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Population genetic structure in Atlantic and Pacific Ocean common murres (*Uria aalge*): natural replicate tests of post-Pleistocene evolution

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Abstract

Understanding the factors that influence population differentiation in temperate taxa can be difficult because the signatures of both historic and contemporary demographics are often reflected in population genetic patterns. Fortunately, analyses based on coalescent theory can help untangle the relative influence of these historic and contemporary factors. Common murres (*Uria aalge*) are vagile seabirds that breed in the boreal and low arctic waters of the Northern Hemisphere. Previous analyses revealed that Atlantic and Pacific populations are genetically distinct; however, less is known about population genetic structure within ocean basins. We employed the mitochondrial control region, four microsatellite loci and four intron loci to investigate population genetic structure throughout the range of common murres. As in previous studies, we found that Atlantic and Pacific populations diverged during the Pleistocene and do not currently exchange migrants. Therefore, Atlantic and Pacific murre populations can be used as natural replicates to test mechanisms of population differentiation. While we found little population genetic structure within the Pacific, we detected significant east–west structuring among Atlantic colonies. The degree that population genetic structure reflected contemporary population demographics also differed between ocean basins. Specifically, while the low levels of population differentiation in the Pacific are at least partially due to high levels of contemporary gene flow, the east–west structuring of populations within the Atlantic appears to be the result of historic fragmentation of populations rather than restricted contemporary gene flow. The contrasting results in the Atlantic and Pacific Oceans highlight the necessity of carefully considering multilocus nonequilibrium population genetic approaches when reconstructing the demographic history of temperate Northern Hemisphere taxa.

Keywords: common murre, genetic equilibrium, phylogeography, Pleistocene, population differentiation, *Uria aalge*

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Introduction

The factors affecting the extent of population differentiation are well understood in theory and include gene flow, effective population size, mutation, and selection (Wright 1978). In practice, however, population differentiation is a complex process and the most important factors causing differentiation can be difficult to identify for any given population (Gavrilets 2003; Coyne & Orr 2004; Friesen *et al*. 2007). Understanding the factors that drive population divergence in temperate Northern Hemisphere species can be particularly challenging as many of these taxa underwent multiple cycles of range expansion and contraction during glacial episodes of the Pleistocene (e.g. invertebrates, Arndt & Smith 1998; songbirds, Burg *et al*. 2006; mammals, Harlin-Cognato *et al*. 2006).

Many contemporary seabird colonies in the Northern Hemisphere occur in areas that were ice-covered during

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major glacial advances of the Pleistocene (Pielou 1991). As such, the demographic history of these populations may be complex. Following periods of range expansions, population bottlenecks, or fragmentation, populations require time to establish equilibrium between mutation, migration, and genetic drift (Whitlock & McCauley 1999). If mutation–migration–drift equilibrium is not yet established, observed patterns of population genetic structure may reflect past rather than contemporary conditions (Whitlock & McCauley 1999). Therefore, to understand mechanisms of population differentiation in temperate seabirds, it is necessary both to quantify population genetic structure and to determine the relative contribution of historical vs. contemporary processes to genetic structure. Fortunately, recently developed analyses based on coalescent theory and maximum likelihood are able to tease apart these relative influences (e.g. Nielsen & Wakeley 2001).

Many temperate seabirds that live in both the Atlantic and Pacific exhibit significant population differentiation between ocean basins (e.g. thick-billed murre *Uria lomvia*, Birt-Friesen *et al*. 1992; black-legged kittiwake *Rissa tridactyla*, Patirana 2000); however, the extent of population genetic structure within ocean basins is more variable (Friesen *et al*. 2007). At this scale, there are few obvious physical barriers, but philopatry may still significantly restrict gene flow. Indeed, red-legged kittiwakes *Rissa brevirostris* (Patirana *et al*. 2002), razorbills *Alca torda* (Moum & Árnason 2001), and marbled murrelets *Brachyramphus marmoratus* (Friesen *et al*. 2005) all exhibit population differentiation within basins. In contrast, population genetic patterns in other species suggest panmixia within ocean basins (e.g. crested auklet *Aethia cristatella,* least auklet *A. pusilla*; Walsh *et al*. 2005). Moreover, some taxa exhibit different population genetic patterns (panmixia vs. population differentiation) in different ocean basins. For example, black-legged kittiwakes are genetically structured in the Atlantic, but not within the Pacific (Patirana 2000).

Common murres (*Uria aalge*; Charadriiformes: Alcidae) breed in large colonies (10^3 to 10^6 breeding pairs) in the northern Atlantic and Pacific Oceans between 35°N and 70°N, with a worldwide population size estimated at 10^7 breeding pairs: approximately 4 million in the Atlantic and 6 million in the Pacific (Nettleship & Evans 1985; Gaston & Jones 1998). They disperse long distances in the nonbreeding season (Storer 1952; Brown 1985), but some evidence suggests that they are also philopatric. For instance, banding studies indicate that a high proportion of banded adults return to the same colony to breed each year (Harris *et al*. 1996a). In contrast, other band returns suggest that immature birds can disperse from their natal colony and occasionally breed at other colonies (Halley & Harris 1993; Lyngs 1993).

Common murres are one of the most morphologically variable alcids (Bedard 1985). In the Pacific, two subspecies have been defined based on morphology (primarily the colour of the mantel, the extent of streaking on the sides, and morphometrics) and geography: *U. a. californica* along the US coast from Washington southwards and *U. a. inornata* everywhere else (Gaston & Jones 1998). In the Atlantic, as many as six subspecies have been defined (Tuck 1961; Bedard 1985). Further, the Atlantic population exhibits a cline in 'bridling' (a white eye ring and auricular groove), as well as in wing and culmen lengths (Bedard 1985). Due to unsuitable breeding habitat in the Arctic Ocean, the Atlantic and Pacific populations of common murres are physically disjunct and likely do not interbreed, and previous genetic analyses detected significant population genetic differentiation between Atlantic and Pacific common murres (Friesen *et al*. 1996). Therefore, common murre populations from the Atlantic and Pacific may form natural replicates to test mechanisms of population differentiation.

Little evidence exists for population genetic structuring within ocean basins. Previous analyses using either mitochondrial DNA (mtDNA) (Moum *et al*. 1991; Moum & Árnason 2001) or microsatellites (Riffault *et al*. 2005) all failed to detect significant population differentiation with in the Atlantic. Similarly, the only study to investigate genetic structure within Pacific common murres did not detect any population differentiation (Friesen *et al*. 1996). However, these studies were limited by small sample sizes, incomplete geographic coverage, and/or the use of only a single genetic marker.

Here we employ the mitochondrial control region and four microsatellite loci (plus four intron loci for Pacific populations) to investigate population genetic structure throughout most of the global range of common murres. First, we use this new suite of loci to investigate if Atlantic and Pacific common murres are genetically independent. Then we use traditional population genetic methods and more recently developed analyses based on coalescent theory and maximum likelihood to determine the extent that population genetic structure reflects historical contingencies or current demographics. By teasing apart these two factors, we infer the mechanisms which have shaped genetic diversity within each ocean and make a comparison between the Atlantic and Pacific to determine if similar evolutionary or ecological forces have influenced differentiation within each basin.

Materials and methods

Sample collection

We obtained tissue samples from 617 common murres from throughout their breeding range (Table 1; Fig. 1). Samples from Bjørnøya, British Columbia, Oregon, and most of Alaska consisted of solid tissue (heart, liver and/or striated muscle) collected from adults in breeding condition in close proximity to colonies. Samples from the

Table 1 Sampling site locations and numbers of individuals (N) included in mitochondrial and nuclear analyses*. Subspecies designations are taken from Gaston & Jones (1998) and Tuck (1961)

						N		
Colony	Geographic location	Abbreviation	Subspecies	Latitude	Longitude	mtDNA	Microsatellites	Introns
Witless Bay	Newfoundland	WB	*aalge*	47°16′N	52°49′W	27	26	0
Gannet Islands	Newfoundland	GA	*aalge*	53°56′N	56°32′W	19	19	0
Funk Island	Newfoundland	FU	*aalge*	40°45′N	53°11′W	27	27	0
Látrabjarg	Iceland	IC	*aalge*	65°38′N	24°19′W	19	19	0
Shetland Islands	Scotland	SH	*aalge*	60°19′N	1°25′W	26	27	0
Isle of May	Scotland	IM	*aalge*	56°11′N	2°33′W	31	32	0
Runde	Norway	RU	*aalge*	62°24′N	5°39′E	6	6	0
Bornholm	Denmark	BO	*intermedia*	55°37′N	14°35′E	18	18	0
Skomer Island	Wales	SK	*albionis*	51°45′N	5°11′W	30	31	0
Hornøya	Norway	HO	*hyperborea*	70°22′N	31° 8′E	30	30	0
Bjørnøya	Norway	BJ	*hyperborea*	74°26′N	19°21′E	10	10	0
Novaya Zemlya	Russia	NZ	*hyperborea*	74°23′N	56°25′E	5	5	0
Sea of Okhotsk	Eastern Russia	OK	*inornata*	47°14′–58°55′N	153°25′–169°47′E	24	25	25
Cape Lisburne	Chukchi Sea	CH	*inornata*	68°52′N	166°14′W	26	27	28
Fairway Rock	Bering Strait	BE	*inornata*	65°40′N	168°58′W	5	6	6
Pribilof Island	Bering Sea	PR	*inornata*	56°53′N	169°59′W	32	12	43
Attu Island	Western Aleutian Islands	WAI	*inornata*	52°55′N	172°26′W	13	13	13
Bogoslof Island	Central Aleutian Islands	CAI	*inornata*	53°56′N	168°03′W	6	8	3
Aiktak Island	Eastern Aleutian Islands	EAI	*inornata*	54°11′N	164°50′W	27	27	23
Midun Island	Western Alaskan Peninsula	WPE	*inornata*	54°51′N	162°11′W	7	7	7
Big Koniuji Island	Central Alaskan Peninsula	CPE	*inornata*	55°05′N	159°30′W	10	12	12
Chowiet Island	Eastern Alaskan Peninsula	EPE	*inornata*	56°04′N	156°42′W	17	19	19
East Amatuli Island	Lower Cook Inlet	LCO	*inornata*	58°53′N	152°00′W	22	23	23
Chisik Island	Central Cook Inlet	CCO	*inornata*	60°08′N	152°33′W	43	48	48
Middleton Island	Prince William Sound	PWS	*inornata*	59°26′N	146°18′W	20	0	30
Triangle Island	British Columbia	BC	*inornata*	50°52′N	129°05′W	22	24	24
Cape Flattery	Washington	WA	*californica*	48°38′N	124°71′W	11	11	11
Newport	Oregon	OR	*californica*	44°63′N	124°05′W	21	21	21
Farallon Island	California	CA	*californica*	37°70′N	123°00′W	21	21	15
Total	29 populations					575	554	351

*Samples from Runde and Bjørnøya were used by Moum *et al*. (1991), samples from Witless Bay, Funk Island, Hornøya, Fairway Rock, Big Koniuji Island, and a subset of samples from Attu Island, Bogoslof Island and Midun Island were used by Friesen *et al*. (1996), samples from Funk Island, Iceland and Bjørnøya were used by Moum & Árnason (2001), and samples from Witless Bay and Skomer Island were used by Riffault *et al*. (2005).

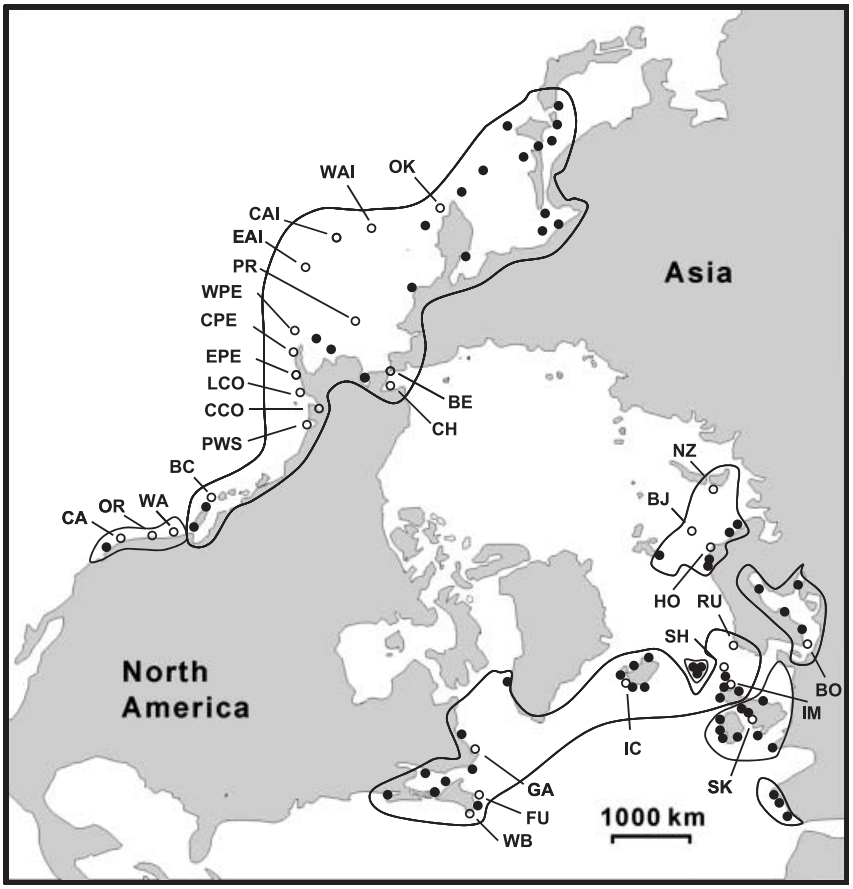

Fig. 1 Approximate breeding distribution of common murres. Open circles represent sampled colonies and black circles represent other large breeding colonies. Subspecies ranges are shown with solid black lines. Abbreviations and sample sizes are given in Table 1.

Sea of Okhotsk consisted of tissue from birds in breeding condition caught in gill nets during the breeding season; although these birds likely bred close to the site of collection, the exact breeding colony is not known. Therefore, results relating to birds from the Sea of Okhotsk should be treated with appropriate caution. Samples from elsewhere consisted of blood from adults or from chicks caught at nests. We extracted DNA using a standard proteinase-K phenol/chloroform technique (Friesen *et al*. 1997). For samples from Bjørnøya and Runde, mitochondrial-enriched DNA was extracted from heart and liver tissue as detailed in Moum *et al*. (1991). Many of the samples used in the present study were also included in previous genetic analyses (details are given in Table 1). For all these samples, we sequenced or genotyped new genetic regions.

Mitochondrial control region

We designed a murre-specific light strand primer for the control region (UaL50, situated near the 5′ end of the control region; 5′-CCATTAATACACACACAGACATAACC-3′) from previously published mtDNA sequences of murres (Moum & Johansen 1992), and a general heavy strand primer for birds (ADH1452, situated in the $tRNA^{phe}$ gene; 5′-TGGCTAAAGCAAGGCGTC-3′) from previously published sequences of several other species of vertebrates. We then amplified an approximately 1-kb fragment of DNA including most of the control region and the entire gene for $tRNA^{phe}$ from one common murre and one thick-billed murre using these primers under standard conditions (10 mM Tris pH 8.5, 50 mM KCl, 1.5 mM $MgCl_2$, 1.6 μM bovine serum albumin, 2% gelatin, 0.2 mM each of the four dNTPs, 0.4 mM each of the heavy and light strand primers, and 0.5 U of *Thermus aquaticus* (*Taq*) DNA polymerase (Boehringer-Mannheim) in a PTC-100 thermal cycler (Bio-Rad) with annealing at 55 °C. We subjected amplified DNA to electrophoresis through 2% agarose gels, purified it using Gene Clean II kits (Bio 101 Inc.) and sequenced the product with Amplicycle cycle sequencing kits (Applied Biosystems) following the manufacturer's suggested protocol. Based on this sequence, we designed one new primer (UaH389, 5′-CGGGTGAGATGGTGAT-GTATAGCCG-3′) to be used with UaL50 to amplify parts of Domain I and Domain II, and two additional primers (UaL750, 5′-CAATAAACCCTTCCAGTGCACCG-3′; UaH900, 5′-CGTTCGAGTATATGAACGTAGGTTG-3′) to amplify parts of Domain II and Domain III. We then used these primer pairs for population-level analyses (see below)

under the above conditions with annealing at 54 °C and 58 °C, respectively.

We initially screened population-level sequence variation using single-stranded conformational polymorphisms (SSCPs, Hayashi 1991; Lessa & Applebaum 1993) with direct incorporation of α-^{33}P-dATP (detailed in Friesen *et al*. 1997). Because haplotype detection using SSCPs becomes less efficient as fragment length increases (Hayashi 1991), we cut each fragment in two using restriction enzymes to allow more accurate separation of fragments on the SSCP gel. We assigned tentative haplotypes on the basis of banding profiles on autoradiograms, and to determine the exact nature of variation, we directly sequenced one or more representative of each haplotype using either (i) the Thermosequenase radiolabelled Terminator cycle sequencing kit (Amersham) according to the manufacturer's recommendations, or (iii) an ABI PRISM 373 Automated Sequencing System (Mobix, McMaster University) with M13F-tailed primers. We aligned control region sequences manually using BIOEDIT (version 7.0.5.3, Hall 1999).

Microsatellites and introns

We screened all samples for length variation in four dinucleotide (CA) repeats (Uaa5-8, Uaa1-23, Ulo12a22, and Ulo14b29) following standard protocols (Ibarguchi *et al*. 2000). Because no genetic structure was initially found in Pacific common murres, we also surveyed sequence variation at four nuclear introns to confirm this pattern. Introns in the genes for crystallin, α-enolase, lactate dehydrogenase, and ribosomal protein 40 (Table S1, Supporting Information) were screened using a combination of SSCPs and direct sequencing (Friesen *et al*. 1997).

Genetic variation within populations

We calculated standard population genetic indices [haplotypic diversity (*h*; Nei 1987) and nucleotide diversity (π; Tajima 1983)] for each colony using ARLEQUIN (version 3.1, Excoffier *et al*. 2005). To test if control region sequences were evolving under neutral expectations, and to examine historical demographic processes, we performed Ewens–Watterson (Ewens 1972; Watterson 1978) and Chakraborty's amalgamation tests (Chakraborty 1990). We calculated allelic richness and allele frequencies for all nuclear loci using FSTAT (version 2.9.3, Goudet 1995), and performed exact tests to determine if nuclear loci deviated from Hardy–Weinberg and linkage equilibrium using ARLEQUIN and FSTAT, respectively.

Isolation of Atlantic and Pacific common murres

To confirm Friesen *et al*.'s (1996) findings that Atlantic and Pacific common murre populations are genetically isolated, we tested for genetic differentiation between samples from different oceans in the following three ways (see next section for details of each analysis):

1 By using hierarchical analyses of molecular variance (AMOVA tests; Excoffier *et al*. 1992) to calculate the proportion of genetic variation that is explained by ocean (Φ_{CT} and F_{CT} for the mitochondrial and nuclear data, respectively);
2 By calculating pairwise F_{ST} analogues and mean pairwise sequence distances (δ, Wilson *et al*. 1985) between all pairs of colonies from different oceans; and
3 By estimating minimum and maximum divergence times between Atlantic and Pacific common murres as $t = \delta/r$ (Wilson *et al*. 1985), where δ is the average of all pairwise δs between Atlantic and Pacific colonies and r is the sequence divergence rate for the entire control region (minimum 5% per million years, and maximum 20% per million years; Wenink *et al*. 1994).

Population genetic structure within ocean basins

We used AMOVA tests to determine the extent of population genetic structure within each ocean basin using ARLEQUIN. Specifically, we estimated Φ_{ST} for control region data and F_{ST} for nuclear data. We determined genetic differentiation between all pairs of colonies by calculating Φ_{ST} and F_{ST}, and used hierarchical AMOVA tests to determine the significance of a priori geographic subdivisions (based on subspecies designations or the geographic proximity of colonies) and *post hoc* subdivisions based on results from pairwise comparisons. We conducted all analyses of mitochondrial haplotypes using Kimura's 2-parameter model of substitution (Kimura 1980) with a shape parameter (α) for the gamma distribution of 0.42 (based on estimates of α for control region sequences that include Domains I, II, and III in other avian species; Marshall & Baker 1997). We tested all results for significance using 10 000 random permutations of the data, and a significance level of $\alpha = 0.05$ with sequential Bonferroni corrections applied to correct for multiple tests (Rice 1989). Due to small sample sizes, we excluded individuals from Fairway Rock, Bogoslof Island, Midun Island, Runde and Novaya Zemlya from all population level analyses, and individuals from Middleton Island from analyses involving nuclear loci.

We also analysed population structure in microsatellite and intron variation using the program STRUCTURE (version 2.1, Pritchard *et al*. 2000). We performed all analyses with an admixture model, sampling location not set as prior information, a burn-in of 15 000 cycles, an additional 150 000 cycles after the burn-in, and the correlated allele frequency setting with $\lambda = 1$. For $K = 1$ to 20 genetic populations, we repeated each analysis 20 times. Following the suggestions of Evanno *et al*. (2005), we used ΔK to infer the

most probable number of genetic populations. However, their method is unable to make inferences if the actual number of populations is 1 or if it is equal to the maximum value of K tested ($K = 20$), and thus, we also used the posterior probability, Ln[$P(X \mid K)$], directly to infer the number of populations (Pritchard *et al*. 2000).

Estimates of demographic parameters

Many traditional population genetic approaches (for example, calculating migration rate from Φ_{ST} values; Whitlock & McCauley 1999) assume that populations are in migration-drift and mutation-drift equilibrium; however, some temperate seabird populations are likely not in equilibrium (e.g. Friesen *et al*. 2005). Therefore, we used approaches based on coalescent theory and maximum likelihood that do not assume genetic equilibrium to estimate demographic parameters. Our general approach was to identify possible trends using noncoalescent-based approaches (e.g. pairwise Φ_{ST} estimates), and then test these trends using coalescent-based approaches.

We used MDIV (Nielsen & Wakeley 2001) to simultaneously estimate Θ ($2N_f\mu$, where N_f is the female effective population size and μ is the mutation rate per gene per year — based here upon three different control region-specific divergence rates spanning a range of possible values; 5%, 10%, and 20% per million years; Wenink *et al*. 1994), T (the divergence time in N_f generations), and M (the number of effective female migrants per generation) between groups of colonies within ocean basins that were found to be genetically different in other analyses or that represented a priori groupings (e.g. subspecies in the Pacific). For each run, we used a Markov chain of 5 million cycles with a burn- in of 500 000 cycles. Due to high levels of homoplasy in the mitochondrial data, we assumed a finite site mutation model (Hasegawa–Kishino–Yano model; Hasegawa *et al*. 1985). We set M_{max} and T_{max} at 5–100 and 0.1–5, respectively, to generate posterior probability distributions that approximated a Poisson distribution, and determined point estimates of M and T by taking the mode of the respective posterior probability estimates. Where possible, we calculated 95% credibility intervals around each point estimate using likelihood ratio tests. However, it is often not possible to estimate the upper bound of T as the likelihood curve approaches zero very slowly (Nielsen & Wakeley 2001). In these cases, we refer to the upper bound of T as undefined. We repeated each analysis at least six times with different random seeds to ensure convergence of the Markov chains.

To test if populations had experienced growth or decline, we first analysed the combined mitochondrial and nuclear DNA data using the software LAMARC (Kuhner 2006). Although LAMARC performed well on the mtDNA data alone, we had difficulty achieving convergence of the Markov chains for the combined nuclear and mitochondrial DNA data set. Therefore, we used LAMARC's predecessor, FLUCTUATE (Kuhner *et al*. 1998), to simultaneously estimate a population growth parameter (g) and θ ($2N_f\mu$, where μ is the mutation rate per site per generation) from control region variation. We used an initial population growth rate of 1 and an initial theta of 0.2 (based on results from preliminary runs); population size was allowed to vary. We ran 30 short chains of 1000 steps and 4 long chains of 100 000 steps, sampling from the chain every 20 steps. To ensure stability of parameter estimates, we performed three independent runs with different random seeds.

Last, we used BOTTLENECK (Version 1.2.02, Cornuet & Luikart 1996) to test for past population bottlenecks and expansions using microsatellite and intron data. For intron data, we assumed an infinite alleles model; because the exact mutation model for microsatellite loci was unknown, we repeated this analysis using a range of combinations of the infinite alleles and the stepwise mutation models. We based inferences on the sign test, which is the most conservative test implemented in BOTTLENECK but also has the lowest power of the three tests provided by the program (Cornuet & Luikart 1996).

To test for concordance between geographic and genetic distance within oceans, we performed Mantel's tests for the control region data alone and for the nuclear data alone (Smouse *et al*. 1986). We measured geographic distance conservatively as log-transformed shortest sea surface distance between colonies and indexed genetic distance using Slatkin's linearized Φ_{ST} or F_{ST}, respectively.

Results

Characterization of variation

For Pacific common murres, we obtained sequence data from 761 base pairs (bp) of the control region, including 400 bp from the 5′ end and 361 bp from the 3′ end. Readable sequence at the 3′ end from Atlantic common murres was 39 bp shorter and yielded a total length of 722 bp. Although nuclear copies of the control region have been reported in many charidriiform birds, several lines of evidence suggested that we amplified the true mitochondrial sequence: (i) sequences from the flanking tRNA^{phe} region folded into a cloverleaf secondary structure (Desjardin & Morais 1990); (ii) regions with high similarity to the avian conserved blocks were found in the expected locations in Domain II and the 5′end of Domain III (C, D, and F Boxes, and CSB-1; Baker & Marshall 1997); (iii) base-pair composition of the L-strand was biased against Gs (26% C, 32% T, 27% A, 15% G;Baker & Marshall 1997); (iv) variable sites were distributed as expected (Baker & Marshall 1997), with 54 in Domain I, 38 in Domain II, and 14 in Domain III (Fig. S1; Table S2, Supporting Information); and (v) sequences from purified

mtDNA samples (Bjørnøya and Runde) did not differ fundamentally from sequences amplified from blood or solid tissue samples.

One-hundred and thirty-eight haplotypes were defined by 106 variable sites including 86 transitions, 17 transversions, and 12 insertions or deletions. Eight sites involved both transitions and transversions, and one site involved a transition and an indel (Fig. S1; Table S2). One insertion involved 28 bp in one individual, which we treated as a single mutational event. Haplotypes differed by a mean of 6.04 ± 2.88 substitutions, or 0.83 ± 0.44%, with a range between one and 23 substitutions (Table S2).

The length of most microsatellite alleles differed by multiples of 2 bp with very few alleles missing from the sequence. Therefore, all loci likely followed a stepwise mutation model. The microsatellite loci had between five and 24 alleles each (Table S3, Supporting Information). All four introns were highly variable in common murres, with between 8 and 19 alleles (Fig. S2, Supporting Information; Table S3). Most differences between alleles involved transitions, but several involved insertions or deletions (not shown).

Tests of assumptions

Within colonies, Ewens–Watterson's tests of neutrality were not significant in any cases and Chakraborty's amalgamation tests were significant only for populations at the Isle of May, Iceland, Aiktak Island and Chisik Island (Table 2). However, significantly large Chakraborty's amalgamation tests can also reflect recent population growth rather than selection (see Results; Chakraborty 1990). Six populations had microsatellite or intron genotype frequencies that significantly differed from Hardy–Weinberg expectations, and linkage disequilibrium was observed between three of the eight loci; however, except for locus *Uaa1-23*, there were no consistent deviations either for any locus across populations or across loci for any population. We repeated all analyses both with and without locus *Uaa1-23*, and results were consistent in all cases.

Table 2 Indices of population genetic variation and results of neutrality tests for the mitochondrial control region data. * indicates significance after Bonferroni corrections. Population abbreviations are given in Table 1

Colony	*n*	*h*	π (× 10–3)	Chakraborty's†
Atlantic				
WB	27	0.75 ± 0.07	3.76 ± 2.29	10/6.44
GA	19	0.81 ± 0.06	5.20 ± 3.07	8/6.78
FU	27	0.81 ± 0.08	3.51 ± 2.17	10/7.80
IC	19	0.74 ± 0.11	3.26 ± 2.08	10/4.42*
SH	26	0.66 ± 0.10	2.29 ± 1.55	8/4.86
IM	31	0.84 ± 0.06	3.79 ± 2.30	15/9.41*
SK	30	0.77 ± 0.08	2.95 ± 1.88	11/7.21
RU	6	0.93 ± 0.12	4.61 ± 3.18	5/5.07
BO	18	0.86 ± 0.06	3.12 ± 2.01	8/7.96
HO	30	0.90 ± 0.04	3.98 ± 2.40	16/12.9
BJ	10	0.84 ± 0.10	4.62 ± 2.92	6/5.59
NZ	5	1.00 ± 0.13	4.71 ± 3.38	N.A.
All Atlantic	248	0.85 ± 0.02	3.86 ± 2.26	64/19.6*
Pacific				
OK	24	0.80 ± 0.05	2.08 ± 1.45	7/7.4
CH	26	0.88 ± 0.05	2.78 ± 1.83	14/10.59
BE	5	0.60 ± 0.18	0.83 ± 0.91	2/2.38
PR	32	0.68 ± 0.06	1.39 ± 1.07	8/5.39
WAI	13	0.73 ± 0.10	1.14 ± 0.98	5/4.60
CAI	6	0.80 ± 0.17	0.46 ± 0.61	4/3.72
EAI	27	0.74 ± 0.08	2.49 ± 1.65	10/6.09*
WPE	7	0.67 ± 0.16	1.19 ± 1.08	3/3.08
CPE	10	0.98 ± 0.05	6.27 ± 3.81	9/9.07
EPE	17	0.82 ± 0.08	2.15 ± 1.51	9/6.84
LCO	22	0.83 ± 0.07	2.91 ± 1.88	11/7.77
CCO	43	0.82 ± 0.05	2.82 ± 1.79	17/9.58*
PWS	20	0.77 ± 0.08	2.54 ± 1.70	8/6.19
BC	22	0.73 ± 0.07	1.80 ± 1.31	6/5.62
WA	11	0.85 ± 0.09	2.43 ± 1.72	6/6.09
OR	21	0.73 ± 0.07	1.89 ± 1.36	7/5.48
CA	21	0.74 ± 0.08	1.73 ± 1.27	7/5.72
All Pacific	327	0.78 ± 0.02	2.28 ± 1.49	74/14.1*
All	575	0.90 ± 0.01	8.29 ± 4.37	138/34.9*

†First number indicates the number of observed haplotypes; second number represents the number of haplotypes expected under neutral evolution.
N.A., test was not possible because all gene copies were different.

Comparison of Atlantic and Pacific samples

When colonies were grouped by ocean basin for a hierarchical AMOVA, the amount of variation explained by ocean basin was highly significant for both the control region and microsatellites ($\Phi_{CT} = 0.79$, $P < 0.0001$; $F_{CT} = 0.08$, $P < 0.0001$, respectively). Between colonies from different ocean basins, pairwise estimates of Φ_{ST} averaged 0.79, and δ-values averaged 8.26 (data not shown). All pairwise estimates of Φ_{ST} and δ were significant after Bonferroni corrections. Pairwise estimates of F_{ST} for microsatellites averaged 0.083 and most were significant. No control region haplotypes were shared between the two ocean basins, and Atlantic and Pacific Ocean haplotypes were separated by a minimum of five mutations (Table S2; Table S4, Supporting Information).

From δ-values, the estimated divergence time between the Atlantic and Pacific was between 56 000 and 226 000 years before present (BP). Because all results confirmed that Atlantic and Pacific common murre populations are genetically distinct, all subsequent analyses were conducted on Atlantic and Pacific samples separately.

Table 3 Estimates of Φ_{ST} based on mitochondrial control region variation (above the diagonal), and of F_{ST} based on microsatellite variation (below the diagonal) between Atlantic colonies of common murres. Results significant before Bonferroni corrections are indicated with *, and those after Bonferroni corrections with **. Population abbreviations are given in Table 1

	WB	GA	FU	IC	SH	IM	SK	BO	HO	BJ
WB		–0.008	–0.012	0.110**	0.143**	0.073**	0.143**	0.153**	0.049	0.063
GA	0.000		–0.003	0.155**	0.201**	0.126**	0.193**	0.191**	0.091**	0.096*
FU	0.028*	0.024*		0.153**	0.190**	0.115**	0.179**	0.197**	0.072**	0.105*
IC	0.041*	0.022	–0.003		0.003	0.004	–0.010	0.068*	0.035*	0.025
SH	0.027*	0.021*	0.025*	0.044*		0.010	–0.014	0.053*	0.029	0.027
IM	0.012	0.011	0.006	0.015	–0.001		0.013	0.066*	0.026*	0.021
SK	0.014	0.010	0.024*	0.037*	0.004	–0.004		0.023	0.036*	0.028
BO	0.002	0.004	0.004	0.014	0.003	–0.012	–0.007		0.037	0.015
HO	0.034*	0.037*	0.001	0.003	0.031*	0.009	0.016	0.007		–0.017
BJ	0.008	0.009	–0.007	0.004	0.019	0.001	0.010	–0.011	–0.001	

Atlantic Ocean: population genetic structure

Haplotype diversity ranged from 0.66 (± 0.099) at the Shetland Islands to 1 (± 0.13) in Novaya Zemlya (Table 2). Nucleotide diversity was also lowest at the Shetland Islands (0.0023 ± 0.0016), and reached a maximum of 0.0052 (± 0.0031) at Gannet Islands (Table 2). We found a significant negative correlation between longitude and haplotype diversity (linear regression; $R^2 = 0.37$, $P < 0.05$), which suggests that haplotype diversity increases along a cline from the western Atlantic towards the eastern Atlantic. None of the relationships between longitude and nucleotide diversity (linear regression; $R^2 = 0.005$, $P > 0.05$), or between latitude and either haplotype ($R^2 = 0.20$, $P > 0.05$) or nucleotide diversity ($R^2 = 0.11$ $P > 0.05$) were significant.

Haplotype A was the most common in the Atlantic, present in 35% of individuals and every population. The second most numerous, haplotype B, was found in 14% of individuals but was generally restricted to the western Atlantic (eastern North American colonies). Only four individuals with this haplotype were found in the eastern Atlantic, one at Iceland, one at the Isle of May, and two at Hornøya (Table S4). Forty-six haplotypes (or 72% of all haplotypes) were found in only one population each. Seven nonprivate haplotypes were found only in colonies in Iceland or farther east. The global estimate of Φ_{ST} for control region sequences indicated significant genetic structuring within the Atlantic ($\Phi_{ST} = 0.072$, $P < 0.0001$). Pairwise Φ_{ST} estimates ranged from –0.097 to 0.20, and 17 were significant after sequential Bonferroni correction (Table 3). All significant estimates were between colonies on opposite sides of the Atlantic (i.e. North American colonies compared to Iceland, Europe, and Russia). Although no pairwise comparisons within the eastern Atlantic or within the western Atlantic were significant after Bonferroni corrections, six comparisons involving eastern Atlantic colonies were significant before correction. All six comparisons were between one colony located in low-arctic waters and one colony in boreal waters. Generally, pairwise Φ_{ST} estimates within the eastern Atlantic were higher between colonies from different ocean regions (i.e. low-arctic vs. boreal; Table 3).

Division of populations into groups based on subspecies (Tuck 1961) did not yield a significant global Φ_{CT} value ($\Phi_{CT} = 0.005$, $P = 0.5$); instead, maximum global Φ_{CT} was obtained by dividing the populations into three groups: (i) all northwest (NW) Atlantic colonies located in low arctic waters (Witless Bay, Gannet Islands, Funk Island), (ii) all northeast (NE) Atlantic colonies located in boreal waters (Shetland, Isle of May, Skomer Island, Runde, Bornholm) plus Iceland, and (iii) all NE Atlantic colonies located in low arctic waters with the exception of Iceland (Hornøya, Bjørnøya, Novaya Zemlya; $\Phi_{CT} = 0.102$; $P < 0.001$). This arrangement explained 10.2% of the variation among groups. Although the Iceland population is located in low-arctic waters, pairwise Φ_{ST} values indicate that Iceland shows more affinities with NE boreal colonies (Table 3). To be sure, we also grouped populations as above but with Iceland as part of the NE Atlantic low-arctic group; however, Φ_{CT} was lower when colonies were grouped this way ($\Phi_{CT} = 0.089$; $P < 0.001$).

Sixteen per cent of microsatellite alleles were shared across all populations and 18% of alleles were private but always at low frequency. Private alleles were found only at Iceland, the Shetland Islands, Hornøya, and Bjørnøya. Mean allelic richness was similar at all colonies.

Results from nuclear analyses revealed patterns of differentiation similar to the control region. The global estimate of F_{ST} was significant ($F_{ST} = 0.013$, $P < 0.01$); however, no pairwise estimates of F_{ST} were significant after Bonferroni correction. Those significant before correction followed the same pattern as the mitochondrial data (Table 3). When populations were grouped by subspecies,

Table 4 Estimates of theta, gene flow (*M*, average number of female migrants per generation), time since population divergence (*T*, in N_f generations), and divergence time in years (assuming three different mtDNA mutation rates) from MDIV. Ninety-five per cent credibility intervals are in brackets. Question marks represent upper bounds of divergence time that could not be estimated. Populations are defined in the text

Ocean Basin	Population 1	Population 2	Theta	*M*	*T*	Population divergence time (Myr, million years) 5% Myr	10% Myr	20% Myr
Atlantic	'West'	'East'	13.65 (10.18–17.86)	5.48 (2.76–9.68)	0.19 (0.06–?)	65 700 (20 760–?)	32 900 (10 380–?)	16 400 (5190–?)
	'West'	'East–Low Arctic'	9.94 (6.79–13.97)	2.7 (0.94–8.78)	0.12 (0.05–?)	30 200 (12 600–?)	15 100 (6300–?)	7600 (3150–?)
	'West'	'East–Boreal'	11.24 (8.38–15.70)	4.1 (2.04–7.58)	0.13 (0.09–?)	37 000 (25 600–?)	18 500 (12 800–?)	9300 (6400–?)
	'East-Low Arctic'	'East–Boreal'	10.6 (7.71–14.67)	7.8 (4.70–39.00	0.07 (0.03–?)	18 800 (8 100–?)	9400 (4000–?)	4700 (2000–?)

F_{CT} was not significant ($F_{CT} = -0.006$, $P = 0.84$). The most probable number of genetic populations as determined in STRUCTURE was one [Pr ($K = 1$) > 0.9999].

Atlantic Ocean: demographic history

Despite significant population genetic differentiation among Atlantic Ocean populations, results from MDIV supported high levels of contemporary migration among all Atlantic Ocean populations. As suggested by Φ_{ST} and F_{ST} estimates, we first divided Atlantic Ocean colonies into western Atlantic (Witless Bay, Gannet Islands, Funk Island) and eastern Atlantic (all others) populations for analysis in MDIV. Migration between the east and west was significantly different from zero and high (5.48 female migrants per generation; Table 4), and the time since initial population divergence, *T*, was 0.19 and significantly different from zero. Conversion of this value of *T* to divergence time in years is highly dependant on the mutation rate assumed; however, assuming a control region divergence rate of 10% per million years (Wenink *et al*. 1994), western and eastern Atlantic populations diverged approximately 33 000 years ago [95% credibility interval: (10 380 years – undefined); please see Table 4 for divergence times estimated using other divergence rates spanning the range suggested by Wenink *et al*. 1994]. As MDIV was not able to estimate an upper bound on population divergence time, we cannot rule out initial population divergence estimates much greater than 33 000 years ago.

The models implemented in MDIV assume that no further population genetic structure exists within populations. In cases where there is uncertainty about whether population substructuring will affect parameter estimation in MDIV, it is recommended to run the analysis both with and without further population division to determine if the results are qualitatively consistent (R. Nielsen, personal communication). Because some evidence suggested further population genetic differentiation among eastern Atlantic populations, we re-ran all MDIV analyses dividing colonies into the three populations suggested by the hierarchical AMOVA: (i) 'West': Witless Bay, Gannet Islands, Funk Island; (ii) 'East-Low Arctic': Hornøya, Bjørnøya, Novaya Zemlya; and (iii) 'East-Boreal' — all other colonies. Although the parameter estimates differed (Table 4), qualitatively the results are consistent with high contemporary migration between all populations and historical divergence of eastern and western populations.

We detected significant correlation between genetic and geographic distance within the Atlantic with the control region data (Mantel's test, $r = 0.64$, $P < 0.01$; Fig. 2), suggestive of isolation by distance. However, analysis of the nuclear data provided no evidence of isolation by distance (Mantel's test, $r = 0.01$, $P > 0.1$).

Because significant population genetic structure was found in Atlantic common murres, groups of colonies which yielded the highest value of Φ_{CT} in hierarchical AMOVA tests (i.e. West, East–Low Arctic and East–Boreal, as above) were analysed separately in FLUCTUATE. All three groups exhibited significantly positive growth rates ($P < 0.0001$). Results from BOTTLENECK indicated that there was a significant heterozygosity deficiency at microsatellite loci, indicative of population growth ($P < 0.03$).

Pacific Ocean: population genetic structure

Haplotype diversity ranged from 0.60 (± 0.18) at Fairway Rock to 0.98 (± 0.05) at Big Koniuji Island (Table 2). Nucleotide diversity was lowest at Bogoslof Island (0.00023 ± 0.00061), and reached a maximum of 0.0063 (± 0.0038) at Big Koniuji Island (Table 2). Because Pacific Ocean common murre colonies are distributed along a continuous tract from California to the Sea of Okhotsk (north to south along the coast of North America and then

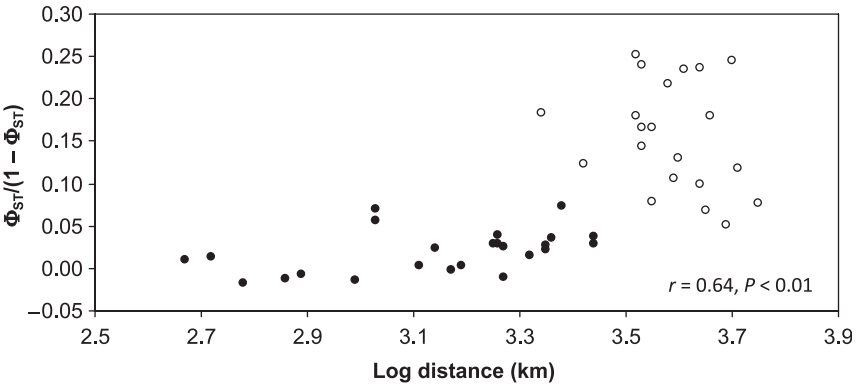

Fig. 2 The relationship between geographic and genetic distance among Atlantic colonies of common murres. Closed circles represent comparisons between colonies on the same side of the Atlantic and open circles represent comparisons between colonies on opposite sides of the Atlantic. The Mantel correlation coefficient and *P* value are given in the lower right corner.

east to west along the Aleutian Islands), we tested whether haplotype or nucleotide diversity vary along this transect. We found no correlation between distance from California for either haplotype or nucleotide diversity (linear regression; $R^2 = 0.00008$, $P = 0.97$ and $R^2 = 0.0015$, $P = 0.88$, respectively).

Haplotype 01 was the most common, present in every population (37.6% of individuals in the Pacific, Table S4). Haplotype 02 was also present in all populations and in 27.2% of individuals. Fifty-three haplotypes (or 71.6% of all haplotypes) were found in only one population. Unlike in the Atlantic, haplotypes were not distributed in an obvious geographic pattern. The global estimate of Φ_{ST} for Pacific control region sequences was not significant ($\Phi_{ST} = 0.009$, $P = 0.08$), suggesting little to no population genetic structure. No pairwise estimates of Φ_{ST} were significant after sequential Bonferroni corrections (Table 5). When populations were grouped by subspecies into a hierarchical AMOVA, the amount of variation explained by differences between subspecies was not significant ($\Phi_{CT} = -0.004$, $P = 0.70$).

Of the microsatellite and intron alleles, 10.7% and 4.8%, respectively, were present in every population. Private alleles accounted for 25% of all microsatellite alleles and 39% of all intron alleles, and were found in most populations but in low frequency (typically one individual; Table S3).

Results from nuclear analyses revealed a similar absence of population differentiation: the global estimate of F_{ST} was not significant ($F_{ST} = 0.003$, $P = 0.27$), and the most probable number of genetic populations as determined using STRUCTURE was one [Pr ($K = 1$) > 0.9999].

Pacific Ocean: demographic history

Preliminary analyses with MDIV revealed that most colonies in the Pacific form a single panmictic population (i.e. *T* was not significantly different from zero in most pairwise comparisons). Therefore, we grouped Pacific Ocean colonies into two groups based on subspecies for further analyses in MDIV. Divergence time between these two groups was not significantly different from zero (not shown). When *T* is not different from zero, estimates of *M* are not reliable (R. Nielsen, personal communication). As a result, accurate estimates of migration between these colonies could not be made; however, it is clear that contemporary migration is high between subspecies.

Mantel's tests for both mitochondrial and nuclear data sets did not detect any significant association between genetic and geographic distances ($P = 0.12$, $P = 0.42$, respectively).

Because little to no population structure was found in Pacific common murres, all colonies were grouped together for analyses in FLUCTUATE and BOTTLENECK. FLUCTUATE estimated a growth rate that was significantly greater than 0 ($P < 0.01$), and results from BOTTLENECK indicated that there was a significant heterozygosity deficiency in both the intron and microsatellite data sets, indicative of population growth (all $P < 0.03$). These results were robust over a wide variety of mutational models.

Discussion

Concordance of mitochondrial and nuclear data

Recently, emphasis has been placed on using multiple nuclear loci rather than mtDNA in population genetic and phylogeographic studies (e.g. Edwards *et al*. 2005; but see Zink & Barrowclough 2008). Here, we have included multiple nuclear loci to (i) corroborate trends suggested by mtDNA, and (ii) enable the use of multilocus coalescent-theory-based methods that can reveal contemporary demographic processes. Population genetic structure as revealed by mtDNA and nuclear DNA was widely concordant in all of our analyses, both in the comparison between the Pacific and Atlantic oceans and within ocean basins. Although the mtDNA and nuclear DNA results were qualitatively similar, in all cases the magnitude of population genetic structure at nuclear DNA markers was lower than at mtDNA. Previous studies have attributed similar differences to processes such as male-biased dispersal (e.g. Burg & Croxall 2001) or to the difficulty of

Table 5 Estimates of Φ_{ST} based on mitochondrial control region variation (above the diagonal), and F_{ST} based on nuclear variation (below the diagonal) between Pacific colonies of common murres. Results significant before Bonferroni corrections are indicated with *, and those significant after Bonferroni corrections with **. Population abbreviations are given in Table 1. Dashes indicate pairwise comparisons that were not possible due to low sample size

	OK	CH	PR	WAI	EAI	CPE	EPE	LCO	CCO	PWS	BC	WA	OR	CA
OK		0.026	0.078*	0.067	0.007	0.003	0.091*	0.013	0.027*	0.002	0.029	0.044	0.054*	0.002
CH	–0.013		0.009	–0.017	0.012	0.010	0.002	0.012	0.002	0.013	0.005	0.012	0.003	0.037
PR	–0.561	–0.708		–0.040	0.023	0.059	–0.014	0.028	–0.006	0.040	0.009	0.014	–0.014	0.067*
WAI	–0.015	–0.002	–1.269		0.014	0.016	–0.042	0.011	–0.022	0.024	0.002	0.007	–0.033	0.077
EAI	–0.026	–0.006	–0.875	–0.001		0.010	0.028	–0.017	–0.007	–0.014	–0.018	–0.005	0.006	–0.015
CPE	–0.033	–0.014	–1.636	–0.010	–0.004		0.025	0.008	0.022	0.006	0.027	–0.012	0.024	0.017
EPE	0.003	–0.011	–0.738	0.005	–0.008	–0.027		0.027	–0.008	0.029	0.017	–0.010	–0.008	0.074*
LCO	–0.023	0.004	–0.986	–0.007	–0.010	0.009	–0.008		–0.003	0.000	–0.005	–0.003	0.006	0.007
CCO	–0.033	–0.007	–0.636	–0.005	–0.010	–0.014	–0.021	0.006		–0.009	–0.016	–0.009	–0.011	0.010
PWS	—	—	—	—	—	—	—	—	—		–0.010	0.015	0.020	0.000
BC	–0.074	–0.044	–1.641	–0.035	–0.015	–0.032	–0.070	–0.017	–0.034	—		–0.005	–0.018	0.006
WA	–0.119	–0.082	–4.101	–0.047	–0.043	–0.063	–0.113	–0.029	–0.063	—	–0.017		–0.015	0.029
OR	–0.019	–0.003	–1.093	0.022	0.019*	–0.003	–0.024	0.040**	0.004	—	–0.022	–0.066		0.046
CA	–0.041	–0.026	–1.267	0.012	0.002	0.001	–0.048	0.002	–0.008	—	–0.008	–0.048	0.007	

comparing molecular markers with different levels of genetic variation (Hedrick 2005). However, no evidence has been found for sex-biased dispersal in common murres (Harris *et al*. 1996b). Moreover, lower levels of differentiation at nuclear loci are expected due to the approximately four times greater effective population size compared to mtDNA (Zink & Barrowclough 2008). Regardless, a relatively small number of microsatellite loci were able to detect the signal of population genetic structure in the Atlantic (in concordance with mtDNA). In the Pacific Ocean, no population genetic structure was present at mtDNA or microsatellite loci, and the inclusion of multiple intron loci to verify this trend also did not reveal population genetic structure.

Isolation of Atlantic and Pacific common murres

Similar to previous morphological (Storer 1952) and genetic studies (Friesen *et al*. 1996), we found evidence based on both mitochondrial and nuclear loci that Atlantic and Pacific common murre populations are genetically isolated. The divergence time estimated from δ-values was between 56 000 and 226 000 years BP, which means that Atlantic and Pacific populations diverged long before the end of the Pleistocene. Since the populations diverged, gene flow between ocean basins has likely been restricted by a combination of sea ice, the Bering Landbridge, and unsuitable breeding habitat throughout the Arctic Ocean. Interestingly, Atlantic and Pacific common murre populations remain isolated today although the Bering Landbridge is open and sea-ice does not represent an impassable barrier through the arctic. Although Pacific common murres are generally larger and have significantly longer bills, tarsi and wings than Atlantic murres (Storer 1952; Gaston & Jones 1998), there is no evidence that Atlantic and Pacific populations have evolved different mating calls or behaviours. Therefore, unsuitable breeding habitat throughout the arctic has probably played, and continues to play, a significant role isolating the Atlantic and Pacific. Regardless of the exact nature of the barrier, Atlantic and Pacific common murre populations have clearly experienced independent evolution for a long time, and can be used as natural replicates to test mechanisms of population differentiation.

Population differentiation and demographic history within ocean basins

Contrary to previous studies (Moum *et al*. 1991; Moum & Árnason 2001; Riffault *et al*. 2005; but see Friesen *et al*. 1996), we found evidence of significant population differentiation in Atlantic common murres both from control region data and to a lesser extent from microsatellite data. Specifically, our data suggest an east–west structuring of common murre populations in the Atlantic. Without knowledge of whether Atlantic common murre populations are in genetic equilibrium, two alternative demographic histories can explain the observed structuring: either (i) eastern and western populations differentiated in allopatric glacial refugia during the Pleistocene and have since come into secondary contact, or (ii) differentiation occurred recently due to restricted contemporary gene flow between the east and west.

As previously acknowledged by Friesen *et al*. (1996), the large population size of common murres and the short time since the last glacial cycle suggest that populations may not be in genetic equilibrium. The present results also suggest

that Atlantic common murre populations are not in genetic equilibrium. First, evidence for population expansion was found in Atlantic Ocean populations using a number of different analyses (FLUCTUATE, BOTTLENECK, Chakraborty's amalgamation tests). This population growth was likely also accompanied by northward range expansion following the retreat of Pleistocene sea-ice. Second, evidence from coalescent analyses that do not assume that populations are in genetic equilibrium suggests that contemporary gene flow is high between all Atlantic populations, despite the existence of population genetic structure. This contrasting pattern could occur if populations are not in migration-drift equilibrium. Specifically, the lack of population genetic structure found by STRUCTURE (although only four loci were used and so power was low) and results from MDIV are indicative of contemporary processes, while Φ_{ST} and F_{ST} values are lagging indicators of historical population structure (Whitlock & McCauley 1999).

Although a Mantel's test revealed a significant association of genetic and geographic distances within the Atlantic, suggesting isolation by distance, this pattern can also result from secondary contact of previously allopatric lineages in a nonequilibrium situation. Indeed, under the framework of Hutchison & Templeton (1999), regional equilibrium should only be inferred if the scatter plot of genetic and geographic distances shows a positive and monotonic relationship over all geographic distances. Inspection of the scatter plot suggests that these conditions are not met in Atlantic common murres (e.g. contrast the closed and open circles in Fig. 2).

Therefore, most evidence suggests that the observed population genetic differences in Atlantic common murres are the result of historical fragmentation into multiple refugia followed by secondary contact, rather than recent differentiation. Although MDIV was unable to estimate upper bounds on the divergence time between eastern and western Atlantic lineages, the estimated times are consistent with historical isolation in multiple Pleistocene refugia (Table 4). Additionally, if populations had diverged in multiple refugia during the Pleistocene, we would expect to find the highest levels of genetic diversity at the zone of secondary contact. In fact, haplotype diversity increased linearly from the west to the east, consistent with a zone of secondary contact in the low-arctic waters of the eastern Atlantic. This is further supported by the presence of 'western-like' haplotypes (e.g. haplotype B) in the eastern Atlantic low-arctic.

During the last glacial maximum, land and sea-ice extended as far south as the northeastern USA and across the Atlantic in a broad band to Portugal (Hewitt 2000). Other phylogeographic studies have identified marine Pleistocene refugia south of the ice sheets both in North America and in Europe. Specifically, Moum & Árnason (2001) found evidence that razorbills (close relatives of common murres) expanded from a single western Atlantic refugium after the Pleistocene. Similarly, many European animal taxa are known to have expanded from an Iberian refugium south of the ice sheet in Portugal (Hewitt 2000). Therefore, contemporary Atlantic murres may be descended from two Pleistocene refugia, one in the west and one in the east. In fact, common murres still breed and overwinter in both of the proposed areas (Brown 1985; Nettleship & Evans 1985). As glacial ice receded, western and eastern refugial populations presumably expanded northward to eastern North America and Europe, respectively, and are now in secondary contact, perhaps in the eastern Atlantic low-arctic.

One of the most striking features of the contemporary Atlantic common murre distribution is that almost all western Atlantic colonies are located in low-arctic waters, while most eastern Atlantic colonies are located in boreal waters (Nettleship & Evans 1985). During the Late Pleistocene, the marine polar front was much farther south and all Atlantic murre colonies would have been in relatively homogenous cool water (Bradley 1999). However, as the Pleistocene ended, the Gulf Stream was re-established and the polar front was pushed north, segregating western and most eastern common murres into low-arctic and boreal waters, respectively (Bradley 1999). However, the colonies of the eastern Atlantic that show the strongest signal of secondary contact are in low-arctic waters that are more similar to the western Atlantic (Bradley 1999). Therefore, we posit that secondary contact of refugial lineages has been facilitated by the similar oceanography of the western and eastern low-arctic, the prevailing west-to-east ocean currents (i.e. the Gulf Stream), and the presence of 'stepping-stone' islands (Greenland and Iceland) that were not available during the Pleistocene for dispersal.

Long-term banding initiatives have documented dispersal consistent with this scenario. Although adult common murres are typically philopatric (Harris *et al*. 1996a), natal dispersal of juveniles within the eastern Atlantic is common (Halley & Harris 1993; Harris *et al*. 1996b). Therefore, the high levels of contemporary migration among common murre populations are likely the result of juvenile dispersal. Interestingly, no trans-Atlantic dispersal has been recorded, despite the large number of birds banded in Europe (Brown 1985). However, if dispersal is mostly unidirectional from the western Atlantic into the eastern low-artic (as would be expected based on ocean currents), it is likely that these movements would not have been recorded, as relatively few common murres have been banded at North American breeding sites. Indeed, evidence from both thick-billed and common murres suggests that long-distance movements are possible. Specifically, thick-billed and common murres banded as juveniles have been found breeding more than 2000 and 2400 km away from their natal colonies, respectively (Kampp & Falk 1998). Moreover,

the geographic pattern of dispersal in common murres is consistent with indirect west-to-east dispersal through the low-arctic. Specifically, common murres banded in Newfoundland have been recovered in Greenland and murres from Iceland have been recovered in Europe (Brown 1985).

In contrast to the Atlantic, all analyses suggested that little genetic differentiation exists among Pacific common murre colonies. Similar to Atlantic common murres, two lines of evidence suggest that Pacific murres are not in genetic equilibrium. First, results from BOTTLENECK and FLUCTUATE indicate that, like Atlantic populations, Pacific murres have experienced a recent population expansion. Second, assuming a stepping-stone model of colonization, populations are expected to exhibit an isolation-by-distance pattern of genetic structure once migration-drift equilibrium has been reached (Hutchison & Templeton 1999). Therefore, in the Pacific Ocean (where colonies are distributed in an obvious stepping-stone distribution), more geographically proximate populations should be more genetically similar if equilibrium has been reached (Hutchison & Templeton 1999); however, Mantel's tests failed to detect concordance between geographic and genetic distances in Pacific common murres. As such, genetic variation within Pacific common murres might still harbour some signature of historical association. Nevertheless, most evidence suggests high levels of contemporary gene flow among Pacific colonies. STRUCTURE identified the most likely number of genetic populations to be one (although only eight loci were used), and high contemporary gene flow was supported by MDIV. Therefore, regardless of the extent that traditional population genetic estimates (e.g. Φ_{ST}) reflect historical association of colonies, Pacific common murres clearly constitute a single genetic population maintained by contemporary migration between colonies.

Unlike Atlantic common murres, we found no evidence that contemporary Pacific Ocean populations are descended from multiple refugial populations. Additionally, recent evidence from a range of marine taxa suggests that there could have been refugial habitat suitable to common murres in at least three locations in the Pacific: (i) south of the ice sheets in Eurasia, (ii) south of the ice sheets in continental North America, and (iii) in Beringia (Harlin-Cognato *et al*. 2006). Although the genetic signature suggests that Pacific murres are descended from a single refugium, common murres may have been fragmented into multiple refugia but the signal is not strong enough to be detected. For instance, murres may have been fragmented in multiple Pacific refugia, but due to the large population size of Pacific common murres, large- scale differentiation never occurred. Alternatively, although genetic equilibrium has not yet been reached, Pacific populations may be close to equilibrium (i.e. any signal of potential historical fragmentation is diminished). In fact, the time it takes for populations to reach genetic equilibrium is strongly influenced by migration rate (Birky *et al*. 1989). Therefore, all else being equal, populations in the Pacific would reach genetic equilibrium faster than Atlantic populations if migration rate was higher in the Pacific. Migration in the Pacific could easily be facilitated by the stepping-stone distribution of colonies from California to the Sea of Okhotsk (Fig. 1) and by the uniformly cool Alaska Current that flows north along much of the North American coast and east along the Aleutian Islands (Colling 2001).

While patterns of genetic differentiation were markedly different within Atlantic and Pacific common murres, both populations appear to be characterized by high contemporary migration among colonies. Specifically, low differentiation among colonies in the Pacific is maintained by high contemporary gene flow, and high differentiation between different sides of the Atlantic appears to be the result of historical fragmentation rather than a contemporary restriction in gene flow. Although few studies have investigated population differentiation in temperate seabird species that live in both the Atlantic and Pacific, black-legged kittiwakes have a similar distribution to common murres and also are differentiated within the Atlantic but not within the Pacific (Patirana 2000).

Taken together, these results suggest that particular care must be taken when inferring demographic history from patterns of population genetic structure in temperate Northern Hemisphere taxa. We have found that, even within a single species, the extent that population genetic structure reflects contemporary demographics can vary among regional populations.

Taxonomy of common murres

The present study partially supports existing subspecific designations for common murres. In every subspecies classification proposed for common murres (reviewed in Tuck 1961; Bedard 1985), Atlantic and Pacific populations have been separated; the genetic data strongly support this division. However, further subspecific divisions within ocean basins (see Introduction) were not concordant with neutral genetic variation. Specifically, estimates of Φ_{CT} and F_{CT} based on groupings of colonies into subspecies were not significant in either the Atlantic or Pacific, although the most morphologically divergent populations in the Atlantic (Cabo Villano and Ilhas Berlengas in Spain and Portugal, respectively) were not sampled. Although the genetic evidence does not seem to support existing designations, ultimately morphological, behavioural and genetic evidence should all be considered when delineating subspecies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Partial sequence of the mitochondrial control region of Pacific Ocean common murre haplotype 01. Conserved sequence blocks are in capitals. Sequence begins 141 bp from the beginning of the control region (Moum and Johansen 1992). Asterisks mark the beginnings and ends of the three Domains, and slashes mark the beginnings and ends of the two fragments that were amplified (sequence is not continuous between these fragments). X represents a 28-bp insertion present in a single individual (5′-TGTAATGGGGTAAAGACATGGCCCTCCA-3′). Variable sites are in bold. Dashes indicate insertion or deletion sites.

Fig. S2 Sequences of the most common alleles for four nuclear introns for common murres. Variable sites are highlighted in bold.

Table S1 Intron primers surveyed and PCR annealing temperatures for Pacific common murres

Table S2 Table of variable sites between common murre control region haplotypes. Dots indicate identity to Pacific Ocean haplotype 01. Indels are indicated by a dash and X represents a 28-bp insertion in haplotype 27

Table S3 Frequencies of alleles at four microsatellite and four intron loci for common murres and estimates of allelic richness (R), and observed (H_O) and expected (H_E) heterozygosities at each locus

Table S4 Distribution and frequency of haplotypes

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