The Condor 106:229–240

LACK OF SPATIAL GENETIC STRUCTURE AMONG NESTING AND WINTERING KING EIDERS

JOHN M. PEARCE[1,5], SANDRA L. TALBOT[1], BARBARA J. PIERSON[1], MARGARET R. PETERSEN[1], KIM T. SCRIBNER[2], D. LYNNE DICKSON[3] AND ANDERS MOSBECH[4]

[1]*U.S. Geological Survey, Alaska Science Center, 1011 E. Tudor Rd., Anchorage, AK 99503*
[2]*Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824*
[3]*Canadian Wildlife Service, Edmonton, AB T6B 2X3, Canada*
[4]*National Environmental Research Institute, Department of Arctic Environment, Frederiksborgvej 399, DK4000 Roskilde, Denmark*

Abstract. The King Eider (*Somateria spectabilis*) has been delineated into two broadly distributed breeding populations in North America (the western and eastern Arctic) on the basis of banding data and their use of widely separated Pacific and Atlantic wintering areas. Little is known about the level of gene flow between these two populations. Also unknown is whether behavioral patterns common among migratory waterfowl, such as site fidelity to wintering areas and pair formation at these sites, have existed for sufficient time to create a population structure defined by philopatry to wintering rather than to nesting locations. We used six nuclear microsatellite DNA loci and cytochrome *b* mitochondrial DNA sequence data to estimate the extent of spatial genetic differentiation among nesting and wintering areas of King Eiders across North America and adjacent regions. Estimates of interpopulation variance in microsatellite allele and mtDNA haplotype frequency were both low and nonsignificant based on samples from three wintering and four nesting areas. Results from nested clade analysis, mismatch distributions, and coalescent-based analyses suggest historical population growth and gene flow that collectively may have homogenized gene frequencies. The presence of several unique mtDNA haplotypes among birds wintering near Greenland suggests that gene flow may now be more limited between the western and eastern Arctic, which is consistent with banding data.

Key words: gene flow, King Eider, microsatellites, mitochondrial DNA, population genetic differentiation, seaduck, Somateria spectabilis.

Ausencia de Estructura Genética Espacial entre Áreas de Nidificación e Invernada en *Somateria spectabilis*

Resumen. Con base en datos de anillamiento y en el uso de áreas de invernada separadas en el Pacífico y el Atlántico, la especie *Somateria spectabilis* ha sido separada en dos poblaciones reproductivas de amplia distribución en Norte América (las del Ártico este y oeste). Se conoce poco sobre los niveles de flujo génico entre estas dos poblaciones. También se desconoce si patrones de comportamiento comunes entre aves acuáticas migratorias, como la fidelidad a los sitios de invernada y la formación de parejas en dichos sitios, han existido por suficiente tiempo como para crear estructura poblacional definida por la filopatría a las áreas de invernada en lugar de a las áreas de nidificación. Utilizamos seis loci nucleares de ADN microsatelital y secuencias del gen mitocondrial citocromo *b* para estimar el grado de diferenciación genética espacial entre áreas de nidificación e invernada de *S. spectabilis* a través de Norte América y regiones adyacentes. Los estimados de la varianza interpoblacional en la frecuencia de alelos de microsatélites y de haplotipos de ADNmt fueron bajos y no significativos con base en muestras de tres áreas de invernada y cuatro de nidificación. Los resultados de un análisis de clados anidados, de las distribuciones ''mismatch'' y de análisis basados en coalescencia sugieren la existencia de crecimiento poblacional histórico y flujo génico, eventos que colectivamente podrían haber homogeneizado las frecuencias génicas. La presencia de varios haplotipos exclusivos entre aves que invernan cerca de Groenlandia sugiere que el flujo génico podría ser ahora más limitado entre el Ártico oeste y este, lo que es consistente con los datos de anillamiento.

Manuscript received 12 May 2003; accepted 11 December 2003.
[5] E-mail: john_pearce@usgs.gov

INTRODUCTION

For species of conservation or management concern, it is important to know whether populations are demographically independent. Studies of waterfowl populations typically focus on nesting areas when defining spatial genetic structure since most waterfowl exhibit a pattern of female-biased breeding and natal philopatry, while males are more prone to disperse (Anderson et al. 1992). Such behaviors can isolate female breeding populations, even though these populations may share wintering areas and migratory routes (Scribner et al. 2003).

Robertson and Cooke (1999) suggested that wintering-area philopatry has been overlooked in studies of waterfowl population structure. Waterfowl of both sexes may exhibit winter philopatry, possibly to form or reform breeding pairs (Anderson et al. 1992). Juveniles may also migrate with adult females to wintering areas, such as Regehr et al. (2001) observed in Harlequin Ducks (*Histrionicus histrionicus*). Thus, philopatry by males, females, and associated young to isolated wintering areas, followed by pair formation, may lead to population-genetic structure among birds of different wintering rather than nesting areas. The hypothesis of wintering-area population structure deserves examination because anthropogenic impacts to seaduck populations are increasing on the wintering grounds, potentially affecting genetically unique groups. For example, on 17 February 1996, an oil spill near St. Paul Island, Alaska (Fig. 1) killed hundreds of King Eiders (*Somateria spectabilis*; Flint et al. 1999).

The King Eider is one of the first waterfowl species to appear in the Arctic each spring, often migrating in flocks of >10 000 individuals (Suydam 2000). The King Eider breeds in coastal arctic tundra and has a Holarctic distribution that includes nesting areas in northern Alaska, Canada, Greenland, Svalbard, and Russia. Wintering birds occur throughout the Bering Sea and Pacific Ocean near Russia and Alaska, along the Atlantic coast of Canada and the United States, in southern Greenland, and along the coast of Norway (Fig. 1). Two breeding populations, from the western and eastern Arctic, are recognized in North America (Suydam 2000). Population counts of breeding birds (Dickson et al. 1997, Gratto-Trevor et al. 1998) and fall migrants (Suydam et al. 2000) suggest that both populations are in decline, although negative population trends observed at Greenland molting areas may be partly the result of changes in distribution due to human disturbance (Frimer 1995, Mosbech and Boertmann 1999).

Population delineation of King Eiders is based on the geographic isolation of wintering areas in the Pacific and Atlantic Oceans and recoveries of birds in the eastern Canadian Arctic that were initially banded during autumn in Greenland (Salomonsen 1968, 1979, Lyngs 2003). Recent satellite telemetry studies confirmed that King Eiders nesting in western arctic areas of Alaska and Canada remain in this region for wintering, moving to locales in the Bering Sea and Pacific Ocean (DLD, unpubl. data). The boundary separating these two populations (Fig. 1) is thought to extend southeast through Victoria Island and Queen Maud Gulf (Dickson et al. 1997). Little is known about the population-genetic structure and gene flow among populations of King Eiders, either within or between the populations of the western and eastern Arctic (Suydam 2000). Female nest-site fidelity, which is common among King Eiders in the central Canadian Arctic (Kellett 1999), and the geographic isolation of King Eider wintering areas, suggest that limited female gene flow may occur between the western and eastern Arctic. Only a single banded individual (a female) is known to have moved between the western and eastern Arctic from Prudhoe Bay, Alaska, to western Greenland (Lyngs 2003). While banding and telemetry data suggest that there may be little interchange between the western and eastern Arctic, recent analyses of stable isotopes (Mehl et al. 2004), suggests limited movement between these populations.

In this study, we examined the variation of polymorphic nuclear microsatellite DNA loci (Scribner and Pearce 2000) and site substitutions of mtDNA sequence data (Randi 2000) from the mitochondrial cytochrome *b* gene to test the null hypothesis of panmixia among King Eiders nesting within the western and eastern arctic populations. We also examined the hypothesis that population structure exists among wintering areas, using samples from Pacific and Atlantic wintering locales. Since the population-genetic structure of arctic species may have been influenced by Pleistocene vicariant events, such as

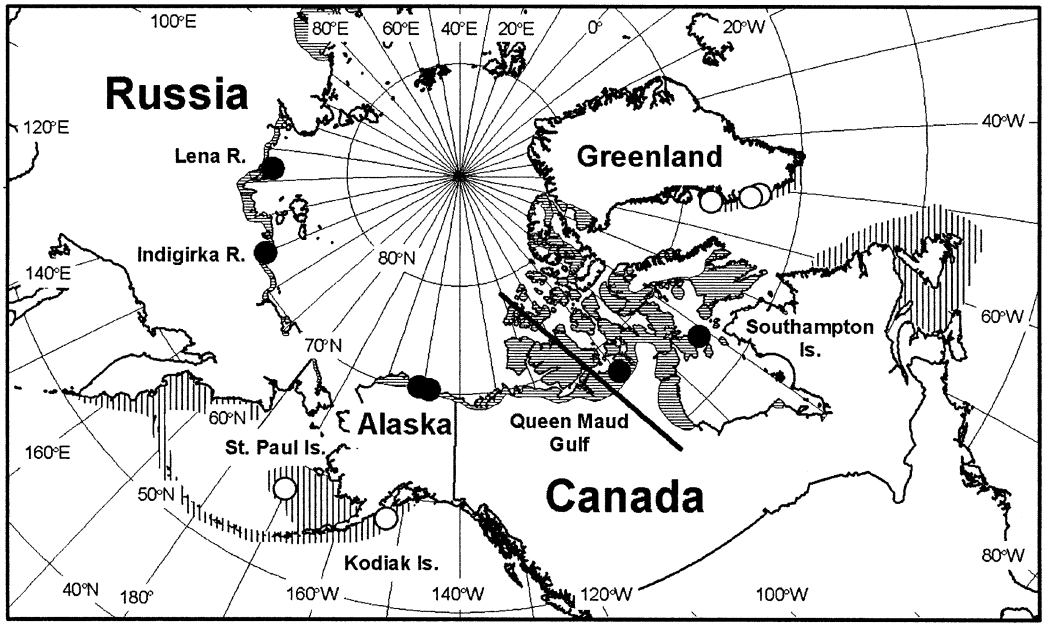

FIGURE 1. Sampling locations within breeding (filled circles) and wintering (unfilled circles) areas of King Eiders. Horizontal lines = breeding areas; vertical lines = wintering areas. The bold line represents the hypothesized boundary between the western and eastern arctic breeding populations of North America (Salomonsen 1968, 1979, Dickson et al. 1997, Lyngs 2003).

isolated refugia and subsequent deglaciation (Hewitt 2000), we used analytical methods that test for the intrapopulation variance of allele frequencies as well as methods that consider historical population change and phylogeography.

METHODS

SAMPLING

Samples were collected across the Holarctic distribution of King Eiders (Fig. 1), and included the Lena (73°30′N, 126°30′E) and Indigirka (71°20′N, 150°20′E) Rivers, Russia, the Colville (70°15′N, 151°05′W) and Kuparuk (70°10′N, 148°20′W) Rivers, Alaska, Queen Maud Gulf (67°00′N, 99°30′W) and Southampton Island (64°00′N, 81°50′W), Canada, St. Paul (57°15′N, 170°15′W) and Kodiak (57°45′N, 152°30′W) Islands, Alaska, and three locations in western Greenland (67°30′N, 54°00′W), which were grouped for analysis. Samples from the six nesting areas included contour feathers from active nests and blood from captured birds. Samples from the three wintering areas included blood and tissue samples from captured and harvested individuals. Nesting-area samples were composed primarily of females to reduce the possible inclusion of dispersing males. However, due to low sample sizes from Southampton Island and Russia, we included males from these areas in microsatellite DNA analyses. MtDNA analyses of nesting areas were based only on females. Due to their geographic proximity and low numbers, samples from the Lena and Indigirka Rivers, Russia, and the Colville and Kuparuk Rivers, Alaska, were combined, yielding two western-arctic nesting areas (Russia and Alaska), one eastern-arctic nesting area (Southampton Island), and one intermediate area (Queen Maud Gulf). Queen Maud Gulf is thought to represent an area where individuals from both the western and eastern Arctic occur during nesting (Mehl et al. 2004). Two wintering areas were sampled in the western Arctic (St. Paul Island and Kodiak Island, Alaska) and one wintering area in the eastern Arctic, which was composed of three sampling sites in western Greenland. Samples from wintering areas included both males and females.

LABORATORY ANALYSES

We extracted DNA from blood and tissue using the PUREGENE DNA extraction kit (Gentra

Systems, Inc., Minneapolis, Minnesota). DNA from feathers was extracted following the general methodology of Pearce et al. (1997). However, instead of Chelex we used an overnight digest in a 700-μL volume of Tris-NaCl-EDTA, 1M Tris-HCl (pH 8.0), 20 mg per mL Proteinase K, 25% sodium dodecyl sulfate, and 1M dithiothreitol, followed by a salt extraction (Medrano et al. 1990). We screened 24 pairs of biparentally inherited waterfowl microsatellite primers for amplification and allele variation in King Eiders. Six loci yielded clear and polymorphic DNA products: Alaμ1 (Fields and Scribner 1997), Bcaμ1, Bcaμ11, Hhiμ5 (Buchholz et al. 1998), and Sfiμ9 and Sfiμ11 (Libants et al., unpubl. data, GenBank accession numbers AF180501 and AF180499). Genotypes at each locus were generated using a Li-Cor 4200 DNA system (Lincoln, Nebraska). One of each primer pair was either directly labeled with a 5′-end Li-Cor 700 or 800 infrared fluorescent dye or synthesized with an M13 forward or reverse primer sequence on the 5′-end and accessed during the polymerase chain reaction (PCR) using the appropriate infrared-dye-labeled universal primer (Oetting et al. 1995). PCR amplification of these loci was conducted on a Stratagene 96 Robocycler (La Jolla, California). Reagents were heated to 94°C for 2 min, then cycled 35–40 times through the following temperatures: 94°C for 2 min, locus-specific annealing temperatures (47°C for Alaμ1, Sfiμ9, and Sfiμ11, and 54°C for Bcaμ1, Bcaμ11, and Hhiμ5) for 1 min, and 72°C for 1 min. Following PCR, 4 μL of loading buffer were added before placing 1–2 μL of PCR products onto 6% polyacrylamide gels and electrophoresed using the Li-Cor 4200 sequencer. We designated allele sizes with an M13 DNA sequence ladder. Samples of scored individuals were then used on subsequent gels to size new genotypes.

We amplified a 510-base-pair fragment within the cytochrome *b* gene of mtDNA using PCR primers H16064 (5′–CTTCAGTYTTTGGTTTACAAGA–3′) and L14990 (5′–AACATCTCAGCATGATGAAA–3′; Sorenson, Ast et al. 1999) for each of 182 King Eider samples. Amplifications were conducted with a Perkin Elmer Cetus DNA Thermal Cycler (Norwalk, Connecticut). PCR amplification involved a 50-μL reaction volume consisting of 25 μL DNA (diluted 1 μL of 50 ng per μL DNA:24 μL dH_20), PCR buffer (67 mM Tris-HCL, pH 8.0, 6.7 mM $MgCl_2$, 0.01% Tween-20), 1 mM each dNTP, 1 μM each primer, and 1 U Taq DNA polymerase. Thermocycler conditions consisted of 40 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 2.25 min. PCR products were electrophoresed through 1.5% agarose, stained with ethidium bromide and visualized using ultraviolet transillumination. Samples yielding a product of expected size were cleaned with Bio-Rad Quantum Prep PCR Kleen-spin columns (Hercules, California) and sequenced directly using Epicentre Technologies SequiTherm EXCEL II DNA sequencing kit (Madison, Wisconsin). Sequencing products were analyzed on a 3.7% polyacrylamide gel on the Li-Cor DNA 4200 system and aligned using the Li-Cor AlignIR program. Due to the common occurrence of nuclear pseudogenes in avian species (Lopez et al. 1994, Sorenson and Quinn 1998), we verified that amplified sequences were of mtDNA origin by comparing sequences obtained from heart, blood, and muscle samples from two King Eiders. In birds, heart tissue is relatively rich in mtDNA, while blood and muscle tissue are relatively rich in nuclear DNA. Thus, any differences between tissues from the same individual reflect amplification of both mtDNA and pseudogenes. PCR primers are redesigned until all tissues yield identical sequences. This method has proven useful for differentiation of true mtDNA from nuclear inserts of mitochondrial origin (numts) in Canada Geese (*Branta canadensis*; Pearce et al. 2000) and Harlequin Ducks (Lanctot et al. 1999). King Eider sequences were also compared to homologous mtDNA regions for Harlequin Duck (GenBank accession number AF173766; Sorenson, Cooper et al. 1999) to ensure similarity. Tissue and DNA samples of each haplotype are archived at the Alaska Science Center, Anchorage, Alaska.

STATISTICAL ANALYSES

For each microsatellite locus, we calculated allele frequencies, number of alleles per locus (*Na*), and observed (H_o) and expected (H_e) heterozygosity using GENEPOP (version 3.2a, Raymond and Rousset 1995). GENEPOP was also used to conduct exact probability tests for deviations from Hardy-Weinberg equilibrium in each nesting and wintering area using the method of Guo and Thompson (1992) and to test genotypic linkage disequilibrium for each pair of loci in each sampling area. To estimate popula-

tion variance in allele frequencies among nesting and wintering areas, we used GENEPOP and the program FSTAT (Goudet 2001) to generate *F*-statistics (Weir 1996) and R_{ST}, an analog of Nei's (1973) *G*-statistic (Michalakis and Excoffier 1996). Nesting and wintering areas were tested separately and were not grouped with respect to location within the western or eastern arctic populations. Per-locus significance levels for tests of Hardy-Weinberg equilibrium and pairwise population tests of F_{ST} among all areas were adjusted using the sequential Bonferroni correction procedures of Rice (1989) with $\alpha = 0.05$.

To further examine population structure of nesting and wintering areas we used the Bayesian clustering method of Pritchard et al. (2000), implemented in the program STRUCTURE. The program uses multilocus genotypes to infer population structure and to assign individuals probabilistically to populations. Even with few microsatellite markers (<10), this method has produced reliable inferences regarding population structure (Galbusera et al. 2000). We examined the probability that nesting and wintering King Eiders originated from *K* populations (where *K* is unknown), each of which is characterized by a set of multilocus allele frequencies. In separate analyses of nesting and wintering data, we estimated the probability of each *K* from 1 to 5. No prior population information is used, but posterior probabilities or the model likelihood of each *K* are estimated for each hypothetical *K*. Model likelihood scores are similar to those derived through Akaike's Information Criterion (AIC) weights (Burnham and Anderson 1998). Improvement in goodness of fit for each *K* was evaluated using a likelihood-ratio test. Results are based on 100 000 Markov chain Monte Carlo iterations following a burn-in period of 30 000 iterations and five repetitions of each value of *K*.

The program ARLEQUIN (Schneider et al. 2000) was used to estimate mtDNA nucleotide diversity (π; Nei and Tajima 1983), haplotype diversity (*h*; Nei 1987) and examine the selective neutrality of mtDNA by generating values for Tajima's *D* (Tajima 1989). Values of Tajima's *D* and Fu's F_s (Fu 1997) were also used to infer patterns in population size because they are sensitive to departures from demographic equilibrium (Aris-Brosou and Excoffier 1996, Fu 1997). Significantly large negative F_s values can be interpreted as evidence for population expansion. Evidence for population expansion were also examined by deriving the mismatch distribution of pairwise genetic differences in the program DNASP (version 3.51, Rozas and Rozas 1999). The distribution tends to be multimodal when populations are at equilibrium and unimodal in cases of recent demographic expansion (Rogers and Harpending 1992). To graphically display the observed mismatch distribution compared to the expected distributions for populations in equilibrium and expansion, we used Roger's method of moments (Rogers 1995) as calculated in DNASP. Historical population dynamics were further examined by generating a maximum-likelihood estimate of the growth parameter *g* and its standard deviation using the approach of Kuhner et al. (1998) in the program FLUCTUATE. Positive values of *g* are indicative of population expansion, while negative values indicate retraction. Values of the genetic diversity parameter, θ, remained fixed at 1.0 and we used 99% confidence intervals to test the significance of *g* from zero. Five independent runs were used to generate a mean value.

ARLEQUIN was used to generate estimates of intrapopulation variance in mtDNA haplotype frequency (Φ_{ST}) among nesting and wintering areas separately. Estimates of Φ_{ST} were generated using molecular information according to the most appropriate model and gamma shape parameter as identified by MODELTEST (Posada and Crandall 1998). Estimates of population differentiation (Φ_{ST}) in ARLEQUIN were tested for statistical significance using 10 000 randomizations of the data. We evaluated the mtDNA haplotype genealogy of all King Eider sampling areas by generating an unrooted haplotype network or cladogram using a statistical parsimony criterion (Templeton et al. 1992) in the program TCS (version 1.13, Clement et al. 2000). The network graphically displays the substitutional relationships among haplotypes and can then be used to test for phylogeographic signal (Templeton 2002). Following the method of Templeton (1998), we partitioned the haplotype network into a series of nested clades (Templeton et al. 1987) to infer genetic and demographic processes associated with the geographical distribution of haplotypes. Clades were tested for significantly small or large geographic distribution by analysis of variance using GEODIS (version 2.0, Posada et al. 2000a) with 10 000 randomizations to assess confidence. The program inference key

TABLE 1. Estimates of observed (H_o) and expected (H_e) heterozygosity and number of alleles (*Na*) per locus for six microsatellite loci assayed in four breeding and three wintering areas of King Eiders (Fig. 1).

		Breeding area (*n*)				Wintering area (*n*)		
Locus		Russia (29)	Alaska (66)	Queen Maud Gulf (47)	Southampton Island (32)	Kodiak Island (38)	St. Paul Island (60)	Greenland (52)
Alaμ1	H_o/H_e	0.19/0.21	0.45/0.49	0.30/0.32	0.19/0.19	0.25/0.25	0.33/0.39	0.30/0.32
	Na	7	9	8	8	10	10	7
Bcaμ1	H_o/H_e	0.08/0.09	0.19/0.19	0.10/0.09	0.13/0.13	0.16/0.15	0.20/0.21	0.15/0.15
	Na	4	4	3	4	4	3	5
Bcaμ11	H_o/H_e	0.21/0.21	0.56/0.54	0.42/0.38	0.23/0.24	0.33/0.29	0.44/0.45	0.34/0.37
	Na	5	5	5	7	6	6	6
Hhiμ5	H_o/H_e	0.15/0.15	0.28/0.25	0.15/0.22	0.16/0.16	0.13/0.18	0.26/0.32	0.21/0.26
	Na	4	6	5	5	7	9	7
Sfiμ9	H_o/H_e	0.22/0.22	0.20/0.31	0.28/0.32	0.22/0.21	0.27/0.27	0.40/0.43	0.29/0.40
	Na	8	9	7	6	9	10	8
Sfiμ11	H_o/H_e	0.22/0.25	0.66/0.66	0.44/0.42	0.26/0.25	0.32/0.34	0.43/0.48	0.40/0.42
	Na	10	12	9	10	9	10	8

(Posada et al. 2000b) was used to attribute possible biological processes to the observed pattern of clade distribution. Although nested clade analysis does not distinguish statistically among alternative interpretations of population history, and inferences drawn from analyses have been shown to be incorrect based on simulation studies (Knowles and Maddison 2002), it provides an additional inferential method for interpreting genealogical patterns.

RESULTS

GENETIC VARIATION

Allelic variation for the six nuclear microsatellite loci ranged between 3 and 12 alleles (Table 1). The number of alleles in each population was similar across areas, and estimates of observed (and expected) heterozygosity ranged from 0.08 to 0.66 (0.09–0.66). Only one locus (Hhiμ5) exhibited a departure from Hardy-Weinberg expectation among wintering birds in Greenland ($P < 0.001$) due to heterozygote deficiency. MtDNA cytochrome *b* sequences were identical for different tissue types within the same individual and corresponded to expected amino acid residues when compared to mtDNA cytochrome *b* sequences published for Harlequin Duck, indicating that DNA fragments amplified in this study represent mtDNA sequences and not nuclear pseudogenes. Eleven unique cytochrome *b* haplotypes (GenBank accession numbers AY124077–AY124080, AY124082, AY124084, AY124086, AY124087, AY124089–AY124091) defined by 11 variable sites were identified among 182 King Eiders (Table 2). Variable sites included one nonsynonymous first-position transition (haplotype C) and 10 synonymous third-position transitions. No deletions or insertions were observed. The minimum spanning tree involved the basal position of the two most common haplotypes (A and B) from which all the singleton haplotypes appear to be derived (Fig. 2). The small number of site substitutions and haplotypes per sampling area led to low estimates of nucleotide diversity (Table 2). Statistical tests of the neutral mutation hypothesis for mtDNA were negative (Tajima's $D = -1.53$, Fu's $F_s = -10.9$), but only Fu's F_s was significantly different from zero ($P < 0.02$), suggesting population growth. The mismatch distribution among haplotypes was unimodal (Fig. 3) and tests for goodness-of-fit of the observed data to a simulated model of population growth revealed no significant differences (sum of squared deviations = 0.04, $P = 0.1$; Harpending's raggedness index = 0.14, $P = 0.14$). The mean ($\pm$ SE) estimate of the growth parameter g was large (2559.6 $\pm$ 60.9) and in each of five runs of the program, we obtained similar values that were within the 95% confidence limits of the mean.

POPULATION STRUCTURE

Based on an analysis of both nuclear microsatellite allele and mtDNA haplotype frequencies, little evidence was observed for genetic differentiation among nesting or wintering areas within or between the western and eastern arctic pop-

TABLE 2. Number, nucleotide (π) and haplotype (h) diversity, and sequence differences of 11 cyt *b* mtDNA haplotypes in 182 King Eiders. Position numbers (read vertically) refer to the location of each variable site in the sequence. Dots indicate similarity with haplotype A. ? = sequence ambiguity.

	Sampling location																
	Breeding				Wintering			Position									
Haplotype	Russia	Alaska	Queen Maud Gulf	Southampton Island	Kodiak Island	St. Paul Island	Greenland	1 0 5 5	1 2 5 9	1 4 2 4	1 4 4 1	2 0 1 4	2 0 4 4	2 6 4 5	3 2 5 8	3 7 8 5	4 0 3 4	4 5 3
A	19	25	18	11	14	17	36	G	C	T	T	C	G	G	G	C	A	G
B		4		3	9	8	9	.	.	.	.	.	A	.	.	.	.	.
C				1				.	.	C	.	.	.	.	.	.	.	.
D				1				A	.	.	.	T	.	.	.	T	.	.
F					1			.	.	.	C	.	.	.	.	.	.	.
H							1	.	.	.	.	.	.	.	A	.	.	.
J							1	.	.	.	.	.	A	.	.	.	G	.
K							1	A	.	.	.	.	A	.	.	.	.	.
M							1	?	T	.	.	.	.	.	.	.	.	.
N							1	.	.	.	.	.	A	.	.	.	.	A
O							1	.	.	.	.	.	A	A	.	.	.	.
Total	19	29	18	16	24	25	51											
π	0.000	0.001	0.000	0.003	0.001	0.001	0.003											
h	0.000	0.246	0.000	0.516	0.539	0.453	0.477											

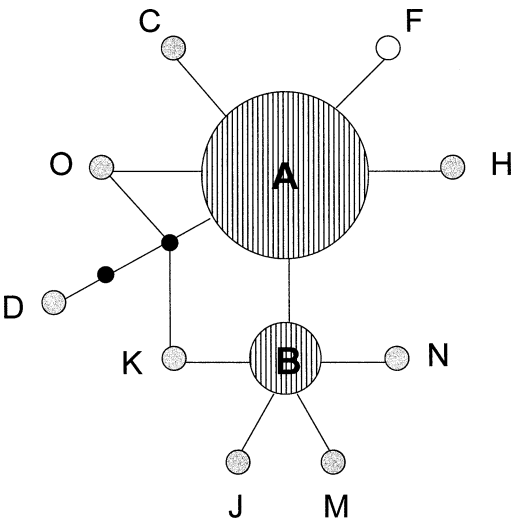

FIGURE 2. MtDNA minimum spanning tree for breeding and wintering areas of King Eiders. Circle size represents the frequency of each haplotype and connecting lines reflect a single mutation. White circles = haplotypes observed only in western-arctic breeding and wintering areas; gray circles = haplotypes observed only in eastern-arctic breeding and wintering areas; hatched circles = haplotypes observed in both areas; black circles = unsampled haplotypes.

ulations of King Eiders. The distribution of microsatellite alleles among all areas was similar. Two mtDNA haplotypes (A and B) were found in all wintering areas in similar frequencies (Table 2). Haplotype A was also observed among all nesting areas, but haplotype B was distributed at low frequency and inconsistently among nesting areas. Unique haplotypes were observed in most areas, but were particularly prevalent among wintering birds in western Greenland (Table 2).

None of the indices of population structure differed significantly from zero among nesting

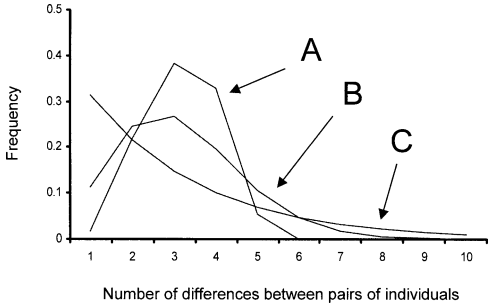

FIGURE 3. Mismatch distributions of (A) observed mtDNA haplotype variation in King Eiders compared to theoretical distributions under scenarios of (B) sudden population growth and (C) demographic equilibrium. Curves represent the frequency distribution of differences between pairs of mtDNA sequences.

TABLE 3. Estimated log-likelihood values (log *P*) and model likelihoods for different values of *K*, the number of hypothesized King Eider populations. Estimates were averaged over five repetitions for each value of *K* using the program STRUCTURE (Pritchard et al. 2000).

	Breeding		Wintering		Pacific vs. Atlantic[a]	
K	log *P*	Likelihood	log *P*	Likelihood	log *P*	Likelihood
1	–3097	1.0	–2547	1.0	–4868	1.0
2	–3123	0.0	–2602	0.0	–4927	0.0
3	–3149	0.0	–2623	0.0	–5291	0.0
4	–3145	0.0	–2766	0.0	–5378	0.0
5	–3116	0.0	–2774	0.0	–5463	0.0

[a] Pacific = Russia, Alaska, St. Paul Island, and Kodiak Island. Atlantic = Southampton Island and western Greenland (Fig. 1).

areas using information from microsatellite allele variation ($F_{ST} = 0.01$, $R_{ST} = 0.01$, $P > 0.05$) or mtDNA haplotypes ($\Phi_{ST} = 0.03$, $P = 0.37$) using the Tamura-Nei model of evolution as identified by MODELTEST ($\Gamma = 0.01$). The range of among-nesting-area pairwise estimates was low ($F_{ST} = -0.03$ to $+0.01$, $\Phi_{ST} = -0.01$ to $+0.08$). Variance estimates among wintering areas were similarly low and nonsignificant for both microsatellite ($F_{ST} = 0.00$, $R_{ST} = 0.01$, $P > 0.05$,) and mtDNA ($\Phi_{ST} = -0.03$, $P = 0.98$). The range of among-wintering-area pairwise estimates was low ($F_{ST} = -0.01$ to $+0.01$, $\Phi_{ST} = -0.04$ to $+0.02$). Results from program STRUCTURE revealed that the model likelihood of nesting and wintering area data was at a maximum with *K* equaling a single population (Table 3), suggesting that the species is genetically panmictic across the geographically disparate areas sampled. A similar result was observed when sampling areas were grouped into Pacific and Atlantic regions as proposed by banding data (Table 3). Nested clade analysis indicated that the geographic range of haplotype B (found in Alaska, Southampton Island, and all wintering areas), haplotype A (and associated tip clades), and haplotype D were significantly smaller than expected ($D_c = 2091$, $P = 0.03$, $D_c = 1393$, $P = 0.01$, and $D_n = 1767$, $P = 0.01$, respectively). Biological inferences based on these results using the program inference key suggested that the clade patterns of haplotypes B and D were the result of long-distance colonization and restricted gene flow, respectively. Inferences regarding haplotype A and associated tip clades were deemed inconclusive using the program key.

DISCUSSION

Microsatellite and mtDNA data offered congruent evidence for a lack of spatial genetic structure among western and eastern arctic populations of King Eiders. Similar microsatellite allele and mtDNA haplotype frequencies were observed among all nesting and wintering areas. Population structure was not detected by the Bayesian clustering method of Pritchard et al. (2000) either among nesting or wintering areas. Lack of structure among wintering areas was not due to the presence of both males and females within winter samples as sampling areas were also undifferentiated when we analyzed only a single sex (not shown). Tests of genotypic linkage disequilibrium for each pair of loci within wintering areas were nonsignificant, suggesting that admixtures of distinct genetic units were not present at these sites. Overall, our genetic data contrast with banding data that suggest a split between western and eastern arctic populations in North America. King Eiders exhibit fidelity to nesting areas (Kellett 1999), which over sufficient time should lead to genetic differentiation among areas, especially for a maternally inherited marker such as mtDNA. However, a recent analysis of stable-isotope ratios in nesting King Eiders (Mehl et al. 2004) suggests low levels of movement between western and eastern arctic populations, which could maintain a homogeneity of genetic characteristics. The lack of spatial variation in King Eider mtDNA haplotype frequency also contrasts with results for Spectacled Eiders (*S. fischeri*; Scribner et al. 2001), which show significant spatial variation of mtDNA haplotype frequency among their three primary arctic nesting areas. This disparity in

results suggests a smaller evolutionary effective population size in Spectacled Eiders and differences in levels of breeding philopatry. These differences are also evident in contemporary breeding distributions, with the Spectacled Eider nesting in three disparate regions of Beringia as compared to the Holarctic King Eider.

Several recent studies have attributed the lack of intraspecific phylogeographic structure to recent population growth following Pleistocene deglaciation (e.g., Great Tit [*Parus major*], Kvist et al. 1999; Marbled Murrelet [*Brachyramphus marmoratus*], Congdon et al. 2000; Lesser Black-backed Gull [*Larus fuscus*], Liebers and Helbig 2002; and Great Spotted Woodpecker [*Dendrocopus major*], Zink et al. 2002). We draw a similar conclusion for King Eiders, observing that the estimate of the exponential growth rate, *g*, significantly exceeded zero and the mismatch distribution was unimodal, both of which tend to occur in cases of a sudden population growth. Similarly, both Fu's F_s and Tajima's *D* were negative, which is expected under a scenario of demographic expansion (Fu 1997, Aris-Brosou and Excoffier 1996).

We observed no clear indication of the origin or direction of growth for King Eiders from the nested clade analysis. The biological inference key suggested long-distance dispersal to explain the presence of haplotype B in all wintering areas, but in only one nesting area in each of the western and eastern populations. We hypothesize that restricted gene flow caused the isolated occurrence of haplotype D in Southampton Island. Results were inconclusive with respect to haplotype A and its associated tip clades (haplotypes O, C, F, and H). Nearly all tip clades were situated in the eastern Arctic, predominantly among wintering birds near Greenland. These private haplotypes appear to be derived from the basal (and inferred ancestral) common haplotypes, suggesting that novel mutations detected in these samples have occurred primarily within the eastern arctic population. The restriction of these haplotypes to the eastern Arctic may indicate limited current gene flow. Additional banding of western-arctic King Eiders and satellite telemetry studies of eastern-arctic birds are needed to examine if the delineation of these two populations is a more recent trend than can be inferred with genetic markers.

In summary, results suggest that historical gene flow and population growth have homogenized gene frequencies among nesting and wintering areas compared in this study. Banding recoveries, recent stable-isotope data, and our observation of several private mtDNA haplotypes in the eastern Arctic suggest that exchange between western and eastern arctic populations may presently occur, but on a limited basis. If limited gene flow is evidence of a trend toward population delineation, this suggests a shift in behavior. Ploeger (1968) concluded that the lack of morphological differentiation among North American King Eiders, as compared to the codistributed races of Common Eiders, was due to the nomadic nature of King Eiders and their sharing of common wintering areas, which may have promoted movement among Pleistocene refugia during the last ice age. Population delineation of King Eiders may also be an ephemeral phenomenon on an evolutionary time scale. Effective population sizes may have also differed greatly between the two species in the past. Although banding data have suggested that some seaducks, such as Harlequin Ducks (Esler et al. 2000), demonstrate high levels of winter site fidelity, return rates are not 100%, and unpaired adult and juvenile Harlequin Ducks are known to disperse among Pacific wintering areas (Cooke et al. 2000). Thus, natal and adult dispersal can lead to the homogenization of gene frequencies, which Lanctot et al. (1999) observed among wintering Harlequin Ducks in south-central Alaska using microsatellite and mtDNA markers.

Anthropogenic effects on King Eiders may more greatly impact population size and distribution (Frimer 1995, Mosbech and Boertmann 1999) than overall genetic diversity. However, this may not be true for the wintering population in western Greenland, where several single unique mtDNA haplotypes were observed. Additional sampling of eastern North America and Greenland nesting and wintering areas is required to better assess the low frequency and distribution of these haplotypes. Nearly all of the seaduck species of North America are experiencing population declines, yet basic biological information is lacking to pinpoint the direct causes for these declines. This study highlights how the combination of data from multiple markers (banding, DNA, stable isotopes) can provide a more comprehensive picture of demographic patterns through time and aid in population assessment.

ACKNOWLEDGMENTS

We thank the following individuals for assistance with sampling: P. Flint and A. Fowler (U.S. Geological Survey), B. Anderson (ABR, Inc.—Environmental Research & Services), D. Troy (Troy Ecological Research Associates), R. Alisauskas, D. Kellett, C. James, G. Gilchrist, M. Wayland (Canadian Wildlife Service), R. Bromley (Northwest Territories Department of Renewable Resources), A. Degtyarev (Yakutsk Institute of Biology), D. Solovieva (Lena Delta State Nature Reserve), P. Chervenak, S. Phelps, P. Squartsoff (Kodiak Island), and F. Merkel (Greenland Institute of Natural Resources). P. Flint, D. Esler, G. Sage, K. Mehl, D. Derksen, D. Dobkin, and two anonymous reviewers provided helpful reviews of this manuscript. Financial support was provided by the U.S. Fish and Wildlife Service, the National Resource Damage Assessment, the U.S. Geological Survey, and the Danish Environmental Protection Agency.

LITERATURE CITED

ANDERSON, M. G., J. M. RHYMER, AND F. C. ROWHER. 1992. Philopatry, dispersal, and the genetic structure of waterfowl populations, p. 365–395. *In* B. D. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu [EDS.], Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis, MN.

ARIS-BROSOU, S., AND L. EXCOFFIER. 1996. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Molecular Biology and Evolution 13:494–504.

BUCHHOLZ, W. G., J. M. PEARCE, B. J. PIERSON, AND K. T. SCRIBNER. 1998. Dinucleotide repeat polymorphisms in waterfowl (family Anatidae): characterization of a sex-linked (Z-specific) and 14 biparentally inherited loci. Journal of Animal Genetics 29:323–325.

BURNHAM, K. P., AND D. R. ANDERSON. 1998. Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York.

CLEMENT, M., D. POSADA, AND D. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657.

CONGDON, B. C., J. H. PIATT, K. MARTIN, AND V. L. FRIESEN. 2000. Mechanisms of population differentiation in Marbled Murrelets: historical versus contemporary processes. Evolution 54:974–986.

COOKE, F., G. J. ROBERTSON, C. M. SMITH, R. I. GOUDIE, AND W. S. BOYD. 2000. Survival, emigration, and winter population structure of Harlequin Ducks. Condor 102:137–144.

DICKSON, D. L., R. C. COTTER, J. E. HINES, AND M. F. KAY. 1997. Distribution and abundance of King Eiders in the western Canadian Arctic, p. 29–39. *In* D. L. Dickson [ED.], King and Common Eiders of the western Canadian Arctic. Canadian Wildlife Service Occasional Paper No. 94, Ottawa, ON, Canada.

ESLER, D., J. A. SCHMUTZ, R. L. JARVIS, AND D. M. MULCAHY. 2000. Winter survival of adult female Harlequin Ducks in relation to history of contamination by the Exxon Valdez oil spill. Journal of Wildlife Management 64:839–847.

FIELDS, R., AND K. T. SCRIBNER. 1997. Isolation and characterization of novel waterfowl microsatellite loci: cross-species comparisons and research applications. Molecular Ecology 6:160–164.

FLINT, P. L., A. C. FOWLER, AND R. F. ROCKWELL. 1999. Modeling bird mortality associated with the *M/V Citrus* oil spill off St. Paul Island, Alaska. Ecological Modeling 117:261–267.

FRIMER, O. 1995. Adaptations by the King Eider *Somateria spectabilis* to its molting habitat: review of a study at Disko, West Greenland. Dansk Ornitologisk Forenings Tidsskrift 89:135–142.

FU, Y. X. 1997. Statistical tests on neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

GALBUSERA, P., L. LENS, E. WAIYAKI, T. SCHENCK, AND E. TATTYSEN. 2000. Effective population size and gene flow in the globally, critically endangered Taita Thrush, *Turdus helleri*. Conservation Genetics 1:45–55.

GOUDET, J. [ONLINE]. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). <http://www.unil.ch/izea/softwares/fstat.html> (10 March 2003).

GRATTO-TREVOR, C. L., V. H. JOHNSTON, AND S. T. PEPPER. 1998. Changes in shorebird and eider abundances in the Rasmussen Lowlands, NWT. Wilson Bulletin 110:316–325.

GUO, S. W., AND E. A. THOMPSON. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372.

HEWITT, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

KELLETT, D. 1999. Causes and consequences of variation in nest success of King Eiders (*Somateria spectabilis*) at Karrak Lake, Northwest Territories. M.Sc. thesis, University of Saskatchewan, Saskatoon, SK, Canada.

KNOWLES, L. L., AND W. P. MADDISON. 2002. Statistical phylogeography. Molecular Ecology 11:2623–2635.

KUHNER, M. K., J. YAMATO, AND J. FELSENSTEIN. 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149:429–434.

KVIST, L., M. RUOKONEN, J. LUMME, AND M. ORELL. 1999. The colonization history and present-day population structure of the European Great Tit (*Parus major major*). Heredity 82:495–502.

LANCTOT, R. L., B. GOATCHER, K. SCRIBNER, S. TALBOT, B. PIERSON, D. ESLER, AND D. ZWIEFELHOFER. 1999. Harlequin Duck recovery from the *Exxon Valdez* oil spill: a population genetics perspective. Auk 116:781–791.

LIEBERS, D., AND A. J. HELBIG. 2002. Phylogeography and colonization history of Lesser Black-backed Gulls (*Larus fuscus*) as revealed by mtDNA sequences. Journal of Evolutionary Biology 15: 1021–1033.

LOPEZ, J. V., N. YUHKI, R. MASUDA, W. MODI, AND S. J. O'BRIEN. 1994. Numt, a recent transfer and tandem amplification of mitochondrial DNA to the

nuclear genome of the domestic cat. Journal of Molecular Evolution 39:174–190.

LYNGS, P. 2003. Migration and winter ranges of birds in Greenland. An analysis of ringing recoveries. Dansk Ornitologisk Forenings Tidsskrift 97:1–168.

MEDRANO, J. F., E. AASEN, AND J. SHARROW. 1990. DNA extraction from nucleated red blood cells. Biotechniques 8:43.

MEHL, K. R., R. T. ALISAUSKAS, K. A. HOBSON, AND D. K. KELLETT. 2004. To winter east or west? Heterogeneity in winter philopatry in a central-arctic population of King Eiders. Condor 106:241–251.

MICHALAKIS, Y., AND L. EXCOFFIER. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics 42:1061–1064.

MOSBECH, A., AND D. BOERTMANN. 1999. Distribution, abundance and reaction to aerial surveys of post-breeding King Eiders (*Somateria spectabilis*) in western Greenland. Arctic 52:188–203.

NEI, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70:3321–3323.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

NEI, M., AND F. TAJIMA. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction site data. Genetics 105:207–217.

OETTING, W. S., H. K. LEE, D. J. FLANDERS, G. L. WEISNER, T. A. SELLERS, AND R. A. KING. 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. Genomics 30:450–458.

PEARCE, J. M., R. L. FIELDS, AND K. T. SCRIBNER. 1997. Nest materials as a source of genetic data for avian ecological studies. Journal of Field Ornithology 68:471–481.

PEARCE, J. M., B. J. PIERSON, S. L. TALBOT, D. V. DERKSEN, D. KRAEGE, AND K. T. SCRIBNER. 2000. A genetic evaluation of morphology used to identify harvested Canada Geese. Journal of Wildlife Management 64:863–874.

PLOEGER, P. L. 1968. Geographical differentiation in arctic Anatidae as a result of isolation during the last glacial period. Ardea 56:1–59.

POSADA, D., AND K. A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818.

POSADA, D., K. A. CRANDALL, AND A. R. TEMPLETON. 2000a. GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Molecular Ecology 9:487–488.

POSADA, D., K. A. CRANDALL, AND A. R. TEMPLETON [ONLINE]. 2000b. Nested clade analysis inference key. Version 24 October 2001. <http://inbio.byu.edu/Faculty/kac/crandall_lab/geodis.htm> (10 March 2003).

PRITCHARD, J. K., M. STEPHENS, AND P. J. DONNELLY. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

RANDI, E. 2000. Mitochondrial DNA, p. 136–167. *In* A. Baker [ED.], Molecular methods in ecology. Blackwell Science Limited, London.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248–249.

REGEHR, H. M., C. M. SMITH, B. ARQUILLA, AND F. COOKE. 2001. Post-fledging broods of migratory Harlequin Ducks accompany females to wintering areas. Condor 103:408–412.

RICE, W. R. 1989. Analyzing tests of statistical tables. Evolution 43:223–225.

ROBERTSON, G. J., AND F. COOKE. 1999. Winter philopatry in migratory waterfowl. Auk 116:20–34.

ROGERS, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608–615.

ROGERS, A. A., AND H. C. HARPENDING. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

ROZAS, J., AND R. ROZAS. 1999. DNASP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175.

SALOMONSEN, F. 1968. The moult migration. Wildfowl 19:5–24.

SALOMONSEN, F. 1979. Thirteenth preliminary list of recoveries abroad of birds ringed in Greenland. Dansk Ornitologisk Forenings Tidsskrift 73:191–206.

SCHNEIDER, S., D. ROESSLI, AND L. EXCOFFIER [ONLINE]. 2000. ARLEQUIN: a software for population genetics data analysis. Version 2.0. <http://lgb.unige.ch/arlequin> (10 March 2003).

SCRIBNER, K. T., AND J. M. PEARCE. 2000. Microsatellites: evolutionary and methodological background and empirical applications at individual, population, and phylogenetic levels, p. 235–271. *In* A. Baker [ED.], Molecular methods in ecology. Blackwell Science Limited, London.

SCRIBNER, K. T., M. R. PETERSEN, R. L. FIELDS, S. L. TALBOT, J. M. PEARCE, AND R. K. CHESSER. 2001. Sex-biased gene flow in Spectacled Eiders (Anatidae): inferences from molecular markers with contrasting modes of inheritance. Evolution 55: 2105–2115.

SCRIBNER, K. T., S. L. TALBOT, J. M. PEARCE, B. J. PIERSON, K. S. BOLLINGER, AND D. V. DERKSEN. 2003. Phylogeography of Canada Geese (*Branta canadensis*) in western North America. Auk 120: 889–907.

SORENSON, M. D., J. C. AST, D. E. DIMCHEFF, T. YURI, AND D. P. MINDELL. 1999. Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. Molecular Phylogenetics and Evolution 12:105–114.

SORENSON, M. D., A. COOPER, E. E. PAXINOS, T. W. QUINN, H. F. JAMES, S. L. OLSON, AND R. C. FLEISCHER. 1999. Relationships of the extinct moa-nalos, flightless Hawaiian waterfowl, based on ancient DNA. Proceedings of the Royal Society of London Series B 266:2187–2193.

SORENSON, M. D., AND T. W. QUINN. 1998. Numts: a challenge for avian systematics and population biology. Auk 115:214–221.

SUYDAM, R. S. 2000. King Eider (*Somateria spectabilis*). *In* A. Poole and F. Gill [EDS.], The birds of North America, No. 491. The Birds of North America, Inc., Philadelphia, PA.

SUYDAM, R. S., D. L. DICKSON, J. B. FADELY, AND L. T. QUAKENBUSH. 2000. Population declines of King and Common Eiders of the Beaufort Sea. Condor 102:219–222.

TAJIMA, F. 1989. The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. Genetics 143: 1457–1465.

TEMPLETON, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Molecular Ecology 7:381–397.

TEMPLETON, A. R. 2002. Out of Africa again and again. Nature 416:445–451.

TEMPLETON, A. R., E. BOERINKLE, AND C. F. SING. 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. Genetics 117:343–351.

TEMPLETON, A. R., K. A. CRANDALL, AND C. F. SING. 1992. A cladistic analysis of phenotypic association with haplotypes inferred from restriction endonuclease mapping. III. Cladogram estimation. Genetics 132:619–633.

WEIR, B. S. 1996. Genetic data analysis II: methods for discrete population genetic data. Sinauer Associates, Inc., Sunderland, MA.

ZINK, R. M., S. V. DROVETSKI, AND S. ROHWER. 2002. Phylogeographic patterns in the Great Spotted Woodpecker *Dendrocopos major* across Eurasia. Journal of Avian Biology 33:175–178.