MITOCHONDRIAL DNA REVEALS POPULATION GENETIC STRUCTURE WITHIN ATLANTIC BUT NOT PACIFIC POPULATIONS OF A HOLARCTIC SEABIRD

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ABSTRACT

SAUVE, D., PATIRANA, A., CHARDINE, J.W. & FRIESEN, V.L. 2018. Mitochondrial DNA reveals genetic structure within the Atlantic but not Pacific populations of a holarctic seabird, the Black-legged Kittiwake *Rissa tridactyla*. *Marine Ornithology* 47: 199–208.

To predict evolutionary processes, such as speciation and local adaptation, we need to understand the mechanisms causing genetic differentiation of populations. We used mitochondrial control region sequence variation to investigate the genetic structure within and between Atlantic and Pacific populations of Black-legged Kittiwake (*Rissa t. tridactyla* and *R. t. pollicaris*, respectively). We predicted that genetic divergence of these populations, as in other northern hemisphere seabird species, might have been caused by glacial vicariance in the late Pleistocene. Further, because of regional differences in the morphology of kittiwakes, and the hypothesized historical vicariance, we predicted that genetic structure would exist within Atlantic but not Pacific populations. Population genetic and phylogenetic analyses of 756 base pairs of control region sequence for 398 kittiwakes indicated that Atlantic and Pacific populations are genetically differentiated from one another. Phylogenetic analyses indicated historical divergence of two mtDNA clades within the Pacific population and four mtDNA clades within the Atlantic population. Population genetic analyses indicated that colonies within the Atlantic were strongly differentiated from one another, which could be explained by restrictions in contemporary gene flow and historical fragmentation in historical refugia. Population genetic analyses provided little evidence for genetic structure in the Pacific population, which we attributed to longer time since vicariance, allowing more migration between colonies. Our results agree with current subspecies designations of Atlantic and Pacific populations.

Key words: coalescence, gene flow, mitochondrial control region, historical demography, mtDNA, Pleistocene glaciation

INTRODUCTION

Population geneticists have well developed theories on how evolutionary processes—such as selection, genetic drift, gene flow, and mutation—can result in population divergence and speciation (e.g., Endler 1977, Coyne & Orr 2004). However, the relative importance of each of these evolutionary forces in the process of genetic divergence or speciation in the natural world is not well known. For example, contemporary patterns of genetic variation are shaped by both present-day and historical forces, making patterns of genetic variation difficult to interpret. Understanding these processes is particularly important for many seabirds because historical fragmentation of their ranges by Pleistocene glaciations, and the long generation times of seabirds, make it probable that genetic patterns of northern hemisphere species are shaped, at least partially, by historical isolation of refugial populations (e.g., Morris-Pocock *et al*. 2008, Tigano *et al*. 2015).

Seabirds provide interesting study systems to investigate mechanisms of population differentiation. Genetic differentiation commonly occurs between seabird populations separated by land, but genetic differentiation can also occur between populations with no contemporary land barrier (Friesen *et al*. 2007, Friesen 2015). When seabird populations are not separated by land (within an ocean basin), genetic differentiation of populations is expected to be weak because of the high potential for dispersal—and therefore gene flow—in seabirds (Friesen *et al*. 2007, Friesen 2015). However, many seabird populations exhibit restricted gene flow and genetic differentiation within ocean basins. Genetic differentiation among seabird colonies within an ocean basin could be the result of historical fragmentation. Historical demographic influences, such as population bottlenecks and spatial fragmentation by Pleistocene glaciers, are recognized as dominant forces shaping present-day diversity and distributions of many northern hemisphere species (Hewitt 2000). For example, genetic structuring in Atlantic Common Murres *Uria aalge* and Razorbills *Alca torda* likely reflects historical separation in multiple glacial refugia (Moum & Arnason 2001, Morris-Pocock *et al*. 2008). Differentiation among populations within ocean basins could also represent contemporary processes. Many seabirds are highly philopatric (but see Coulson 2016), and philopatry could restrict gene flow among colonies (Quinn & Dittman 1990, Friesen *et al*. 2007, Friesen 2015). Similarly, differences in selective pressures (e.g., due to ocean regime) may deter migration among colonies or reduce the fitness of immigrant individuals. Therefore, genetic differentiation among colonies of seabirds could be the result of a combination of historical fragmentation, natural selection, or philopatric behaviour.

The extent of genetic structure within Atlantic or Pacific seabird populations is difficult to predict. In the Atlantic, genetic structure is found in Common Murres, Razorbills, and Black Guillemots *Cepphus grylle,* whereas little genetic structure is found in Northern Fulmars *Fulmarus glacialis* or Thick-billed Murres *Uria lomvia*

(Kidd & Friesen 1998, Moum & Arnason 2001, Morris-Pocock *et al*. 2008, Kerr & Dove 2013, Tigano *et al*. 2015). Similarly, within the Pacific, genetic structure is found in Thick-billed Murres and Pigeon Guillemots *Cepphus columba* but not in Northern Fulmars or Common Murres (Kidd & Friesen 1998, Morris-Pocock *et al*. 2008, Kerr & Dove 2013, Tigano *et al*. 2015). Reviews of seabird species suggest multiple environmental and ecological factors could interact to produce genetic structure (Friesen *et al*. 2007, Friesen 2015). For example, genetic structure in Black Guillemots is primarily attributed to small population sizes and low dispersal, whereas genetic structure in Atlantic Common Murres is attributed to fragmentation during the Pleistocene glaciation (Kidd & Friesen 1998, Morris-Pocock *et al*. 2008). Determining the relative importance of these factors in shaping genetic structure will require the accumulation of genetic, ecological, and environmental data on multiple species within ocean basins.

Black-legged Kittiwakes *Rissa tridactyla* are small, pelagic, cliff-nesting gulls that have a subarctic and arctic breeding distribution and show regional variation in morphometrics and plumage (Sluys 1982; Chardine 2002). Two subspecies are generally recognized: *R. t. pollicaris*, confined to Alaska and the Bering, East Siberian, and Chukchi Seas; and *R. t. tridactyla*, restricted to arctic Canada, Norway, Western Greenland, Western Russia, and the Northeast Atlantic (Cramp & Simmons 1983). Pacific Black-legged Kittiwakes generally have a longer bill, slightly larger body size, and more black on the primaries (Cramp & Simmons 1983; Chardine 2002).

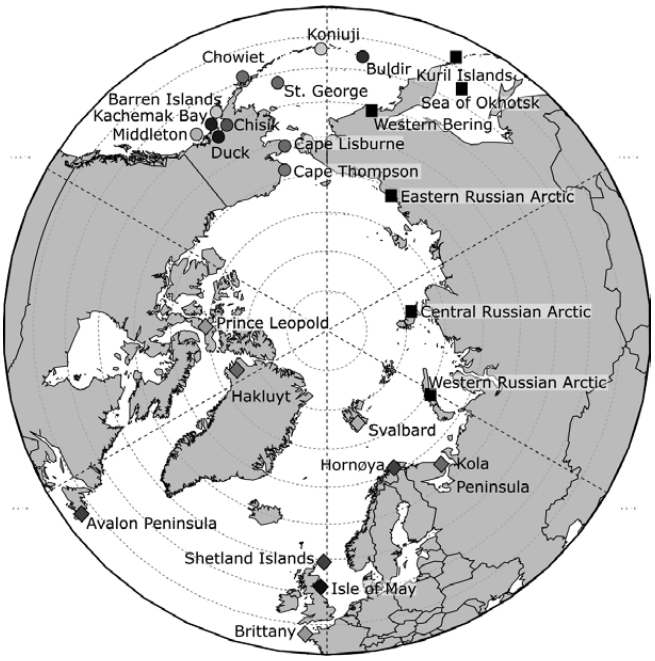

Fig. 1. Map of Black-legged Kittiwake sampling sites. Diamonds depict the Atlantic and circles depict the Pacific subspecies. Black squares indicate major sampling gaps. Sample sizes and coordinates are given in Table 1.

TABLE 1
Locations, coordinates, sample sizes (*n*), haplotype diversities (H_S), nucleotide (π) diversities, Tajima's *D* statistics, and Fu's F_S test of neutrality for Atlantic and Pacific kittiwake colonies[a, b]

Location	Population	Coordinates	*n*	H_S(SE)	π(SE)	Tajima's *D*	Fu's F_s
Alaska, USA	Barren Islands*	58°53′N, 152°00′W	45	0.91(0.03)	0.010(0.005)	-0.54	**-6.0**
	Kachemak Bay*	59°30′N, 151°29′W	16	0.91(0.08)	0.015(0.008)	-0.7	-0.8
	Buldir Island	52°21′N, 175°55′E	12	0.86(0.09)	0.012(0.006)	-0.9	0.6
	Cape Lisburne	68°53′N, 166°21′W	24	0.98(0.02)	0.012(0.006)	-1.1	**-8.2**
	Cape Thompson	68°09′N, 165°58′W	13	0.98(0.01)	0.013(0.007)	-0.6	-3.5
	Chisik Island*	60°08′N, 152°33′W	9	0.97(0.06)	0.012(0.007)	0.4	-1.5
	Chowiet Island	56°02′N, 156°42′W	15	0.96(0.05)	0.009(0.005)	-0.8	-2.0
	Duck Island*	60°09′N, 152°33′W	13	0.96(0.05)	0.011(0.006)	0.0	-2.8
	Koniuji Island*	52°15′N, 175°07′W	16	0.97(0.03)	0.011(0.006)	-0.4	**-5.5**
	Middleton Island	59°26′N, 146°20′W	26	0.96(0.02)	0.011(0.006)	-0.5	-4.2
	St. George Island*	56°39′N, 169°47′W	20	0.95(0.04)	0.010(0.006)	-1.1	**-4.9**
Canada	Avalon Peninsula	47°21′N, 053°19′W	30	0.87(0.04)	0.009(0.005)	-0.6	0.3
	Prince Leopold I.	74°02′N, 090°05′W	7	0.83(0.13)	0.004(0.003)	-0.6	-0.8
Greenland	Hakluyt	77°27′N, 071°48′W	11	0.81(0.08)	0.004(0.003)	-1.5	2.1
United Kingdom	Shetland Islands	60°20′N, 001°14′W	8	0.64(0.18)	0.003(0.002)	-1.5	0.8
	Isle of May	56°11′N, 002°33′W	30	0.71(0.07)	0.003(0.002)	-1.0	-1.1
France	Brittany	48°42′N, 003°48′W	17	0.75(0.09)	0.005(0.003)	-1.1	0.1
Norway	Hornøya	70°23′N, 031°09′E	30	0.80(0.05)	0.005(0.003)	-0.3	0.0
	Svalbard	78°54′N, 012°00′E	29	0.61(0.10)	0.003(0.002)	-1.2	-0.9
Russia	Kola Peninsula	67°18′N, 041°06′E	27	0.85(0.05)	0.007(0.004)	-0.9	0.4
Total			**398**				

[a] Significant Tajima's *D* and Fu's F_s values are in bold. The alpha used for evaluating F_s significance was 0.02 (Fu 1997).
[b] Asterisks indicate birds collected near breeding colonies during the breeding season instead of actively nesting adults or chicks hatched at a colony.

Kittiwakes in the North Atlantic differ regionally in the extent of black on the tips of primary feathers (Chardine 2002) and biometrics (wing, culmen, and tail length; Sluys 1982). Wing-tip patterns of Atlantic kittiwakes suggested that there are two geographic groups: 1) Arctic Canada and West Greenland; and 2) Newfoundland, United Kingdom, and Barents Sea (Chardine 2002). To the best of our knowledge, a regional analysis of the variation of phenotypes present in the Pacific has not been conducted. Variation in plumage between and within ocean basins suggests that significant genetic differences and restricted gene flow may exist among kittiwakes from different ocean basins and to some extent within the Atlantic Ocean (Chardine 2002; Coulson 2016). Variation in microsatellite markers indicate that Atlantic and Pacific colonies are genetically different, whereas colonies within the Atlantic are not (McCoy *et al*. 2005).

In the present study, we analyzed sequence variation in the mitochondrial control region of Black-legged Kittiwakes sampled from colonies throughout most of their range. The mitochondrial control region exhibits a high mutation rate and small effective population size compared to nuclear DNA, and it is therefore sensitive to restrictions in gene flow and population size (Avise 1994). Because many conspecific seabird populations are genetically differentiated between ocean basins (Friesen *et al*. 2007, Friesen *et al*. 2015), and microsatellite markers indicate that Pacific and Atlantic kittiwakes differ genetically (McCoy *et al*. 2005), we predicted that mitochondrial sequences would differ between Black-legged Kittiwakes in the Atlantic versus Pacific Oceans. If so, Atlantic and Pacific populations act as natural replicates for tests of mechanisms of population differentiation within ocean basins.

Evidence from morphometrics suggests that genetic structure exists within the Atlantic but not the Pacific population of Black-legged Kittiwakes. Therefore, we predicted that population genetic structure in mtDNA would contrast with nuclear DNA of Black-legged Kittiwakes because of restrictions in contemporary gene flow.

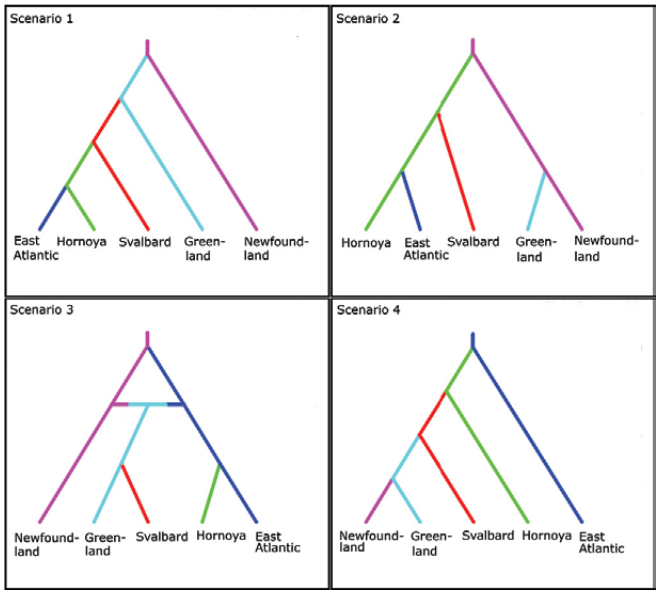

Fig. 2. Population history scenarios tested in DIYABC. Scenarios include a refugial Newfoundland population that successively spread East (Scenario 1), Eastern and Western refugial populations that successively spread to form the Atlantic colonies (Scenario 2), Eastern and Western populations that experienced an admixture event that led to the Svalbard and Greenland populations (Scenario 3), and a refugial European population that successively spread West (Scenario 4).

Because genetic variation may allow species to adapt to changing environmental conditions, estimates of genetic structure and differentiation may have conservation implications (Allendorf *et al*. 2013). If populations differ genetically, then loss of a population may result in loss of overall genetic variation. Therefore, genetic differences should be considered in the assessment of management units. Genetic information guiding management of kittiwakes is pertinent because rapid and sustained declines in population sizes of Black-legged Kittiwakes in the Atlantic have resulted in the species being listed as vulnerable by the International Union for the Conservation of Nature (BirdLife International 2017).

METHODS

DNA extraction and amplification

We extracted DNA from blood samples, muscle tissue, or pin feathers from 398 Black-legged Kittiwakes from 20 colonies (Fig. 1, Table 1) using standard protease-k, phenol-chloroform protocols (Friesen *et al*. 1997). Although samples from Duck Island, Kachemak Bay, and Chisik Island are geographically close to each other, they were analyzed separately to reveal any fine-scale structure.

We assayed variation in the 5′ and 3′ ends of the mtDNA control region in two nonoverlapping fragments following Patirana *et al*. (2002), using the primers RbL20 and RtH400 (Domains I and II), and RtL500mt and RtH900 (Domains II and III). We discarded sequences with more than three ambiguous sites from downstream analyses. We then collated sequences from the two fragments for each bird. We quantified genetic variability using gene diversity (H_s; Nei 1987) and nucleotide diversity (π; Nei & Tajima 1983) in Arlequin (3.5.22; Schneider *et al*. 2000).

We calculated Tajima's D (Tajima 1989) and Fu's F_s (1997) using Arlequin (Excoffier *et al*. 2007) to test whether sequence variation deviated significantly from the assumption of a neutral model of evolution. Tajima's D is expected to be negative under a model of population expansion or under a selective sweep and positive under rate heterogeneity or diversifying selection (Tajima 1989). Fu's F_s is more sensitive to population expansion than Tajima's D, and is expected to be negative following a population expansion or selective sweep; positive values might indicate a population bottleneck (Fu 1997).

Tests of population differentiation

We conducted the analyses described below in Arlequin unless otherwise noted. We used an analysis of molecular variation (AMOVA) to calculate ϕ_{st} statistics (Excoffier *et al*. 1992) and to deduce the statistical significance of geographic variation in mitochondrial haplotypes. We ran these analyses using Kimura two-parameter distances with the alpha (α) parameter of the gamma distribution set to 0.42 (Marshall & Baker 1997). We tested the significance of ϕ_{st} estimates by comparison to values generated from 10 000 random permutations of sequences among populations.

To test for restrictions in gene flow due to distance (Wright 1943), we performed Mantel's tests using the R package "ecodist" (Goslee & Urban 2007) within each ocean basin to determine whether a

positive correlation existed between Slatkin's linearized ϕ_{st} and log-transformed geographic distance.

Population history

We used DIYABC (2.1.0) to test for genetic support for alternative models of population history within the Atlantic population (Cornuet *et al*. 2014). We did not conduct DIYABC analyses for the Pacific because there were no biologically significant population genetic groupings in the Pacific. Populations were clustered based on pairwise ϕ_{st} values. Because the Kola Peninsula, Brittany, Isle of May, and Shetland colonies had significant pairwise ϕ_{st} with all other colonies in the Atlantic except each other (see Results), we combined these colonies into a single population ("East Atlantic"). We excluded the colony at Prince Leopold Island from the analysis due to small sample size. We tested four different population scenarios based on possible Pleistocene refugia in the Atlantic (Fig. 2): 1) an eastward expansion from a single western Atlantic glacial refugium; 2) allopatric divergence followed by expansion of eastern and western Atlantic populations into the Arctic; 3) allopatric divergence, with a gene flow event between eastern and western refugial populations giving rise to the Greenland and Svalbard colonies; and 4) a westward expansion from a single eastern Atlantic glacial refugium.

We generated a reference table containing 1×10^7 simulated datasets for each scenario and used 0.1 % of the simulated datasets closest to the observed genetic dataset to estimate posterior probabilities for each scenario using a logistic approach. All genetic parameters available in DIYABC were simulated for each scenario and compared to the observed genetic parameters of the dataset (Supplementary Table S3, available on the website). To validate confidence in scenario choice, we calculated the posterior predictive error. The posterior predictive error is reported as the proportion of incorrectly identified scenarios out of 500 simulated datasets. Finally, we used a model-checking analysis to evaluate whether our posterior model fit the data.

Phylogeographic structure

To infer the relationships among control region haplotypes and to estimate the divergence time between Atlantic and Pacific mitochondrial lineages, a gene tree was constructed using BEAUti (2.3.1) to import data, BEAST (2.3.1) to perform the analysis, TREEANNOTATOR (2.3.1) to produce a summary tree, TRACER (1.6) to examine trace file output, and FIGTREE (1.4.3) to generate gene tree figures (Heled & Drummond 2010, Bouckaert *et al*. 2014, Drummond & Bouckaert 2015). In BEAST, a burn-in period of 10^5 iterations was used on a total of 10^8 Markov Chain Monte Carlo iterations. Sequence of the closely related Red-legged Kittiwake *Rissa brevirostris* mitochondrial control region was used to root the Black-legged Kittiwake sequences (Patirana *et al*. 2002). Nucleotide substitution models were determined by JMODEL test (Darriba *et al*. 2012), and a strict molecular clock was used. A constant coalescence prior was applied to the gene tree. Convergence of the MCMC process was monitored using TRACER (Version 1.6; Drummond & Rambaut 2007), and we ensured effective sample sizes (ESSs) were all higher than 200. Additionally, a haplotype network was constructed using statistical parsimony (Clement *et al*. 2002) in PopArt (http://popart.otago.ac.nz).

Maximum and minimum divergence times (t) were calculated using δ/r, where δ is the average of all pairwise δs between Atlantic and Pacific colonies (or between clades within the Atlantic or Pacific) and r is the sequence divergence rate for the control region in years (Wilson *et al*. 1985). The divergence rate is not known for the mitochondrial control region of kittiwakes. Quinn (1992) estimated a divergence rate of 20.6 % per million years (Ma) for the hypervariable Domain I of geese, and Wenink *et al*. (1996) determined an overall divergence rate of 14.8 %/Ma for Domains I and II of the Dunlin *Calidris alpina*. Similarly, Vigilant *et al*. (1991) estimated the mean divergence rate for the entire human mitochondrial control region to be between 11.5 %/Ma and 17.3 %/Ma. Thus, we applied conservative maximum and minimum divergence rates of 11.5 %/Ma and 5 %/Ma for the entire control region.

RESULTS

Characterization of control region variation

Analysis of 756 base pairs (bp) of the mitochondrial control region identified 134 haplotypes and 102 variable sites among 398 Black-legged Kittiwakes. One Atlantic kittiwake from Hakluyt, Greenland possessed a haplotype (U2K) that otherwise occurred only among Pacific kittiwakes. Apart from this bird, no other haplotypes were shared among individuals from different ocean basins. Otherwise, 38 haplotypes (defined by 46 variable positions) were found among the 189 Atlantic samples. Among the Atlantic haplotypes, 44 polymorphic sites were found in Domain I (335 bp), with no variable sites in Domain II (100 bp), and two variable sites in Domain III (321 bp). Thirty-nine transitions and 10 transversions were evident, with three insertion/deletions (indels). The number of substitutions between haplotypes varied from one to 26, corresponding to Kimura two-parameter distances of 0.13 % to 3.5 %. The mean pairwise sequence divergence between Atlantic haplotypes was 0.66 % (five substitutions). Each sampling site had either one or two common haplotypes, and two (Greenland) to seven (Newfoundland) private haplotypes, at low frequency. For example, the most common haplotypes within the Northeast Atlantic were BF and EE, which were shared among 16 % and 11 %, respectively, of the 189 individuals. Haplotypes B4F and G2F were unique to Hornøya, where they were found among 36 % and 23 % of individuals, respectively, at that colony. In Svalbard, 62 % of individuals shared haplotype F2F, which was also present in Greenland at a much lower frequency (20 %). Similarly, 51 % individuals in Newfoundland shared haplotype A6F, while 15 % of individuals shared A6F in the Kola Peninsula. Several haplotypes were shared among populations (e.g., K2F, K3F, AF, F1F) but occurred in only one or two birds at each colony.

Of the 134 haplotypes, 96 (defined by 86 variable positions) were identified exclusively among the 209 Pacific kittiwakes (Supplementary Table S2, available on the website). Among Pacific haplotypes, 73 polymorphic sites were in Domain I (335 bp), three were in Domain II (100 bp), and 10 were in Domain III (321 bp). Among these haplotypes, 74 transitions, 10 transversions, and 10 indels were evident. The mean pairwise sequence divergence between Pacific haplotypes was 1.06 % (eight substitutions). Haplotypes differed by one to 23 substitutions, corresponding to Kimura two-parameter distances of 0.13 % to 3.0 %, respectively. The most common Pacific haplotype (U2K) was shared among 14 % of the 203 individuals and was found across all sampling sites except for Kachemak Bay and Duck Island (Supplementary Table S2). The second most common haplotype (UK) was found in 7.6 % of the individuals and was restricted to Gulf of Alaska

samples. All other haplotypes were unique to one or two populations and occurred in only one or two individuals.

The Pacific had higher haplotype diversity than the Atlantic (F = 18.85, P < 0.001), ranging from 0.86 to 0.91 in the Pacific and 0.61 to 0.87 in the Atlantic (Table 1). Nucleotide diversities were also higher in Pacific colonies compared to Atlantic colonies, ranging from 0.005–0.015 in the Pacific and 0.003–0.009 in the Atlantic (F = 30.52, P < 0.0001).

Tajima's D was negative but non-significant for both the Atlantic (D = -1.1, P = 0.12) and Pacific (D = 1.3, P = 0.06), while Fu's F_s was negative and significant for both the Atlantic (F_s = -14.5, P = 0.002) and Pacific (F_s = -24.5, P < 0.0001) samples. Tajima's D and Fu's F_s were mostly negative but not statistically different from zero for any Atlantic colony (Table 1). In Pacific colonies, Tajima's D was negative and significant at the Barren Islands, and Fu's F_s was negative and significant at Cape Lisburne, Koniuji Island, and St. George Island (Table 1).

Test of population differentiation

AMOVA indicated significant population genetic structure within Black-legged Kittiwakes (global ϕ_{st} = 0.53; P < 0.00001). Significant genetic structuring was found among both Atlantic (ϕ_{st} = 0.23; P < 0.0001) and Pacific (ϕ_{st} = 0.06; P < 0.0001) colonies. Mantel's tests did not detect significant associations between log geographic distance and Slatkin's linearized ϕ_{st} within either the Atlantic (Mantel r = 0.25, P = 0.24) or Pacific (Mantel r = -0.14, P = 0.34) colonies.

Population history

DIYABC identified Scenario 3 (historical isolation of eastern and western Atlantic colonies followed by admixture; Fig. 2) as the most likely historical scenario in the Atlantic (Posterior Probability = 0.9; 95 % Confidence Interval = [0.78–1.0]). Scenario 2 (historical isolation of eastern and western Atlantic colonies without admixture) was the next most likely tested scenario (Posterior Probability = 0.2; 95 % Confidence Interval = [0.06–0.30]). Model checking indicated that observed summary statistics for Scenario 3 were not significantly different from statistics simulated from the DIYABC model (P < 0.01; Supplementary Table 3).

Phylogeographic structure

The gene tree for Atlantic and Pacific haplotypes (Fig. 3.) shows six strongly supported monophyletic clades. Within each clade, relationships were poorly resolved; no nodes had greater than 75 % posterior probability. Kittiwakes from the Atlantic comprised four haplotype groups, whereas Kittiwakes from the Pacific comprised two haplotype groups. The statistical parsimony network clearly separated haplotypes from the Atlantic and Pacific Ocean basins. However, the gene tree and haploytype network provide little evidence for strong phylogeographic clustering within ocean basins. Assuming divergence rates of 11.5 %/Ma and 5 %/Ma, respectively, between the Atlantic and Pacific Ocean basins, corrected pairwise sequence divergence (δ = 3.2 %; Fig. 3) between the two ocean basins suggests that their mtDNA lineages diverged 0.64 to 0.28 Ma years ago. Corrected pairwise sequence divergence indicated that major divergences occurred in both the Atlantic and Pacific around

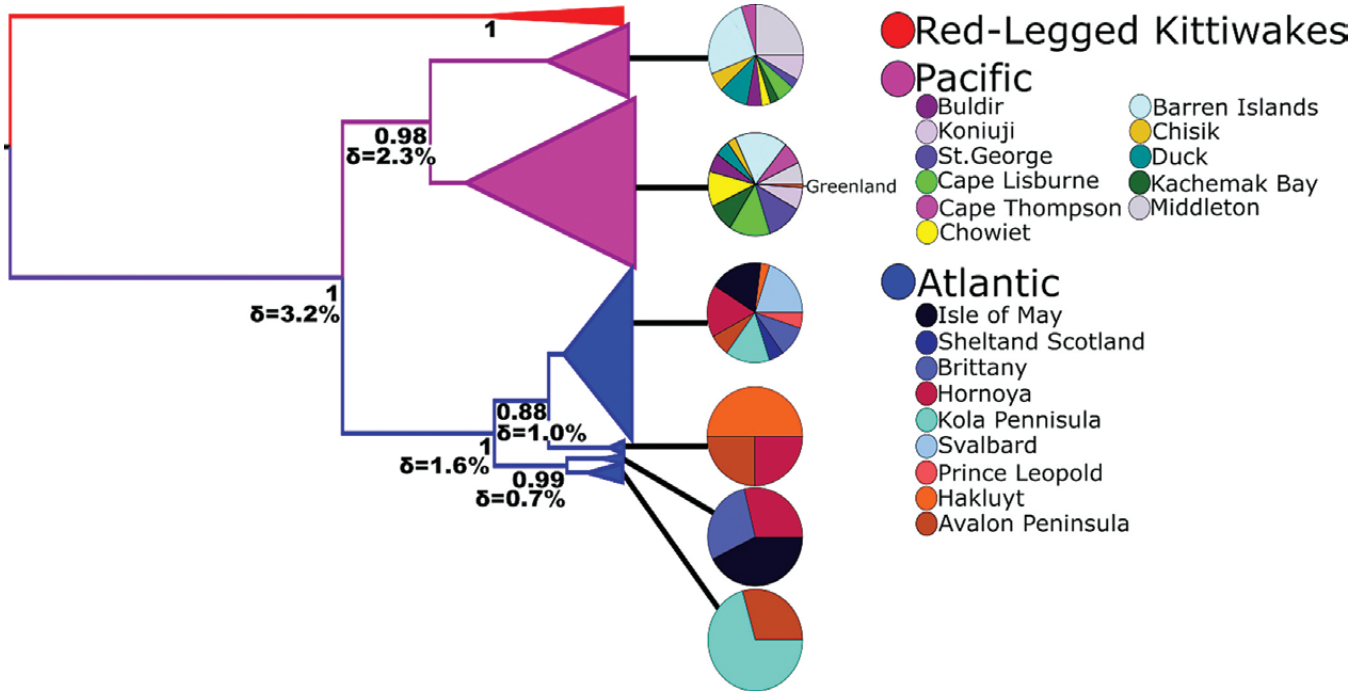

Fig. 3. Bayesian rooted gene tree of Black-legged Kittiwake mitochondrial control region sequences. The collapsed nodes represent the Red-legged Kittiwake outgroup (red), Atlantic (blue), and Pacific (purple) haplotypes. Posterior probabilities are displayed on nodes of the tree and divergence rates (δ) are displayed underneath posterior probabilities. Colony composition of each haplotype clade is displayed in pie graphs.

0.32 to 0.14 Ma (δ = 1.6 %) and 0.46 to 0.20 Ma (δ = 2.3 %), respectively (Fig. 3).

DISCUSSION

Differentiation between Atlantic and Pacific populations

Geographic variation in control region sequences, microsatellite markers (McCoy *et al*. 2005), and phylogenetic relationships among mtDNA haplotypes (Figs. 3, 4) provided evidence for genetic differentiation between Atlantic and Pacific kittiwake subspecies. Other than one common Pacific haplotype that was found in one Atlantic kittiwake, haplotypes of Atlantic and Pacific kittiwakes formed strongly supported reciprocally monophyletic groups separated by at least eight substitutions. An estimated divergence time of 0.64 to 0.28 Ma correlates with the isolation of Atlantic and Pacific oceans by the Bering Landbridge and Pleistocene glaciers (Kúrten & Anderson 1980). Subdivision by Pleistocene glaciers, the Bering Landbridge, or both, is thought to have contributed to population differentiation and speciation in several other arctic and north temperate seabirds (Friesen *et al*. 2007, Morris-Pocock *et al*. 2008; Friesen 2015). Estimates of the divergence time of Atlantic and Pacific mtDNA lineages of kittiwakes in our study are very similar to those found between Atlantic and Pacific Common Murres (Morris-Pocock *et al*. 2008). The similar divergence timing of the Common Murres and Black-legged Kittiwakes could indicate that similar glacial processes drove genetic divergence in these largely co-distributed seabird species.

Population structure of Atlantic kittiwake colonies

Results from AMOVA indicate that significant genetic differences exist among kittiwakes from different Atlantic colonies (Table 2). Furthermore, some Atlantic colonies were characterized by population-specific haplotypes that were found in a substantial proportion of individuals. Kittiwakes from Isle of May, Brittany, Shetland, and the Kola Peninsula appear to be genetically similar to each other, whereas kittiwakes from all other colonies are genetically different. The genetic similarity of European colonies and the Kola Peninsula is surprising given the distance between the Kola Peninsula colony and the sampled European colonies. Overlapping non-breeding distributions or foraging distributions are sometimes a predictor of genetic divergence (Friesen 2015). Geolocation data from Atlantic kittiwakes indicate that some Russian birds may have overlapping winter distributions with British colonies in the North and Labrador seas (Frederiksen *et al*. 2012). However, many Atlantic colonies in our study have overlapping non-breeding distributions, indicating that wintering distributions are not necessarily good predictors of mtDNA structure (Frederiksen *et al*. 2012).

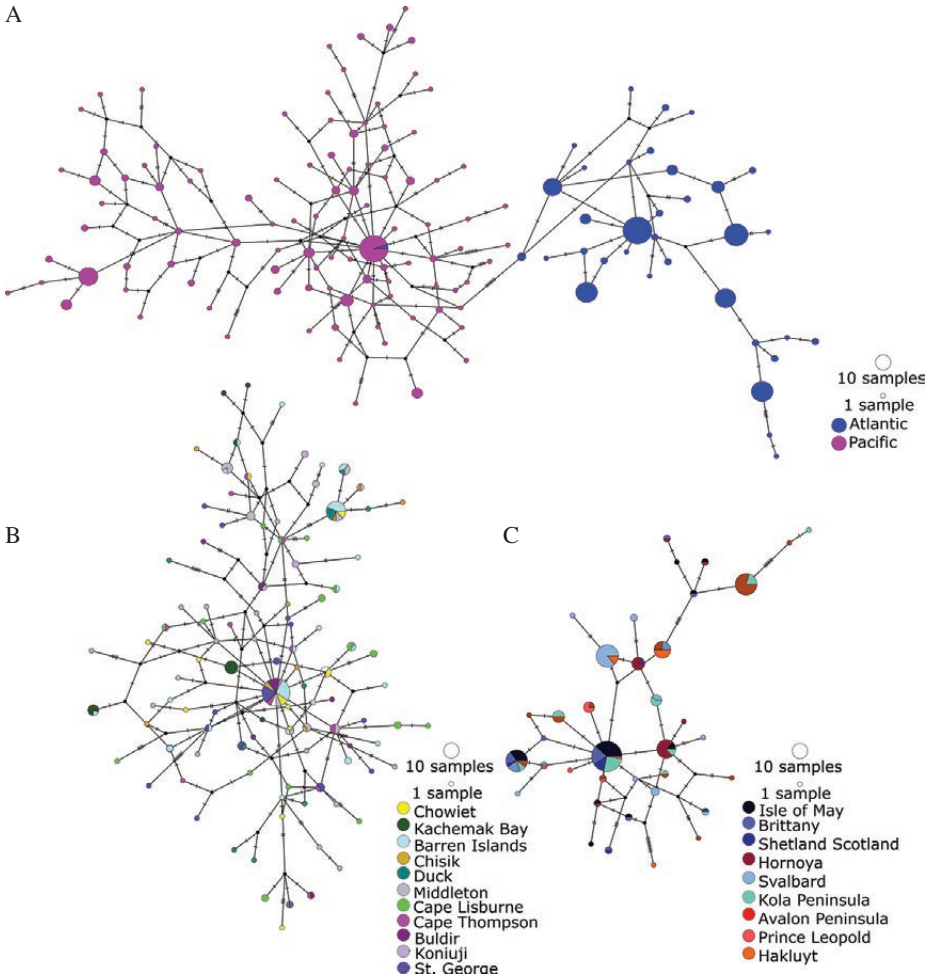

Fig. 4. TCS haplotype network of A) the global, B) Atlantic, and C) Pacific haplotypes. Colours represent different sampled colonies.

Population genetic structure could also be explained by philopatry (Coulson 2016), a behaviour that would reduce gene flow among colonies. Philopatry alone putatively led to population genetic structure in several seabird species, including Stewart Island shags *Leucocarbo chalconotus* (Rawlence *et al*. 2014), Brant Goose *Branta bernicula* (Shields 1990), Fairy Prions *Pachyptila turtur* (Ovenden *et al*. 1991), and Red-legged Kittiwakes (Patirana *et al*. 2002; reviewed in Friesen *et al*. 2007; Friesen 2015). Importantly, mtDNA is maternally inherited, but in Black-legged Kittiwakes females are less philopatric than males. Sex-biased dispersal of female kittiwakes may reduce the speed at which mtDNA markers sort among colonies.

Significant genetic structure may also arise through isolation by distance, wherein gene flow among populations decreases with increasing geographic distance, resulting in greater genetic difference with distance (Wright 1943). Mantel's test for a correlation between ϕ_{st} and distance among Atlantic colonies was not significant; thus, there is either an n-island (random) model of dispersal, or mitochondrial DNA is not yet in migration-drift equilibrium (Hutchison & Templeton 1999). Genetic differentiation metrics assume that the diversifying effects of genetic drift and the homogenizing effects of gene flow are at equilibrium; violation of this assumption means that differentiation metrics are not representative of contemporary processes. Evidence of recent population expansions provided by negative Fu's F_s statistics and DIYABC results suggest that populations in the Atlantic are not in migration-drift equilibrium. Tajima's D values agreed with Fu's F_s in direction but not significance. Simulations of both test statistics indicate that Fu's F_s is more sensitive to population expansion (Fu 1997). Therefore, the observed geographic pattern of genetic variation could be caused by a combination of contemporary and historical processes.

Population structure of Pacific kittiwake colonies

In contrast to Atlantic colonies, little evidence of population genetic structure was found within the Pacific Ocean. Haplotypes did not cluster by site on the gene tree (Fig. 3.), and few pairwise estimates of ϕ_{st} were significant (Table 3). The weak population structure in Pacific kittiwakes is similar to some other species in this region. For example, Shields & Wilson (1987) found little differentiation in mtDNA among populations of Canada Geese *Branta canadensis* in the Aleutian Islands, and Pacific Common Murres have little genetic

TABLE 2
Pairwise ϕ_{st} values between Atlantic kittiwake colonies[a]

	Kola Peninsula	Hornøya	Svalbard	Shetland	Isle of May	Brittany	Hakluyt	Prince Leopold
Hornøya	**0.10**							
Svalbard	**0.22**	**0.19**						
Shetland	0.02	**0.15**	**0.29**					
Isle of May	0.02	**0.15**	**0.32**	0.0				
Brittany	0.02	**0.14**	**0.29**	-0.03	-0.04			
Hakluyt	**0.16**	**0.18**	**0.18**	**0.25**	**0.26**	**0.21**		
Prince Leopold	**0.09**	**0.22**	**0.40**	**0.10**	**0.11**	**0.09**	**0.34**	
Avalon Peninsula	**0.18**	**0.33**	**0.41**	**0.32**	**0.31**	**0.27**	**0.26**	**0.35**

[a] Comparisons that were significant after a Benjamini-Yekutieli correction with an alpha of 0.05 are in bold.

TABLE 3
Pairwise ϕ_{st} values between Pacific kittiwake colonies[a]

	Buldir	Koniuji	St. George	Cape Lisb.	Cape Thomp.	Chowiet	Chisik	Barren Islands	Duck	Kachemak Bay
Koniuji	0.00									
St. George	0.01	0.03								
Cape Lisb.	-0.02	0.01	0.0							
Cape Thomp.	-0.02	-0.02	-0.02	-0.02						
Chowiet	-0.01	0.00	0.0	-0.01	-0.02					
Chisik	0.03	0.02	0.07	0.06	0.03	0.0				
Barren Islands	0.01	0.02	0.03	**0.04**	0.00	0.00	-0.03			
Duck	0.12	**0.12**	**0.18**	**0.14**	0.11	0.10	-0.01	0.07		
Kachemak Bay	0.06	0.09	0.03	0.05	0.06	0.04	0.09	0.06	**0.18**	
Middleton	0.11	0.07	**0.16**	**0.14**	0.09	**0.20**	-0.02	**0.07**	0.03	**0.20**

[a] Comparisons that were significant after a Benjamini-Yekutieli correction with an alpha of 0.05 are in bold.

structuring in the mitochondrial control region (Morris-Pocock *et al*. 2008). In contrast, other seabirds species in this region, such as Marbled Murrelets *Brachyramphus marmoratus* (Congdon *et al*. 2000), Pigeon Guillemots (Kidd & Friesen 1998, Poland *et al*. unpubl. data), and Kittlitz's Murrelets *Brachyramphus brevirostris* (Birt *et al*. 2011), show significant structuring in mtDNA (reviewed in Friesen *et al*. 2007, Friesen 2015).

Overlapping non-breeding distributions between Black-legged Kittiwakes in the Bering Sea and Gulf of Alaska might explain some of the lack of genetic structure found in the Pacific. Tracking data from two colonies in the Bering Sea (St. George & St. Paul) indicate a broad non-breeding distribution of Black-legged Kittiwakes in the Northern Pacific (Orben *et al*. 2015a, 2015b), which potentially overlaps with some individuals from colonies in the Gulf of Alaska (Shoup Bay & Passage Canal; McKnight *et al*. 2011). If the tracking results from these studies are generalizable to other colonies in the Bering Sea and Gulf of Alaska, there may be substantial overlap of non-breeding distributions.

Evolutionary histories of Atlantic versus Pacific kittiwakes

The low genetic structure in the Pacific compared to the Atlantic may be due to differences between ocean basins in refugial histories. We found evidence that clade divergences are more recent in the Atlantic compared to the Pacific, and that contemporary Atlantic populations are derived from the expansions of two refugial populations (Fig. 3). In contrast, our gene tree suggests that Pacific clades may be older and have, therefore, had more time for admixture to occur. Differentiation caused by this separation may have been erased by admixture. Alternatively, the genetic structure could be hidden from our current analysis because of the lack of samples derived from Western Pacific and Siberian populations.

Within both the Atlantic and Pacific, both the gene trees and haplotype networks were characterized by incomplete lineage sorting between colonies (Fig. 4). All lineage divergences within each ocean were estimated to have occurred during the Pleistocene, with the most ancient divergence in the Pacific occurring 0.46 to 0.20 Ma and the most ancient Atlantic divergence occurring 0.32 to 0.14 Ma years ago. During the late Pleistocene, five major and two minor glaciations occurred (Head *et al*. 2008). Much of the present breeding range of kittiwakes was covered by glaciers until about 10 000 years ago (Pielou 1991). Thus, glaciations would have forced kittiwakes into one or more southerly refugia. The most supported scenario from DIYABC involved historical isolation of eastern and western Atlantic populations, followed by admixture and divergence of the admixed population into the Svalbard and Greenland colonies as the eastern Atlantic populations diverged into the British and Hornøya colonies (Fig. 2; Scenario 3). This scenario corresponds to two glacial refugia in the Atlantic Ocean. Geological and paloeclimatic evidence suggests that the coasts of Newfoundland, the Gaspé Peninsula, and Grand Banks remained unglaciated in the late Pleistocene (Pielou 1991), providing one possible refugium. Like other Atlantic taxa (e.g., Common Murres; Morris-Pocock *et al*. 2008), Black-legged Kittiwakes may have had a second, southeastern refugium, possibly off Iberia (Hewitt 2000).

Comparison of nuclear versus mitochondrial variation

Nuclear markers suggest that little genetic structure exists among the Atlantic black-legged kittiwakes (McCoy *et al*. 2005), which contrasts with the strong geographic structure in mtDNA. Greater structure in mtDNA compared to nuclear DNA is common. Assuming a constant mutation rate, mtDNA variation is expected to sort four times faster than nuclear DNA (Birky *et al*. 1989). However, comparing microsatellite markers to mtDNA is difficult because mutation rates are not equivalent. High mutation rates in microsatellite loci could drive fast sorting, while homoplasy might result in decreased genetic structure. The high mutation rate of microsatellites results in a low magnitude of differentiation statistics (Birky *et al*. 1989, Hedrick 1999).

Geographic variation in morphology among Atlantic colonies

Because geographic variation in plumage and morphometrics was previously described for Atlantic kittiwakes (Sluys 1982, Chardine 2002), we expected to find geographically ordered mtDNA variation and restricted gene flow between colonies. Despite only two phenotypic groupings of Atlantic populations (one in Arctic Canada and West Greenland, and one in Newfoundland, United Kingdom, and Barents Sea; Chardine *et al*. 2002), we observed extensive genetic structure in mtDNA. Therefore, although geographic patterns of variation in morphology and mtDNA exhibit some similarities, cryptic genetic variation exists that does not match patterns of morphology among Atlantic kittiwake colonies.

Taxonomy and conservation

Despite earlier skepticism of subspecies designations due to considerable phenotypic overlap (Vaurie 1965, Sluys 1982), mtDNA data correlate with differences in morphology and microsatellite variation between kittiwakes from the two oceans (McCoy *et al.* 2005, Cramp & Simmons, 1983). Original subspecies designations of Atlantic and Pacific Black-legged Kittiwakes are therefore supported by mtDNA, nuclear DNA, and morphological data. For conservation and management, the Atlantic and Pacific subspecies should be considered evolutionarily significant units. Further, mtDNA results indicate that Atlantic colonies should be managed as separate management units. Genetic structuring of these colonies indicates that loss of one colony may result in an overall loss of genetic diversity.

CONCLUSIONS

Analyses of genetic variation in the mitochondrial control region in Black-legged Kittiwakes indicates that Atlantic and Pacific subspecies are highly differentiated and form monophyletic groups that are essentially reciprocal. Within the Atlantic, most colonies differ genetically. In contrast, Pacific colonies have weak genetic structure. This difference may be due to differences in the timing of major clade divergence between ocean basin populations. Genetic structure in the Atlantic could be partially attributed to historical fragmentation, and differentiation between subspecies may have arisen during a period of isolation during the mid to late Pleistocene. Future studies should assess the genome-wide genetic structure of colonies in the Pacific Ocean, and should increase sampling ranges to include more western Pacific and Arctic colonies to determine whether mtDNA and nuclear DNA are concordant. Future studies should also include more detailed analyses in the Pacific Ocean.

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