Conserv Genet
DOI 10.1007/s10592-013-0477-8

RESEARCH ARTICLE

Phylogeography and population genetic structure of double-crested cormorants (*Phalacrocorax auritus*)

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Received: 18 September 2012 / Accepted: 11 March 2013

Abstract We examined the genetic structure of double-crested cormorants (*Phalacrocorax auritus*) across their range in the United States and Canada. Sequences of the mitochondrial control region were analyzed for 248 cormorants from 23 breeding sites. Variation was also examined at eight microsatellite loci for 409 cormorants from the same sites. The mitochondrial and microsatellite data provided strong evidence that the Alaskan subspecies (*P. a. cincinnatus*) is genetically divergent from other populations in North America (net sequence divergence = 5.85 %; Φ_{ST} for mitochondrial control region = 0.708; F_{ST} for microsatellite loci = 0.052). Historical records, contemporary population estimates, and field observations are consistent with recognition of the Alaskan subspecies as distinct and potentially of conservation interest. Our data also indicated the presence of another divergent lineage, associated with the southwestern portion of the species range, as evidenced by highly unique haplotypes sampled in southern California. In contrast, there was little support for recognition of subspecies within the conterminous U.S. and Canada. Rather than genetically distinct regions corresponding to the putative subspecies [*P. a. albociliatus* (Pacific), *P. a. auritus* (Interior and North Atlantic), and *P. a. floridanus* (Southeast)], we observed a distribution of genetic variation consistent with a pattern of isolation by distance. This pattern implies that genetic differences across the range are due to geographic distance, rather than discrete subspecific breaks. Although three of the four traditional subspecies were not genetically distinct, possible demographic separation, habitat differences, and documented declines at some colonies within the regions, suggests that the Pacific and possibly North Atlantic portions of the breeding range may warrant differential consideration from the Interior and Southeast breeding regions.

Keywords Double-crested cormorant · *Phalacrocorax auritus* · Mitochondrial DNA · Microsatellite · Population genetics · Phylogeography

Introduction

The double-crested cormorant (*Phalacrocorax auritus*) is a ubiquitous, colonial, North American waterbird that recently experienced dramatic increases in population numbers. Consequently, management to control numbers of the species has become conspicuous and controversial. Double-crested cormorants rapidly changed from being a species of concern in 1970 to being generally perceived as overabundant and a nuisance throughout parts of its range (Weseloh et al. 1995). The drastic population declines in the 1950s and 1960s were primarily due to pesticide contamination and persecution, and their recovery in the last quarter of the twentieth century was mainly attributed to the banning of DDT and inclusion as protected under the Migratory Bird

Electronic supplementary material The online version of this article (doi:10.1007/s10592-013-0477-8) contains supplementary material, which is available to authorized users.

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Published online: 02 April 2013

Treaty (Hatch 1995; Hatch and Weseloh 1999; Trapp et al. 1995). Consequently, cormorant populations have been increasing in most of central and eastern North America since the mid-1970s and recent estimates put the total population at 1–2 million individuals (about 350,000 breeding pairs; Hatch 1995). The increased abundance raises concern over possible impacts of high cormorant densities on other natural resources, particularly fisheries (Duffy 1995). Currently, management efforts for the species vary across the range depending on local abundance and perceptions regarding its role as a nuisance species, rather than population status and structure. In this study, we used independent nuclear markers and mitochondrial DNA (mtDNA) to describe the genetic structure of this controversial waterbird across its range in North America.

Double-crested cormorants are habitat generalists that occupy diverse aquatic environments including inland and bicoastal waters of North America. Colony sizes can range from a few individuals to several 100,000 pairs (Bent 1922; Palmer 1962; Hatch 1995; Hatch and Weseloh 1999). In North America, four subspecies have been described that occupy five geographic breeding regions (Fig. 1). *Phalacrocorax auritus* occupies the Interior and the North Atlantic regions; *P. a. floridanus* occupies the Southeast region, and breeds from Texas to Florida and north to the Carolinas; *P. a. cincinnatus* inhabits and breeds solely in Alaska; *P. a. albociliatus* occupies the Pacific region, and breeds along the Pacific Coast from British Columbia south to Sinaloa, Mexico and inland, possibly to New Mexico, Utah, and Montana [American Ornithologists' Union (AOU) 1957; Bent 1922; Hatch 1995; Hatch and Weseloh 1999; Palmer 1962; Wires and Cuthbert 2006). These subspecies were primarily based upon allopatry of breeding regions. The geographic distributions are not entirely discrete, however, but overlap and are poorly defined in some locations (Hatch 1995). Subspecies were also differentiated by size and crest character, but there is considerable variation in morphology and the distribution of crest characters is poorly known. In general, average size is described as increasing from southeast to northwest, and plumage varies across regions (Palmer 1962). Specifically, southeast birds are smallest and darkest with black crests, northeast birds have occasional white or partially colored crests, and north Pacific birds are largest and often have all-white crests (Palmer 1962). No detailed morphological analyses have been conducted on the species and no significant phenotypic differences between subspecies have been characterized.

Numbers of breeding pairs in each subspecies were estimated as: Interior and North Atlantic, 257,000–357,000

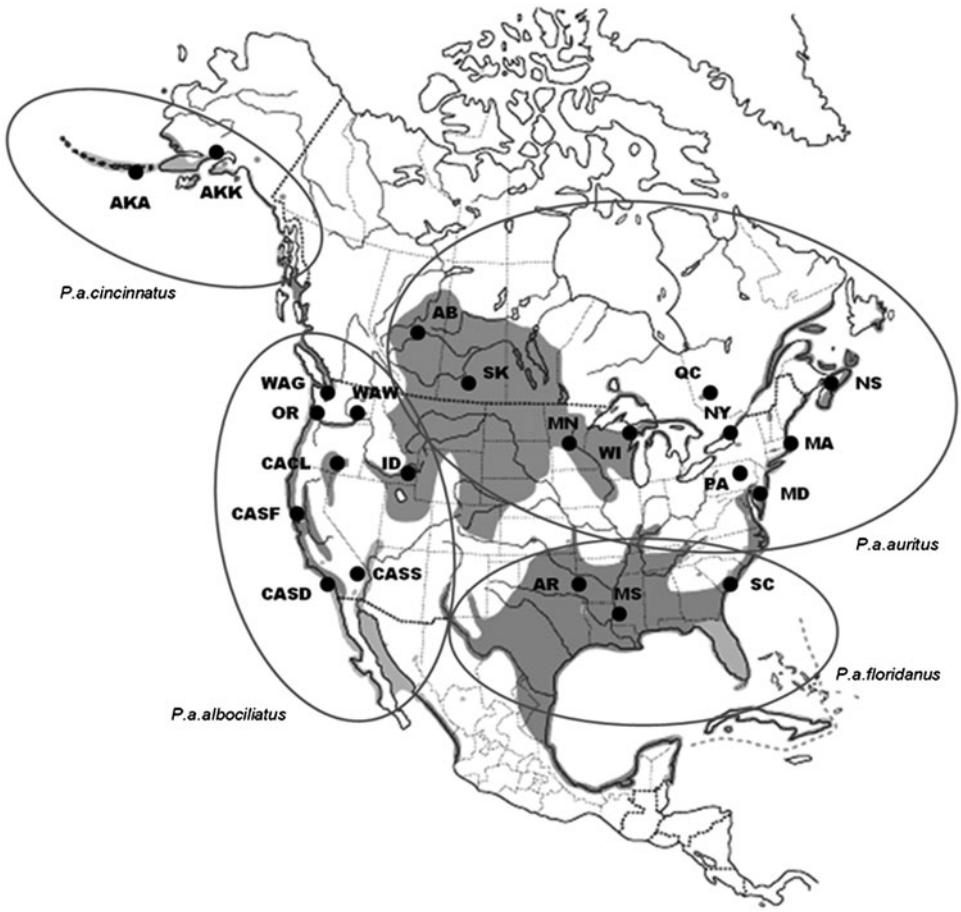

Fig. 1 Map of double-crested cormorant sampling sites included in current study. Sampling site information is provided in Table 1. Species overall range (modified from Hatch and Weseloh 1999) is shown, with approximate distributions of currently recognized subspecies (Alaska, *P. a. cincinnatus*; Pacific, *P. a. albociliatus*; Southeast, *P. a. floridanus*, and Interior/North Atlantic, *P. a. auritus*) *circled*

pairs (170,000–270,000 pairs, and 87,000 pairs, respectively); Southeast, 9,400 pairs; Pacific, 33,000 pairs; and Alaska, 3,029 pairs (Wires and Cuthbert 2006). Interior and North Atlantic cormorants are migratory, mostly wintering along the Gulf of Mexico, while other subspecies are mostly resident within their breeding regions. Explicit information about seasonal movement within and between regions is limited, although, banding data suggest cormorants have some tendency towards breeding site fidelity and geographically restricted dispersal (Clark et al. 2006; Dolbeer 1991; Guillaumet et al. 2011; Hatch and Weseloh 1999; King et al. 2010; Scherr et al. 2010).

Cormorant populations in the eastern United States are currently managed according to the Final Environmental Impact Statement and Record of Decision published by the U.S. Fish and Wildlife Service (USFWS) and the U.S. Department of Agriculture/Wildlife Services (USDA/WS) (USDI/FWS 2003a, b), which established a Public Resource Depredation Order effective in 24 eastern states. Concurrently, large-scale management plans to reduce cormorant numbers have been implemented in several areas, including New England (Krohn et al. 1995), the Great Lakes (Weseloh et al. 1995), and the St. Lawrence River (Bédard et al. 1995). In contrast, cormorants in western states were not included in the Environmental Impact Statement or Depredation Order, and are not actively managed for population control or conservation. In British Columbia the species is designated as "threatened" on the provincial Red List due to declines in most colonies in the province (British Columbia Conservation Data Centre 2008).

An understanding of population structure and status is necessary to better inform conservation and management practices for the species across its range. Previous genetic analyses of the species were limited to two studies that exclusively examined the relationship of southeastern U.S. cormorants relative to northeastern U.S. cormorants (Waits et al. 2003; Green et al. 2006). Both investigations failed to find significant genetic structure. The aim of this study was to examine and describe the genetic structure of double-crested cormorants in North America using a large number of breeding sites, individuals, mtDNA sequences, and microsatellite loci. Specific goals were to characterize the overall pattern of genetic differentiation within the species and identify and evaluate the genetic distinctiveness of individual breeding sites and a priori defined subspecies.

Methods

Sample collection and DNA extraction

Blood or tissue samples were collected from cormorants at 23 breeding sites across the species range during the 2002–2011 breeding seasons (Table 1; Fig. 1). In areas without a depredation order, blood samples were collected following the protocol of the American Ornithologists' Union (Gaunt and Oring 1997), or tissue samples were obtained from birds collected under a scientific collection permit. In areas with a depredation order, pectoral tissue samples were obtained from individuals that had been euthanized. No known close relatives (e.g., parent/offspring, siblings, etc.) were included in the sampling. Samples were stored in 2 ml cryogenic vials containing a buffer solution (100 mM Tris HCl, pH 8.0; 100 mM EDTA, pH 8.0; 10 mM NaCl; 0.5 % SDS) and frozen at −80 °C until analysis. DNA was obtained by standard phenol/chloroform extraction (Haig et al. 2004).

Mitochondrial DNA methods

An approximately 1.8 kb fragment containing ND6 and the control region of the mtDNA was initially obtained by long PCR using the primers L16087 (Desjardins and Morais 1990) and H1251 (Sorenson et al. 1999). Domains I and II of the control region were readily amplifiable, while long repeats in domain III made sequencing problematic. Similar complex repeats have been reported in other seabirds (Abbott and Double 2003; Berg et al. 1995; Burg 2000). Forward primer DC01 (5′-TAGCCCTCAACCACAGGA-3′) and reverse primer DC02 (5′-TTAGAAAGTTAGCGGTGGCG-3′) were subsequently designed to amplify a 900 bp fragment containing sequence from domains I and II.

Amplifications were performed using a PTC 100 thermal cycler (MJ Research). A total reaction volume of 20 µl was used with the following concentrations: 10 mM Tris–HCl at pH 8.3; 50 mM KCl; 3.5 mM $MgCl_2$; 100 µM for each dNTP; 0.2 µM of each primer; 100 ng of template; and 1.5 U AmpliTaq Gold Polymerase (Perkin Elmer). The following parameters were used for amplifications: 12 min denaturation at 93 °C, followed by 35 cycles of 30 s at 93 °C, annealing at 50 °C for 30 s, and elongation at 72 °C for 1 min. A final 10 min elongation period at 72 °C followed the last cycle. Bi-directional sequences were generated on an ABI 3100 automated sequencer located in the Central Services Laboratory at Oregon State University. Use of avian blood as a DNA source could allow the amplification of nuclear homologs (Sorenson and Fleischer 1996). However, there was no relationship between tissue type and haplotypes, which would be expected if amplification of blood samples were resulting in nuclear homologs, and there were no ambiguous peaks that occurred consistently across samples. Alignment, trimming, and manual adjustment of DNA sequences was completed using BIOEDIT version 7.0.5 software (Hall 2001). The final data set contained 700 bp of the control region for 248 double-crested cormorants sampled from 23 breeding sites (Table 1).

Table 1 Sample site information and within population genetic variation for mtDNA control region and eight microsatellite loci for double-crested cormorants. *N* is the number of individuals analyzed, *h* is the haplotype diversity, *s* is the number of polymorphic sites, π_n is nucleotide diveristy, H_E is expected heterozygosity, F_{IS} is the within population inbreeding coefficient, R_S is allelic richness

Site code	Site location	mtDNA					Microsatellites			
		N	*h*	*s*	π_n	Tajima's D	*N*	H_E	F_{IS}	R_s
Alaska (*P. a. cincinnatus*)										
AK-A	Eastern Aleutians, AK	5	0.700	8	0.006	1.028	5	0.600	0.208	4.13
AK-K	Kenai Peninsula, AK	18	0.660	10	0.007	2.122	20	0.593	0.094	3.87
Pacific (*P. a. albociliataus*)										
WA-G	Gray's Harbor Co., WA	10	0.867	77	0.055	1.725	17	0.611	0.061	4.21
WA-W	Walla Walla Co., WA	10	0.867	17	0.008	−0.454	20	0.720	0.002	5.03
OR	Clatsop Co., OR	17	0.882	82	0.042	0.631	20	0.668	0.065	4.79
CA-SF	San Francisco, CA	10	0.933	82	0.038	−0.585	17	0.675	0.096	4.83
CA-SD	San Diego, CA	20	0.895	74	0.045	2.129	20	0.634	0.026	4.59
CA-SS	Imperial Co., CA	10	1.000	80	0.053	1.243	20	0.718	−0.008	5.23
CA-Cl	Modoc Co., CA	10	0.756	13	0.075	0.621	14	0.656	0.088	4.26
ID	Caribou Co., ID	10	0.978	69	0.026	−1.393	20	0.680	−0.002	4.97
Interior/North Atlantic (*P. a. auritus*)										
Interior										
AB	Lakeland, AB	10	1.000	31	0.013	−0.752	18	0.712	0.054	5.16
SK	Regina, SK	8	0.964	22	0.013	−0.409	20	0.709	0.069	4.85
MN	Cass Co., MN	10	1.000	26	0.010	−1.022	20	0.683	0.075	5.02
WI	Door Co., WI	10	1.000	29	0.013	−0.731	20	0.700	0.045	4.99
QC	Albitibi, QC	10	0.933	24	0.012	−0.296	20	0.683	0.048	4.66
NY	Buffalo Co., PA	10	0.978	26	0.013	−0.191	20	0.701	0.001	4.83
North Atlantic										
NS	Pictou Co., NS	10	0.822	13	0.008	0.885	20	0.680	0.072	4.51
MA	Dukes Co., MA	10	0.911	14	0.008	0.829	12	0.693	0.033	4.75
MD	Talbot Co., MD	10	0.933	17	0.007	−1.073	12	0.732	0.047	5.11
PA	Harrisburg Co., PA	10	0.933	26	0.011	−0.883	20	0.656	0.065	4.69
Southeast (*P. a. floridanus*)										
AR	Sevier Co., AR	10	0.867	21	0.011	−0.085	15	0.681	0.132	4.62
MS	Yazoo Co., MS	10	0.978	23	0.011	−0.167	20	0.706	0.098	4.77
SC	Calhoun Co., SC	10	0.889	19	0.001	−0.624	19	0.688	0.034	4.78

Phylogenetic inference was conducted with maximum parsimony criteria, maximum likelihood criteria, and Bayesian analysis. Only unique sequences, i.e., haplotypes, were included in matrices used for phylogenetic analyses. Parsimony analyses were conducted with program PAUP* 4.0b10 (Swofford 2000) using a heuristic search with 100 random addition-sequence replicates and tree-bisection-reconnection (TBR) branch swapping. Nodal support was assessed through nonparametric bootstrap analysis using 1,000 bootstrap replicates with 10 random addition-sequence replicates per bootstrap replicate. The most appropriate model of sequence evolution was selected using Akaike's information criteria (Akaike 1974) with the program Modeltest 3.7 (Posada and Crandall 1998). Likelihood analysis was then conducted in PAUP* using successive iterations with starting parameters based on estimates from the previous tree (Sullivan et al. 2005). Parameters for the first tree were estimated from the most-parsimonious tree with the best likelihood score. Iterations were continued until successive searches yielded identical trees, likelihood scores, and model parameters.

Bayesian analyses were conducted in MRBAYES version 3.1.1 (Huelsenbeck and Ronquist 2001). Two replicate analyses, with four Markov chains each, were conducted simultaneously. Markov chains were run for 7 million generations and sampled once every 100 generations. We used a conservative burn-in of 20,000 generations that was determined by examining stationarity of the likelihood scores and convergence of posterior probabilities between the two runs using the standard deviation of split frequencies. Additionally, we used the computer program NETWORK (available from www.fluxus-engineering.com)

to obtain a median-joining network of the analyzed haplotypes (Bandelt et al. 1999). An initial star-contraction procedure (Forster et al. 2001), with a star connection limit of 2, was used to reduce the data set and provide a clearer presentation.

All control region sequences were used for population genetic analyses and grouped as populations by breeding site (i.e., colony). Summary statistics within and among breeding sites were derived using ARLEQUIN version 3.01 (Excoffier et al. 2005). Genetic variation within breeding sites was measured with a variety of diversity statistics, including haplotype diversity (h), number of polymorphic sites (s), and nucleotide diversity (π_n). Deviations from the assumptions of neutrality (Kimura 1983) were measured using Tajima's (1989) D statistic. Genetic divergence among breeding sites was estimated using F statistics in Arlequin version 3.01, which takes into account the number of mutations between haplotypes. A Tamura-Nei correction (Tamura and Nei 1993) was used for all calculations. Pairwise Φ_{ST} values were calculated among all individual sites and for comparisons between subspecies. One thousand random permutations were used to test the probability of observing Φ_{ST} values as large as or larger than those observed by chance, and the significance level for each test (α) was determined using a sequential Bonferroni adjustment (Rice 1989). We also calculated the corrected average percent sequence divergence, equivalent to net sequence divergence, between phylogenetically identified clades and a priori subspecies in Arlequin.

Population structure was further analyzed by conducting a principal coordinate analysis (PCoA) in the program GenAlEx 6.5 (Peakall and Smouse 2012), using the pairwise genetic distance matrix calculated previously in Arlequin. The presence of an isolation by distance pattern was tested in GenAlEx 6.5 by performing a Mantel test (Mantel 1967), using the genetic distance matrix from Arlequin and a matrix of geographic distances between breeding sites. One hundred random permutations were used to test the probability of observing a correlation (r) as large, or larger, than that observed by chance.

Microsatellite methods

Microsatellite primer sequences were obtained from two sources (Piertney et al. 1998; Mercer et al. 2010). Initially, seven primer pairs isolated from the great cormorant (*P. carbo*; Piertney et al. 1998) were tested for cross-species amplification. From these, three loci (PcD2, PcT3, and PcT4) were polymorphic and gave reproducible results. Additional microsatellite loci were isolated and screened (Mercer et al. 2010), and five novel loci (DCCO-01, DCCO-02, DCCO-03, DCCO-04, DCCO-05) were analyzed. PCR amplifications were performed in 20 µl reactions using the same conditions as described for mitochondrial amplifications, except with a 54 °C annealing temperature. The resulting fragments were analyzed using an ABI 3100 automated sequencer located in the Central Services Laboratory at Oregon State University. ABI Genemapper® software was used to score alleles. MICROCHECKER software (Oosterhout et al. 2004) was used to check for the presence of null alleles and other possible genotyping errors. Linkage disequilibrium between loci across all breeding sites and deviations from Hardy–Weinberg genotype frequency for each locus and breeding site were tested with GENEPOP version 3.4 (Raymond and Rousset 1995). Significance for these analyses was evaluated by Fisher exact tests, where P values were estimated by applying a Markov chain method. Sequential Bonferroni corrections were applied to determine the significance level of each test (Rice 1989).

Genetic variation within breeding sites was quantified based on average gene diversity (H_E), within-population inbreeding coefficient (F_{IS}), and allelic richness (R_S), as calculated in FSTAT version 2.9.3.2 (Goudet 2001). Genetic divergence among breeding sites was estimated with F-statistics using Arlequin version 3.01, as described for mtDNA. In addition, a PCoA and a Mantel test were performed as described for mitochondrial data.

We also used a Bayesian clustering approach implemented in program STRUCTURE version 2.2.3 (Pritchard et al. 2000) to estimate the number of populations (K) and assign individuals to one or more of these populations. We used the admixture model that assumes gene flow among populations and correlated allele frequencies. This model assigns a proportion of each individual's genome to each population. STRUCTURE was run for $K = 1$ to $K = 8$ clusters. Each run was pursued for 1 million Markov chain Monte Carlo (MCMC) iterations, with an initial burn-in of 50,000, which gave consistent results in preliminary runs. Ten independent simulations were run for each K to assess stability and the mean ln P(d) across runs for each K was calculated. An ad hoc statistic (ΔK) was estimated, as it was shown to be a better predictor of the true K at the uppermost hierarchical level (Evanno et al. 2005).

Results

Mitochondrial DNA variation

In the final alignment, 116 of 700 total characters were variable, of which 104 were parsimony informative. In total, 87 haplotypes were detected. Only one haplotype was common and geographically widespread, occurring in 27 individuals sampled in the Pacific, Interior/North Atlantic, and Southeast. All other haplotypes were found in 10 or fewer individuals. Consequently, there were many low-

frequency haplotypes detected in only a single site and thereby subspecies. The haplotypes unique to a subspecies included: 6 in Alaska, 24 in the Pacific, 32 in the Interior/North Atlantic (24 unique to the Interior, 5 unique to the North Atlantic, and 5 shared by the Interior and North Atlantic), and 9 in the Southeast. Finally, there were 16 haplotypes shared among subspecies, although Alaska shared no haplotypes with other sites.

Within-population genetic diversity was generally high, although haplotype and nucleotide diversity exhibited a range of values across breeding sites (Table 1). Haplotype diversity was lowest in Kenai, Alaska (0.66) and the Aleutian Islands, Alaska (0.70) and highest in Alberta, Minnesota, Wisconsin, and Imperial, California (1.00). Nucleotide diversity was also lowest in Kenai, Alaska (0.007), and highest in Imperial, California (0.053) and Grays Harbor, Washington (0.055). At no site did Tajima's *D* statistic differ significantly from expectations under neutrality, providing no evidence of population bottlenecks or expansion. Overall *D* for cormorants was 0.703 ($P > 0.10$).

Maximum-parsimony analysis generated 6,084 most parsimonious trees of 367 steps (CI = 0.624, RI = 0.900). The best model of sequence evolution chosen by Modeltest 3.7 was a GTR+I+G model (general time reversible model, with a proportion of invariant sites, and among-site rate heterogeneity). The maximum likelihood analysis only required two iterations to reach convergence of tree topologies and branch lengths. The negative log-likelihood score of the final tree was 2,824.92 (estimated base frequencies: A: 0.2875, C: 0.1938, G: 0.1733, T: 0.3453; rate matrix: A–C: 2.309, A–G: 36.050, A–T: 1.100e−10, C–G: 1.048, C–T: 30.882, G–T: 1.000; shape parameter for gamma distribution: 0.4144; proportion of invariant sites: 0.4885). In the Bayesian analysis, plots of model parameters and likelihood scores indicated stationarity was reached by generation 100,000. Further, the standard deviation of split frequencies between runs indicated convergence had also been achieved after removal of a conservative burn-in of 20,000 generations. Thus, the last 50,000 sampled trees in each of two runs were combined to yield 100,000 trees for the final Bayesian posterior probabilities (bpp). The 50 % majority rule consensus tree of all sampled Bayesian trees had 41 nodes with >50 % support, 38 of which were also observed in the maximum likelihood tree. Bayesian posterior probabilities for these nodes are shown on the maximum likelihood tree (Fig. 2).

All analyses yielded nearly identical tree topologies with many well-supported nodes and a basal structure of three major clades having geographic affiliation. All Alaskan haplotypes, along with two Pacific haplotypes, formed one distinct clade (clade I; Fig. 2). Clade II consisted of seven haplotypes corresponding to 18 individuals from the Pacific, 13 of which were sampled in southern California. Between these two clades were four intermediary haplotypes, corresponding to five individuals from the Pacific. The third and largest clade, III, contained the remaining 69 haplotypes and included individuals from all subspecies, except for Alaska. Within this large clade, there were many well-supported sub-clades, although there was only shallow divergence and little geographic structure. The only slight geographic structure was the concentration of many North Atlantic samples in one sub-clade. The median-joining network was congruent with the phylogenetic analysis (Fig. 3), and the mutational groups identified were identical to the phylogenetic clades. Additionally, the network illustrated that within the main clade many haplotypes were shared between subspecies and all haplotypes were highly similar, with less than five mutational steps between any adjacent nodes.

The corrected average percent sequence divergence (equivalent to net percent sequence divergence) between clades was substantial, with 8.0 % divergence between clades I and II, and clade III, and 4.0 % divergence between clade I and II. Concordantly, the sequence divergence between Alaskan cormorants and other subspecies was large (5.85 %). In contrast, sequence divergence calculated for each of the other three subspecies was <1 % (Pacific 0.23 %; Interior/North Atlantic 0.77 %; Southeast 0.35 %).

Population genetic analyses revealed a distribution of genetic variation among breeding sites consistent with the phylogenetic analysis (Table 2). The overall Φ_{ST} value among all sites was large and statistically significant ($\Phi_{ST} = 0.44$, $P < 0.001$), while pairwise comparisons had a wide range of values from negative numbers up to 0.929. Of the 253 pairwise comparisons, 74 were significant after sequential Bonferroni correction and nearly all of the significant comparisons involved an Alaskan site (Kenai or Aleutians) or a North Atlantic site (Nova Scotia, Maryland, Massachusetts, or Pennsylvania). Thirty-eight of 42 comparisons (91 %) between Alaska and other sites were significant, with a mean Φ_{ST} of 0.817 (range 0.378–0.929). Thirty of 68 comparisons (44 %) between North Atlantic and other sites, outside Alaska, were significant, with a mean Φ_{ST} of 0.269 (range 0.024–0.460). The remaining 6 significant comparisons were 5 comparisons between Pacific sites and South Carolina, and 1 comparison between San Diego, California and Quebec. Additionally, large Φ_{ST} values were observed in comparisons between San Diego, California and other sites (mean 0.321; range −0.057 to 0.543), but most values were not significant.

Considering a priori groupings by subspecies, the greatest pairwise Φ_{ST} value was observed between Alaska and all other subspecies ($\Phi_{ST} = 0.708$; $P < 0.001$). Comparisons between the Pacific and other subspecies ($\Phi_{ST} = 0.064$; $P < 0.001$), the Interior/North Atlantic and other subspecies ($\Phi_{ST} = 0.204$; $P < 0.001$), and the Southeast and other

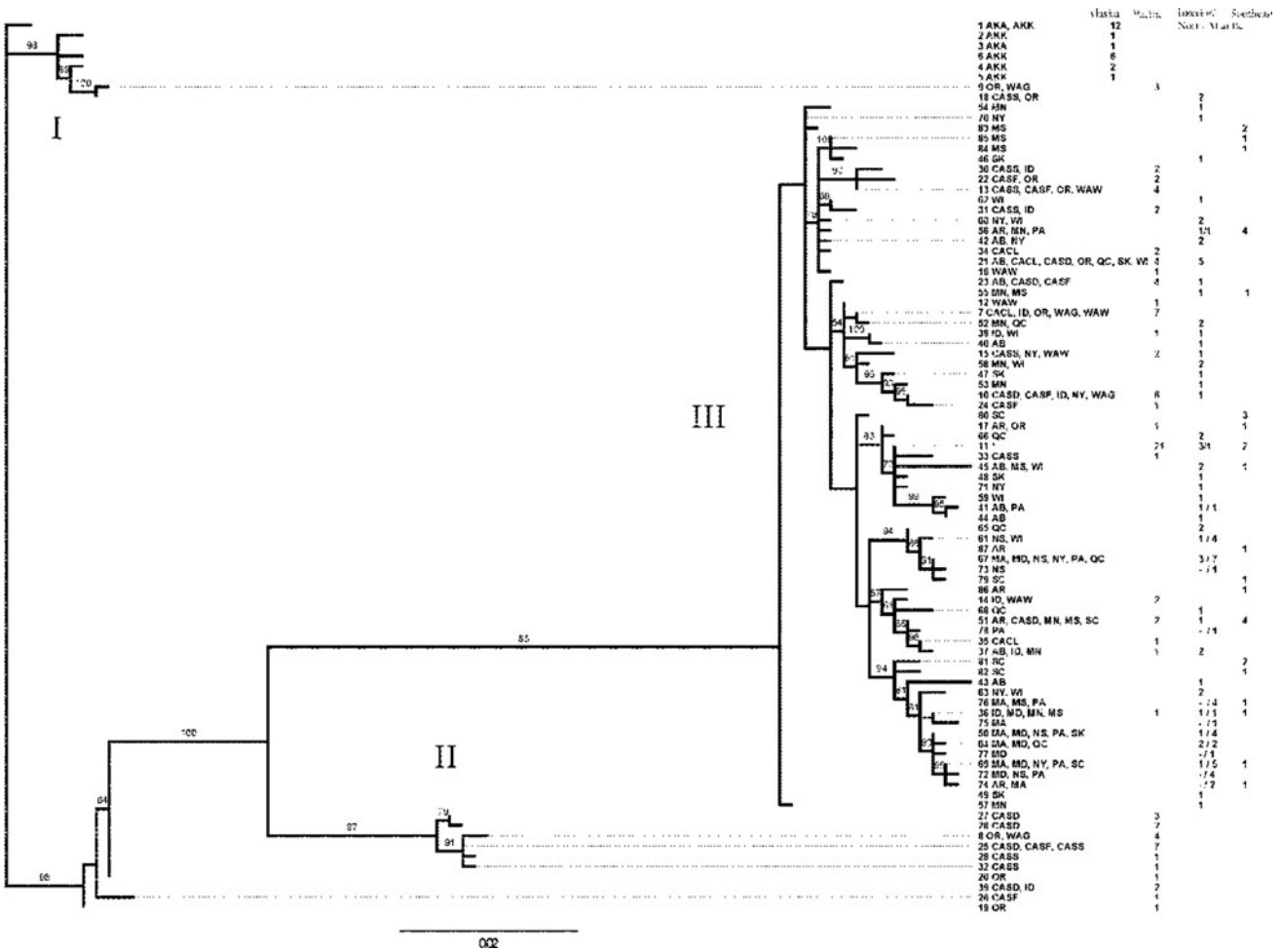

Fig. 2 Maximum likelihood tree from analysis of mtDNA control region for double-crested cormorants. Haplotype numbers are shown in *bold* at branch tips along with site codes (which correspond to codes used in Table 1 and Fig. 1); numbers of individuals per subspecies for each haplotype are given in right-hand columns. *Numbers* shown at nodes are Bayesian posterior probabilities from 100,000 sampled trees. *Roman numerals* identify the three major clades referred to in text. *AB, AR, CACL, CASF, CASS, MD, MS, OR, SK, WAG, WAW, WI

subspecies ($\Phi_{ST} = 0.085$; $P = 0.002$) were notably smaller, although still statistically significant.

The principal coordinate analysis illustrated a relationship between breeding sites that was roughly consistent with geographic relationship (Fig. 4a). Principal coordinate 1, which explained 52 % of the total genetic variance, primarily separated the geographically distant Alaskan sites from all other sites and differentiated some of the Pacific sites. Principal coordinate 2, which explained 23 % of the total genetic variance, primarily separated the North Atlantic sites (Massachusetts, Nova Scotia, Maryland, and Pennsylvania), but did not clearly discriminate the rest of the sites by region or otherwise. Overall, Alaskan and North Atlantic sites were clearly grouped by region, Pacific sites were loosely clustered by region, and Interior and Southeast sites were not separated. Similarly, the mantel test showed pairwise Φ_{ST} values were significantly and positively correlated with geographic distance ($r = 0.378$, $P = 0.01$).

Microsatellite DNA variation

The final microsatellite data set contained 112 alleles in total, with 2–28 alleles per locus (average = 14 alleles/locus). Within nearly all of the breeding sites, genotypic frequencies conformed to expected Hardy–Weinberg proportions for all eight loci and there was no evidence of linkage disequilibrium between any pairs of loci. No loci showed evidence of null alleles across sites, and MICROCHECKER detected no other potential scoring errors. Departures from Hardy–Weinberg equilibrium were found in only two cases: Kenai, Alaska and Grays Harbor, Washington for locus PcT4. Only three tests for linkage disequilibrium were significant after sequential Bonferroni correction: loci DCCO-02 and PcT4,

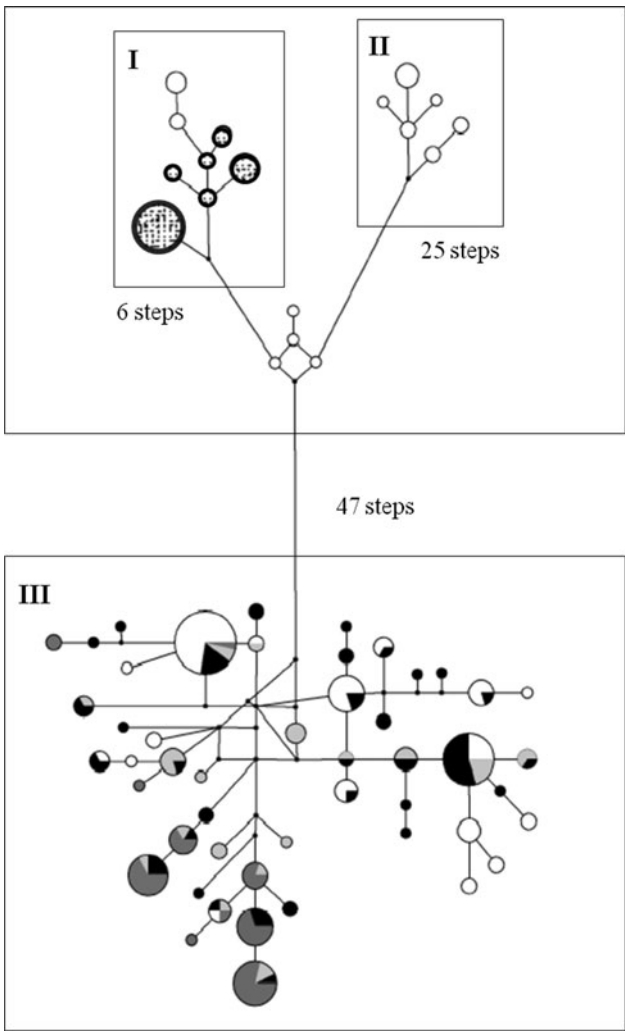

Fig. 3 Median joining network of mtDNA control region haplotypes for double-crested cormorants. *Circle sizes* are proportional to the number of individuals sharing the haplotype. *Shades* refer to the proportion of haplotypes that came from a subspecies: Alaska are *dappled black* and *white* with a *thick black outline*; Pacific are *white*; Southeast are *light gray*; Interior/North Atlantic are *black* (Interior) and *dark gray* (North Atlantic). Haplotype groups, concordant with clades in Fig. 2, are identified by *roman numerals*

loci DCCO-01 and PcD2, and loci PcT3 and PcD2 in Grays Harbor, Washington.

Overall, the eight microsatellite loci yielded similarly high levels of genetic diversity for all breeding sites (Table 1). The mean allelic richness per site was 4.72, ranging from 3.87 (Kenai, Alaska) to 5.23 (Imperial, California), and expected heterozygosity averaged across loci ranged from 0.593 (Kenai, Alaska) to 0.732 (Maryland). Likewise, the population inbreeding coefficient (F_{IS}) ranged from 0.208 (Eastern Aleutians, Alaska) to −0.002 (Caribou, ID), although no F_{IS} values were significant after correction. A total of 21 alleles unique by subspecies were sampled, including: 4 in Alaska; 8 within the Pacific; 8 within the Interior/North Atlantic (4 unique to the Interior, 4 unique to the North Atlantic, and none shared by the Interior and North Atlantic); and 1 within the Southeast. The majority of these alleles were also unique to one breeding site; only four of the alleles unique by subspecies were shared by two or three breeding sites. Concordantly, all unique alleles were low frequency with frequencies ranging from 0.003 to 0.020.

The global F_{ST} value for all breeding sites was 0.028 and was statistically significant ($P < 0.001$), and pairwise F_{ST} values ranged from negative values up to 0.107. Of the 253 pairwise comparisons, 93 were significant after sequential Bonferroni correction (Table 2). In agreement with the mitochondrial Φ_{ST} values, nearly all significant comparisons involved either an Alaskan site or a North Atlantic site. Thirty out of 42 comparisons (71 %) between Alaska and other sites were significant, with a mean $F_{ST} = 0.072$ (range 0.045–0.107). Thirty of 68 comparisons (44 %) between North Atlantic and other sites, outside Alaska, were significant, with mean $F_{ST} = 0.030$ (range 0.004–0.073). The remaining 33 significant comparisons were mainly between Pacific sites and eastern sites. Similar to mitochondrial results, large F_{ST} values were observed in comparisons between San Diego, California and other sites (mean 0.042; range 0.019–0.090). Unlike with the mitochondrial data, 15 of these comparisons (68 %) were significant. In addition, all comparisons with Gray's Harbor (Washington) were significant (mean 0.060; range 0.045–0.73).

Considering groupings by subspecies, the largest significant pairwise F_{ST} value was observed between Alaska and all other subspecies ($F_{ST} = 0.052$; $P < 0.001$). Comparisons between the Pacific and other subspecies ($F_{ST} = 0.012$; $P < 0.001$), the Interior/North Atlantic and other subspecies ($F_{ST} = 0.010$; $P < 0.001$), and the Southeast and other subspecies ($F_{ST} = 0.005$; $P = 0.003$) were small but nonetheless statistically significant.

As with the mtDNA data, the principal coordinate analysis and mantel test indicated a relationship between genetic and geographic distance (Fig. 4b). The total genetic variance explained by the first and second coordinates was 39, and 22 %, respectively. Similar to mtDNA results, the principal coordinate analysis clearly separated the Alaskan sites, along with the Gray's Harbor, Washington, slightly separated the Pacific sites and North Atlantic sites from other regions, and did not distinguish the Interior and Southeast sites. Congruently, pairwise F_{ST} values were significantly and positively correlated with geographic distance ($r = 0.316$, $P = 0.03$).

In the STRUCTURE analysis, the number of clusters (K) with the highest mean log probability of the data [ln P(D) = −11766.3] was K = 2. However, the mean ln P(D) was only 15 lower for K = 3 and ΔK was higher for K = 3 than K = 2 (ΔK = 19.6 and 12.6, respectively).

Table 2 Pairwise genetic distances between sampling sites, ordered by subspecies. Pairwise Φ_{ST} values for mtDNA control region for 248 double-crested cormorants shown below the diagonal. Pairwise F_{ST} values for 8 microsatellite loci and 409 double-crested cormorants shown above the diagonal. Significant values are in bold and highlighted

	Alaska	Pacific								Southeast			Interior/						North Atlantic				
Site	**AK-A**	**AK-K**	**WA-G**	**WA-W**	**OR**	**CA-SF**	**CA-SD**	**CA-SS**	**CA-CL**	**ID**	**AR**	**MS**	**SC**	**AB**	**SK**	**MN**	**WI**	**QC**	**NY**	**PA**	**MD**	**MA**	**NS**
AK-A	---	0.040	0.072	**0.057**	0.049	0.060	**0.090**	0.061	**0.081**	**0.060**	**0.097**	0.067	0.060	0.049	0.061	0.058	0.065	0.062	**0.069**	**0.105**	0.089	**0.106**	**0.071**
AK-K	-0.042	---	**0.049**	**0.058**	**0.048**	**0.065**	**0.082**	**0.071**	**0.045**	**0.061**	**0.106**	**0.071**	**0.073**	**0.078**	**0.076**	**0.077**	**0.065**	**0.058**	**0.075**	**0.093**	**0.094**	**0.107**	**0.095**
WA-G	0.378	**0.544**	---	**0.045**	**0.045**	**0.052**	**0.067**	**0.056**	**0.051**	**0.057**	**0.066**	**0.052**	**0.060**	**0.068**	**0.067**	**0.071**	**0.067**	**0.056**	**0.057**	**0.055**	**0.071**	**0.057**	**0.073**
WA-W	**0.917**	**0.920**	0.321	---	0.005	-0.002	0.021	-0.002	0.010	0.006	0.021	0.006	0.019	0.002	0.003	0.012	0.009	0.013	0.010	0.020	0.019	0.015	0.039
OR	0.530	**0.637**	0.014	0.113	---	0.010	0.025	0.001	0.013	0.006	**0.030**	0.018	0.031	0.016	0.020	0.008	**0.021**	0.018	0.021	**0.044**	**0.044**	0.035	**0.044**
CA-SF	**0.647**	**0.756**	0.055	0.049	-0.044	---	0.020	0.002	0.023	0.013	0.025	0.019	0.025	0.009	0.016	0.019	0.013	0.025	0.022	0.024	0.034	0.027	**0.039**
CA-SD	0.433	**0.543**	-0.025	0.376	0.134	0.145	---	0.009	0.019	0.028	**0.050**	**0.039**	**0.036**	**0.029**	**0.044**	0.021	**0.038**	**0.044**	**0.037**	**0.060**	**0.063**	**0.041**	**0.061**
CA-SS	0.463	**0.617**	-0.075	0.232	-0.018	-0.014	-0.017	---	0.021	0.004	0.014	0.009	0.019	0.000	0.007	-0.006	0.008	0.014	0.013	**0.036**	0.026	0.013	**0.036**
CA-CL	**0.919**	**0.921**	0.328	-0.060	0.118	0.062	0.380	0.242	---	0.022	**0.045**	0.026	**0.038**	**0.030**	0.027	0.030	0.032	**0.031**	0.027	**0.052**	**0.058**	**0.059**	**0.060**
ID	**0.759**	**0.828**	0.164	0.033	0.020	-0.057	0.244	0.089	0.049	---	0.024	0.021	**0.027**	0.008	0.005	0.004	0.023	0.012	**0.027**	**0.034**	**0.045**	**0.033**	**0.045**
AR	**0.896**	**0.908**	0.323	0.061	0.130	0.072	0.369	0.235	0.022	0.024	---	0.013	0.024	0.005	0.009	0.013	0.023	0.011	0.018	0.031	0.029	0.026	**0.036**
MS	**0.890**	**0.905**	0.313	0.063	0.128	0.069	0.360	0.229	0.036	0.012	-0.031	---	0.008	0.009	0.005	0.012	0.005	-0.001	-0.001	0.016	0.006	0.007	0.024
SC	**0.911**	**0.916**	**0.330**	**0.228**	0.160	**0.137**	0.377	**0.268**	**0.222**	0.090	0.110	0.097	---	0.012	0.019	0.014	0.014	0.008	0.012	0.026	0.005	0.004	0.006
AB	**0.876**	**0.899**	0.295	-0.038	0.111	0.041	0.351	0.210	-0.035	-0.005	0.001	-0.017	0.123	---	-0.004	-0.003	0.004	0.005	0.005	**0.027**	0.014	0.013	0.023
SK	**0.893**	**0.908**	0.280	0.031	0.101	0.020	0.334	0.191	-0.006	-0.010	-0.034	-0.066	0.172	-0.024	---	0.008	0.005	0.007	0.011	**0.028**	0.023	0.024	0.032
MN	**0.896**	**0.908**	0.302	0.059	0.126	0.049	0.347	0.217	0.068	-0.035	0.008	-0.018	0.163	-0.006	-0.034	---	0.010	0.006	0.009	**0.043**	**0.036**	0.019	**0.035**
WI	**0.882**	**0.902**	0.304	-0.020	0.117	0.045	0.357	0.217	-0.024	-0.004	-0.030	-0.049	0.118	-0.065	-0.056	-0.023	---	0.006	-0.004	0.020	0.005	0.010	0.021
QC	**0.889**	**0.904**	0.315	0.114	0.138	0.097	**0.372**	0.247	0.108	0.043	0.030	0.027	0.001	0.053	0.075	0.079	0.026	---	0.000	0.013	0.006	0.010	0.017
NY	**0.880**	**0.900**	0.301	0.030	0.122	0.043	0.353	0.217	0.033	-0.011	-0.030	-0.034	0.078	-0.015	-0.042	-0.018	-0.065	-0.020	---	**0.026**	0.000	0.004	0.015
PA	**0.896**	**0.909**	0.335	0.251	0.179	0.149	**0.379**	**0.270**	0.240	0.099	0.116	0.080	0.040	0.123	0.153	0.162	0.125	0.024	0.073	---	0.013	0.002	**0.034**
MD	**0.929**	**0.927**	**0.391**	**0.460**	0.250	**0.252**	0.423	**0.339**	**0.449**	**0.237**	0.323	0.280	0.209	**0.317**	0.357	**0.377**	0.321	0.178	0.256	-0.012	---	-0.013	-0.003
MA	**0.915**	**0.919**	**0.378**	**0.422**	0.238	**0.235**	**0.416**	**0.328**	**0.419**	0.208	0.280	**0.249**	0.152	**0.295**	0.330	**0.337**	0.281	0.105	0.196	-0.006	-0.026	---	-0.005
NS	**0.917**	**0.920**	**0.381**	**0.435**	0.238	**0.241**	**0.420**	**0.331**	**0.439**	**0.230**	0.301	0.313	0.223	**0.328**	**0.373**	**0.371**	**0.281**	0.125	0.190	0.197	0.303	0.137	---

Therefore, we chose K = 3 as the most appropriate number of cormorant population clusters following the recommendation of Evanno et al. (2005). The three clusters identified (Fig. 5) had some relationship with geography. Both Alaskan sites, Aleutians and Kenai, along with the Pacific site of Gray's Harbor, Washington had high mean membership in cluster 1 (68.1, 76.5, and 68.4 %, respectively). Similarly, the four North Atlantic sites, Pennsylvania, Maryland, Massachusetts, and Nova Scotia had high mean membership in cluster 3 (51.5, 63.2, 66.5, and 68.8 %, respectively). Finally, the San Diego, California site was the only site with a large mean membership in cluster 2 (51.0 %). All other sampling sites had <51 % mean membership in a single cluster.

Discussion

Results from this study provided a thorough characterization of genetic variation and differentiation within double-crested cormorants across the species range in the United States and Canada. The phylogenetic analysis identified three main clades that were well-supported and highly divergent. Clade I had the strongest geographic identity, being primarily restricted to Alaska and containing all haplotypes sampled within Alaska. Clade II also had a clear geographic association as it primarily contained individuals sampled in southern California, including 10 of the 20 cormorants sampled in San Diego, California and three of the 10 cormorants sampled in Imperial, California. In contrast, clade III was characterized by shallow divergence between haplotypes and a general lack of association with geography, except for the concentration of North Atlantic individuals in one sub-clade. These three clades were separated by long branches with the only intermediaries being a less well-supported group of four haplotypes from the Pacific, occurring between clade I and II. This structure implies past fragmentation associated with historical vicariance events producing the observed genetic discontinuities (Avise 2000). The deep divergences observed may be explained by late Pleistocene events which have been postulated as driving speciation in many species (Hewitt 1996), as well as substantial microevolutionary genetic diversification in birds (Avise et al. 1998). The lack of monophyly for Alaska and Southern California, particularly the grouping of two haplotypes sampled in Oregon, Washington, and California with clade I and the grouping of one haplotype sampled in Oregon and Washington with clade II, may be the result of incomplete lineage sorting or may simply indicate contemporary gene flow between previously isolated areas.

Population genetic analyses of mtDNA and microsatellite markers were congruent and supported the inferred phylogenetic structure. Specifically, both data sets identified the Alaskan sites as the most genetically distinct and supported some differentiation of the North Atlantic and Pacific sites. The pairwise genetic distance matrices for the two markers were highly correlated (Mantel test for correlation: $r = 0.78$; $P = 0.01$) and revealed significant differences in

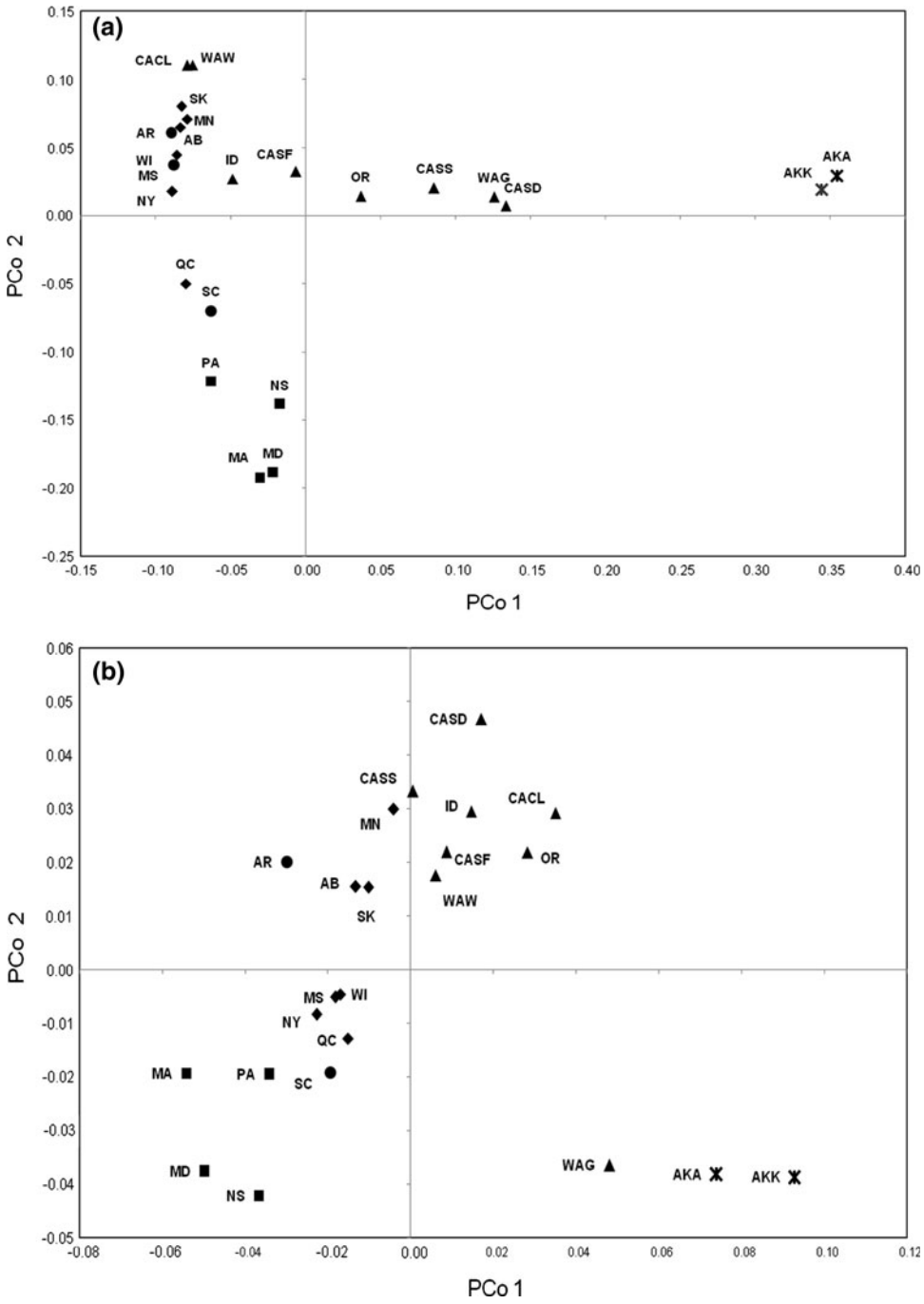

Fig. 4 **a** Principal coordinate (PCo) 1, which explained 52 % of the variation in double-crested cormorant mitochondrial data, versus PCo2, which explained 23 % of the variation. **b** PCo1, which explained 39 % of the variation in double-crested cormorant microsatellite data, versus PCo2, which explained 22 % of the variation. *Symbols* for sampling sites differ by subspecies: Alaska sites are *asterisk*; Pacific are *triangles*; Southeast are *circles*; and Interior/North Atlantic are *diamonds* and *squares*, respectively. Site codes correspond to codes used in Table 1 and Fig. 1

mitochondrial and nuclear allele frequencies for comparisons involving Alaskan or North Atlantic sites, as well as, Gray's Harbor, Washington and San Diego, California for microsatellite data. Likewise, the principal coordinate analyses showed similar patterns for both data sets with breeding sites being roughly grouped by region. Further, although STRUCTURE could not clearly discriminate clusters, the three clusters identified were consistent with the phylogenetic analysis in distinguishing the Alaska sites, the North Atlantic sites, and San Diego, California.

Subspecies and conservation status

The Alaskan sites were characterized by the presence of phylogenetically distinct haplotypes, low genetic diversity, and substantial divergence from other breeding sites. Genetic data indicate Alaskan cormorants, although not strictly monophyletic, meet genetic criteria for avian subspecific status and recognition as a conservation unit, based on their significant variation in microsatellite allele and mtDNA haplotype frequencies, the presence of unique alleles at

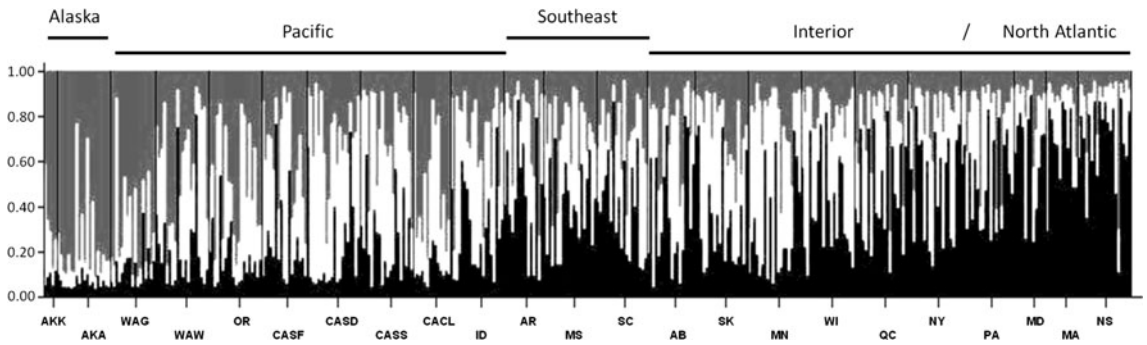

Fig. 5 Population structure inferred by a Bayesian clustering algorithm implemented in STRUCTURE for 409 double-crested cormorants, based on 8 microsatellite loci. Three population clusters were identified cluster 1 (*grey*); cluster 2 (*white*); and cluster 3 (*black*). The figure shows mean individual membership in each of these three clusters, with individuals grouped by sampling site and ordered by subspecies. *Labels* below the figure refer to the 23 sites (see Table 1 for site codes)

multiple loci, and significant net sequence divergence (Funk et al. 2007; Moritz 1994a, b). For example, all pairwise F_{ST} and Φ_{ST} values between Alaska and other sites were large and nearly all were statistically significant. Likewise, all six Alaskan haplotypes were unique to the subspecies and formed a well-supported phylogenetic clade. Consequently, net sequence divergence between Alaskan individuals and all others was substantial (5.85 %) and statistically significant.

Historical records, contemporary population estimates, and field observations are consistent with recognition of Alaskan double-crested cormorants as distinct and potentially of conservation interest. The Alaskan cormorants are the most geographically isolated owing to a disjunct distribution along the Pacific Coast, with no nesting colonies occurring along the coast of British Columbia north of the Strait of Georgia (Carter et al. 1995). Further, Alaskan birds are mostly resident within their breeding range and seasonal movements may only extend to northern British Columbia (Hatch and Weseloh 1999). This geographic separation likely limits dispersal and suggests Alaskan cormorants are demographically isolated. In addition, the Alaskan subspecies currently has the smallest population size of the purported subspecies examined (3,029 breeding pairs), consisting of only 126 colonies, most being <100 pairs (Wires and Cuthbert 2006). This contemporary population is less numerous and less widespread than historically (Wires and Cuthbert 2006) and may still be experiencing declines (Carter et al. 1995; Tyson et al. 1999; Irons pers. comm.).

In contrast to Alaska, the San Diego and Imperial, California sites had high genetic diversity, particularly nucleotide diversity. This result is best explained as a consequence of the confluence of lineages of multiple origins at these sites (Petit et al. 2003). Of 30 individuals analyzed from this area, 13 contained haplotypes found in the more geographically restricted clade II, while the remaining 17 were within the cosmopolitan clade III. From this pattern, it is evident the observed genetic differentiation of the area is characterized by admixture rather than a strict genetic discontinuity. The presence of unique haplotypes at these sampling sites may indicate introgression of southern alleles into more northern regions. Additionally, clade II also contained one haplotype that was sampled in Oregon and Washington. Thus, southern California may represent the primary zone of introgression, while the extent of emigration may be as far north as Washington, or further.

A northward expansion of a southern lineage from the outer coast of Baja California, Mexico to the Southern California Bight is consistent with field observations and historical records. The southern limit of the double-crested cormorant breeding range extends to Baja California Sur and Sinaloa, Mexico, although our sampling did not include birds from northwestern Mexico due to complications of collection and permits. Substantial declines in colonies and productivity were documented in northwestern Mexico during the early to late twentieth century (Gress et al. 1973, 1995), including the complete disappearance of the largest double-crested cormorant colony on record (213,500 pairs; Hatch 1995), at San Martin Island, Baja California Nord, MX. Concurrently, numbers of breeding cormorants in southern California increased strikingly, such that the rate of increase could not be explained by local productivity alone and has been attributed to immigration of birds from Mexico (Carter et al. 1995; Wires and Cuthbert 2006). Thus, it is likely the unique haplotypes observed in southern California may be derived from Mexican cormorants, and thereby represent northward movement and introgression between previously isolated lineages.

There was little genetic evidence for subspecies level differentiation of cormorants within the conterminous U.S. and Canada. Rather than overt subspecific breaks between three of the traditional subspecies (Pacific, Interior/North Atlantic, and Southeast), we observed clinal variation of

haplotypes and allele frequencies, wherein genetic dissimilarity increased with geographic distance. First, there were no mtDNA clades corresponding to subspecies and subsequently there was <1 % sequence divergence for any of these three subspecies. Secondly, none of the three subspecies exhibited consistently significant genetic distances in pairwise comparisons with other subspecific sites. Rather, the genetic distance matrixes showed a range of Φ_{ST} and F_{ST} values wherein the largest genetic distances were comparisons between the most geographically distant sites, while comparisons involving more centrally located sites were notably smaller and mostly not significant. Further, the multivariate analysis (PCoA) and Bayesian clustering analysis (STRUCTURE) did not identify distinct clusters of sites by subspecies. The STRUCTURE analysis did identify three groups within North America, however, individual genomes from across the conterminous U.S. and Canada were split between these groups, suggesting substantial introgression between the purported subspecies. Finally, a Mantel test showed a significant and positive correlation between geographic and genetic distance at mitochondrial and microsatellite loci.

The observed relationship between genetic and geographic distance is consistent with the isolation by distance model, which asserts that as geographic distance increases, genetic dissimilarity increases due to limited dispersal (Wright 1943). Further, peripheral populations are more likely to be genetically isolated than central populations because individuals at the center of a species' range can disperse in many directions, while those at the range edge can disperse in fewer directions (Eckert et al. 2008). This model is likely appropriate for the double-crested cormorant given their life-history traits, demography, and observed movements. Cormorants are colonial nesters with mate selection and pairing occurring at the breeding colony, a system that may be conducive to natal philopatry and breeding site fidelity in stable colonies (Hatch 1995). However, banding and movement data have not supported the presence of strong natal philopatry or breeding site fidelity by colony. Rather, the available data has shown many cases of movement within regions with fewer incidents of long-distance movement between regions, suggesting the tendency for some fidelity to a larger geographic area, but without strict natal philopatry. Clark et al. (2006) specifically noted that movements of cormorants banded in Oregon were nearly restricted to the Pacific Northwest, with only rare occurrences east of the Cascade-Sierra Nevada range. Dolbeer (1991) also noted a pronounced lack of interchange between cormorant colonies east of the Rocky Mountains and colonies in the Pacific. More recent studies in the eastern U.S. have indicated migratory connectivity and observed some site fidelity. Guillaumet et al. (2011), found significant migratory connectivity in Great Lakes cormorants, consistent with a migratory divide across the Great lakes, with western populations mainly using the Mississippi flyway while eastern populations mainly used the Atlantic flyway although, individuals did exhibit variability and flexibility in movement patterns. Scherr et al. (2010) also observed movements consistent with a migratory divide as well as observing fidelity to the previous summer's location in 15 of 20 satellite tagged individuals. Finally, King et al. (2010) analyzed 10,620 cormorant band recoveries and found a majority of bands were recovered in the same region in which the bird was banded. Most notably, there were only 2 cases of birds banded in the Pacific region being recovered east of the Rockies. In contrast, birds banded in the Interior were recovered across the range with most recoveries east of the Rockies but, with several recoveries within the Pacific region.

This suspected low level of interchange between regions, along with the lower density of cormorants within the intermountain western states, may particularly facilitate the differentiation of Pacific sites. In addition, Pacific cormorants tend to be residential while Interior and North Atlantic cormorants are migratory, and these differences in migration may decrease gene flow between regions. In contrast, cormorants at the North Atlantic sites lack any perceivable barriers to gene flow and are migratory in habit like those in the Interior and Southeast. The observed differentiation of the North Atlantic population may be solely attributed to its location at the peripheral northeastern edge of the range, along with some regional fidelity, and possible variation in migratory flyway and seasonal movements. Lack of a genetic discontinuity between the traditional geographic subspecies of double-crested cormorant does not necessarily imply strong demographic connectivity and homogenization across the range. Only a few dispersers per generation are necessary to prevent significant genetic differentiation (Mills and Allendorf 1996; Slatkin 1985; Wright 1931), but the same number of migrants may have no significant demographic impact. For example, although, the Pacific region has exhibited continued gradual increases in total population size, the rate of increase is low relative to changes in the Interior. Furthermore, colony declines have been documented over much of British Columbia, Washington, and Southern California, despite substantial population growth in the Interior region (Anderson et al. 2004; Capitolo et al. 2004; Moul and Gebauer 2002; USFWS unpubl. data). Likewise, growth of the North Atlantic region may have ceased by 1990 (Hatch and Weseloh 1999), with some areas exhibiting recent negative rates of change (Tyson et al. 1999).

Conclusions

Our mtDNA and microsatellite data provided the first extensive view of double-crested cormorant genetic structure.

Consistent with current taxonomy, all analyses strongly supported a genetic distinction between Alaska and other breeding regions, providing evidence for the continued recognition of an Alaskan subspecies (*P. a. cincinnatus*). Contemporary population estimates, in conjunction with historical records and field observations, indicate this subspecies may be of particular conservation interest. In addition, analyses suggested the presence of another unique lineage within the double-crested cormorant associated with the southwestern extent of the species' range in northwestern Mexico. Additional sampling and analysis would be necessary to confirm this supposition and delineate any potentially unique contemporary population. In contrast to current taxonomy, there was little genetic support for recognition of subspecies within the continental U.S. and Canada, outside of Alaska. Rather than distinct subspecific breaks between regions, we observed a pattern of genetic differentiation more consistent with a gradual isolation by distance. We note that while regions are evidently connected by sufficient gene flow to prevent significant genetic divergence, the Pacific and North Atlantic regions, or portions of the breeding region therein, may still warrant differential consideration from populations in the Interior and Southeast due to possible demographic separation, habitat differences, and documented declines at some colonies. Additional information on regional population demographics, breeding site fidelity, and movements throughout the annual cycle, would further clarify the demographic connectivity of these putative regional populations.

Acknowledgments We thank the U.S. Geological Survey (USGS) Forest and Rangeland Ecosystem Science Center (FRESC), U.S. Army Corps of Engineers, Portland District, and Bonneville Power Administration for providing funding. The U.S. Fish and Wildlife Service, the Canadian Wildlife Service, and the U.S. Department of Agriculture's Animal and Plant Health Inspection Service and Wildlife Service were all instrumental in facilitating and obtaining cormorant samples. Many individuals contributed samples to this study, and we are grateful to everyone who provided samples or assisted with sample collection. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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