.25	31.50	186.50	113.50	473.00	172.25
MCZ 246704	M	I	157.00	31.00	203.83	113.25	474.00	160.50
MCZ 246702	M	I	158.00	33.00	200.25	116.00	495.50	177.00
MCZ 246701	M	I	158.75	33.75	210.33	118.50	474.50	169.25
MCZ 101086	M	I	160.00	33.75	211.75	116.75	505.50	188.00
MCZ 246703	M	I	167.75	32.00	209.00	113.00	497.00	183.00
FMNH 370620	M	I	167.50	32.67	208.75	115.50	495.00	184.75
FMNH 385671	M	I	156.75	31.50	205.50	116.50	481.00	173.25
FMNH 360099	M	I	161.00	31.30	188.00	114.50	494.00	187.00
FMNH 33767	M	I	166.25	32.25	220.25	125.25	500.50	187.25
USNM 332552	M	I	139.75	29.00	198.50	111.75	486.00	181.50
USNM 110210	M	I	153.00	34.00	193.50	107.50	472.50	174.25
USNM 8690[c]	M	I	162.25	32.50	201.25	112.75	520.00	177.25
MCZ 246698	M	W	162.75	33.00	215.00	108.75	474.00	172.75
MCZ 229215	M	W	160.00	35.25	199.50	113.75	503.50	187.00
MCZ 246697	M	W	157.75	32.50	195.25	112.50	488.50	172.00
MCZ 219809	M	W	161.75	33.50	190.50	115.00	493.50	182.50
MCZ 246715	M	W	167.25	33.25	191.50	117.50	480.50	186.25
MCZ 101085	M	W	162.75	32.25	226.25	121.25	511.00	179.75
MCZ 207887	M	W	160.50	32.25	199.50	103.00	481.50	173.00

Appendix B. Continued.

Museum ID[a]	Sex	Group[b]	Culmen	Depth of Bill	Tarsus	Middle Toe	Wing Chord	Tail
MCZ 301205	M	W	150.75	32.00	191.25	105.50	482.00	188.50
MCZ 301207	M	W	160.25	31.75	202.50	111.00	498.00	178.00
MCZ 186366	M	W	160.75	33.00	206.75	122.50	502.50	184.50
MCZ 251273	M	W	162.50	34.00	224.50	124.25	485.50	173.25
FMNH 385669	M	W	160.50	31.00	205.25	115.00	504.50	184.00
CM F262	M	W			219.00	122.25	482.00	193.00
CM F263	M	W			214.25	118.75	492.00	183.00
FMNH 111914	M	W	152.00	29.50	191.75	115.75	469.00	173.00
FMNH 128638	M	W	161.50	32.00	199.00	121.25	492.50	192.00
CMNH 11148	M	W	159.25	32.25	198.00	116.50	484.00	181.25
USNM 110695	M	W	162.50	32.50	226.00	126.50	513.00	187.67
USNM 110675	M	W	171.50	32.75	218.25	120.75	504.00	179.75
USNM 89896	M	W	162.25	34.00	199.00	122.50	465.00	186.75
LSUMZ 136102	M	W	152.80	31.30	204.50	118.25		174.30
LSUMZ 136101	M	W	158.30	31.00	223.00	123.50		184.30
LSUMZ 155818	M	W	156.00	34.00	209.30	115.00		178.70
LSUMZ 155816	M	W	161.30	33.00	227.20	115.75		183.70

[a]ANS = Academy of Natural Science (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, MCZ = Museum of Comparative Zoology (Harvard University), USNM = United States National Museum (Washington, D.C.).

[b]I = intermediate Würdemann's heron, W = great white heron.

[c]Type specimen.

Appendix C. Mitochondrial DNA (mtDNA) sequences. Great white and great blue heron mtDNA haplotypes Hap1 (n = 19) and Hap2 (n = 1). Sequences are reported from the 5' to 3' end of the light strand and cover 1,048 bp of the cytochrome *b* gene and 28 bp of the $tRNA^{Thr}$ gene. Numbers in parentheses, which represent the location of the first base pair in each line of text, are referenced to the complete mtDNA sequence of the chicken (Desjardins and Morais 1990). A "." means that the base pair in Hap2 is identical to that of Hap1. See text for details on sample locations.

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       ⇐ cytochrome b ⇒
(14991) Hap1:  C TTT GGA TCC CTC CTA GGC ATC TGC CTA ATG ACA CAA
       Hap2:  . ... ... ... ... ... ... ... ... ... ... ... ...
(15028) Hap1:ATC CTA ACC GGC CTC CTC CTA GCC ATA CAC TAC ACC GCA
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15067) Hap1:GAC ACA ACC CTA GCC TTC TCA TCC GTC GCC CAC ACA TGC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15106) Hap1:CGA AAC GTC CAG TAC GGC TGA CTA CTC CGT AAC CTG CAC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15145) Hap1:GCT AAC GGT GCT TCA CTC TTC TTC ATC TGC ATT TAC CTC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15184) Hap1:CAC ATC GGC CGC GGA CTC TAT TAC GGC TCG TAC CTC TTC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15223) Hap1:AAA GAA ACC TGA AAC ACA GGA GTT ATC CTG CTA CTT ACC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15262) Hap1:CTA ATA GCA ACC GCC TTT GTA GGG TAC GTT CTT CCA TGA
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15301) Hap1:GGA CAA ATA TCC TTC TGA GGG GCT ACA GTC ATC ACC AAC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15340) Hap1:CTA TTC TCA GCT ATC CCC TAT ATT GGA CAA ACC CTA GTA
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15379) Hap1:GAA TGA GCC TGA GGC GGA TTC TCA GTA GAT AAC CCC ACA
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15418) Hap1:CTT ACA CGG TTC TTC GCC CTT CAC TTC CTT CTC CCA TTC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15457) Hap1:GCA ATC GCA GGT CTC ACC CTA ATT CAC CTT ACC TTC CTC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15496) Hap1:CAC GAA TCA GGA TCA AAC AAC CCC CTA GGC ATC GTA TCA
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15535) Hap1:AAC TGC GAT AAG ATT CCA TTC CAC CCC TAC TTC TCC ATA
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15574) Hap1:AAG GAT ATC CTA GGC TTC ATG CTT ATG CTA CTA CCA CTC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15613) Hap1:ACA ACC CTA GCC CTA TTT TCC CCC AAC CTC TTA GGA GAC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15652) Hap1:CCA GAA AAC TTT ACC CCA GCA AAC CCC CTA GTA ACA CCT
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ..C
(15691) Hap1:CCC CAC ATC AAA CCA GAA TGA TAC TTC CTA TTT GCC TAC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15730) Hap1:GCC ATT CTC CGA TCT ATC CCT AAC AAA CTA GGA GGA GTC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15769) Hap1:CTG GCC CTA GCC GCA TCA GTA CTG ATC CTA TTC TTA ATC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15808) Hap1:CCC TTC CTC CAC AAA TCA AAA CAA CGC ACC CTA ACC TTC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15847) Hap1:CGC CCA CTC TCC CAG CTC CTA TTC TGA ACC CTC GTC GCC
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15886) Hap1:AAC CTC CTT ATC CTC ACA TGA GTA GGC AGC CAA CCC GTA
       Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
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Appendix C. Continued.

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(15925) Hap1:GAA CAC CCC TTC ATT ATC ATC GGC CAA CTA GCC TCC ATT
        Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
(15964) Hap1:ACC TAC TTC ACA ATC CTC CTA ATC CTA TTC CCC ATT ACT
        Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...
                                              cytochrome b ⇒|
(16003) Hap1:GGA GGC CTA GAA AAC AAA ATA CTA AAT TAC TAA ACC ACT
        Hap2:... ... ... ... ... ... ... ... ... ... ... ... ...

                  ⇐ tRNA^Thr ⇒
(16042) Hap1:CTA ATA GTT TAT TAA AAA CAT TGG T
        Hap2:... ... ... ... ... ... ... ... .
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Appendix D. The following tables contain allele frequencies for 6 *A. herodias* groups at 12 microsatellite loci (number of individuals genotyped is shown in parentheses). Abbreviations: B-PNW = great blue herons from the Pacific Northwest, B-N = great blue herons from the north-central United States, B-FP = great blue herons from the southern Florida peninsula, B-FB = great blue herons from Florida Bay, W-FB = great white herons from Florida Bay, and W-OK = great white herons from the outer Keys.

Locus Ah 205

Allele	B-PNW (10)	B-N (30)	B-FP (19)	B-FB (35)	W-FB (76)	W-OK (37)
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	

Locus Ah 209

Allele	B-PNW (10)	B-N (28)	B-FP (20)	B-FB (34)	W-FB (71)	W-OK (35)
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

Locus Ah 217

Allele	B-PNW (11)	B-N (30)	B-FP (18)	B-FB (35)	W-FB (77)	W-OK (35)
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

Locus Ah 320

Allele	B-PNW (11)	B-N (30)	B-FP (21)	B-FB (35)	W-FB (77)	W-OK (37)
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				

Locus Ah 341

Allele	B-PNW (11)	B-N (30)	B-FP (22)	B-FB (35)	W-FB (74)	W-OK (37)
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

Appendix D. Continued.

Locus Ah 343

Allele	B-PNW (11)	B-N (30)	B-FP (22)	B-FB (35)	W-FB (77)	W-OK (36)
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				

Locus Ah 414

Allele	B-PNW (11)	B-N (27)	B-FP (22)	B-FB (35)	W-FB (77)	W-OK (37)
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

Locus Ah 421

Allele	B-PNW (10)	B-N (10)	B-FP (20)	B-FB (35)	W-FB (77)	W-OK (35)
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	

Locus Ah 517

Allele	B-PNW (11)	B-N (30)	B-FP (17)	B-FB (34)	W-FB (74)	W-OK (35)
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 D. Continued.

Locus Ah 526

Allele	B-PNW (9)	B-N (29)	B-FP (17)	B-FB (31)	W-FB (70)	W-OK (35)
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

Locus Ah 536

Allele	B-PNW (11)	B-N (30)	B-FP (15)	B-FB (32)	W-FB (74)	W-OK (37)
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

Locus Ah 630

Allele	B-PNW (11)	B-N (30)	B-FP (19)	B-FB (34)	W-FB (77)	W-OK (36)
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

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