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No evidence of divergence at neutral genetic markers between the two morphologically different subspecies of the most numerous Arctic seabird

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Identifying natural populations that might be considered separate units using morphology, genotype or both is important in understanding the process of speciation and for conservation. We examined the relationships between the only two subspecies of the most numerous Arctic seabird, the Little Auk *Alle alle*, using both morphological (wing and head-bill lengths) and genetic data (482 base pairs of the mitochondrial control region and seven nuclear microsatellite loci). We found significant morphological differences between the subspecies, *A. a. polaris* being significantly larger than the nominate *A. a. alle*. However, we did not find the subspecies to be differentiated at either mitochondrial DNA or at microsatellite loci. Consequently, one evolutionary significant unit is proposed. The similarity of the two subspecies at neutral genetic markers may be due to contemporary gene flow between populations, as well as large population sizes both in the present and in the past, combined with recent post-glacial colonization of the Artic.

Keywords: *Alle alle*, Dovekie, gene flow, genetic population differentiation, Little Auk, population structure.

Many avian species are divided into subspecies, the merits of which remain a contentious topic of discussion (Zink 2004, Philimore & Owens 2006, James 2010). Traditionally, subspecies are regarded as breeding populations that occupy different segments of the geographical range of the species and that are measurably different in morphology, ecology and/or behaviour (Mayr & Ashlock 1991). However, several studies have shown that subspecies delineated by morphology are often very similar at neutral genetic markers, probably as a consequence of recurrent gene flow (e.g. Moen 1991, Ball & Avise 1992, Greenberg *et al.* 1998, Chan & Arcese 2003, Whitter *et al.* 2006). Only 36% of avian subspecies represent distinct phylogenetic lineages (Philimore & Owens 2006). In contrast, several studies of morphologically similar populations have found them to be genetically divergent (e.g. Irwin *et al.* 2001, Lohman *et al.* 2010). Identifying natural populations that differ morphologically, genetically or both is important, as population differentiation is often the first step towards speciation (Coyne & Orr 2004). Moreover, recognizing populations that differ morphologically and/or genetically, and that function independently is important for designing appropriate management strategies to preserve biodiversity (Bickford *et al.* 2006, Gill 2014).

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We examine the morphological and genetic relationships between the two described subspecies of a small, colonially breeding seabird, the Little Auk (Dovekie) *Alle alle*. The species is considered to be the most numerous alcid in the Atlantic Ocean and is probably one of the most numerous seabirds in the world (*c.* 37 million pairs, as reviewed in Isaksen & Gavrilo 2000, Stempniewicz 2001, Montevecchi & Stenhouse 2002, and reported in Egevang *et al.* 2003). The largest breeding colonies are located in Greenland and on Spitsbergen; much smaller colonies are situated on the southern (Jan Mayen and Bjørnøya) and eastern (Franz Josef Land, Novaya Zemlya and Severnaya Zemlya) borders of the breeding range (Stempniewicz 2001; Fig. 1). Small numbers of Little Auks have been reported to breed in the Pacific (Day *et al.* 1988, Montevecchi & Stenhouse 2002). The subspecies differ in body size, with the nominate *A. a. alle* being considerably smaller than *A. a. polaris* (Stenhouse 1930, Stempniewicz *et al.* 1996, Wojczulanis-Jakubas *et al.* 2011). The latter inhabits Franz Josef Land and possibly Severnya Zemlya (Golovkin 1990), whereas the nominate subspecies occurs across the remainder of the breeding range (Fig. 1).

Bédard (1985) suggested that morphological divergence between the two subspecies resulted from separation of an ancestral population into two glacial refugia during the Pleistocene. The large body size of birds classified as *A. a. polaris* might be an adaptation to the severity of the environment (the lowest air temperatures among the Little Auk breeding sites; Wojczulanis-Jakubas *et al.* 2011). However, the genetic relationship between the subspecies has not been investigated thoroughly. Wojczulanis-Jakubas *et al.* (2014) found no differences in mitochondrial DNA (mtDNA) between them, but tested only three individuals of *A. a. polaris*. In the present study,

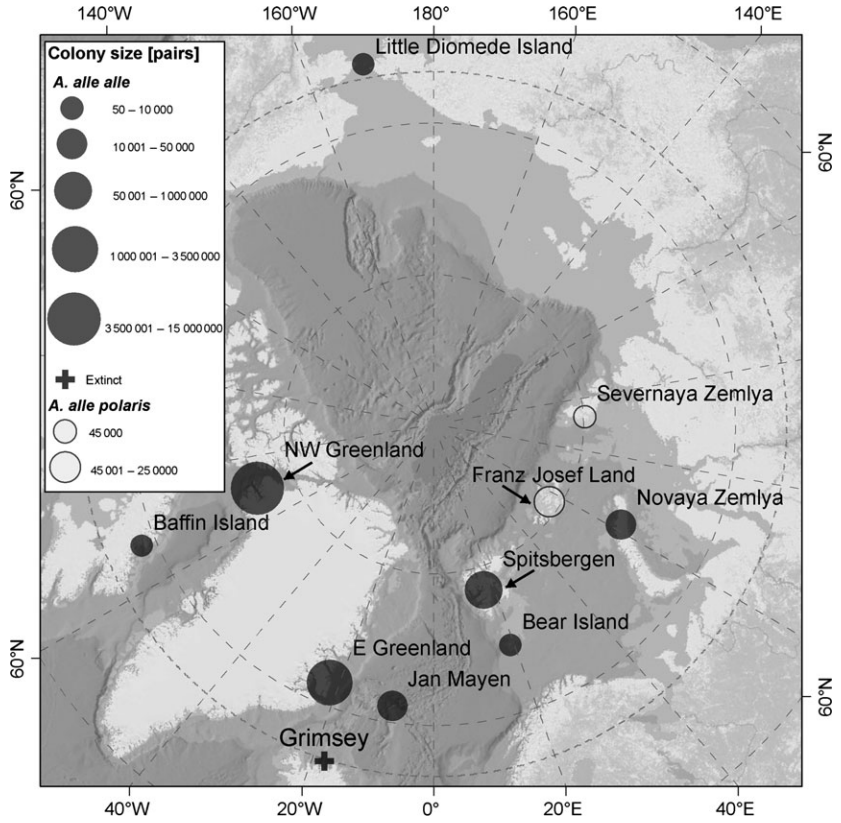

Figure 1. Distribution and relative size of Little Auk breeding populations, indicated by grey circles for nominate subspecies *Alle alle alle*, and white circles for *Alle alle polaris* subspecies: the populations sampled in the present study are indicated by arrows. The map was produced in ArcGIS 9.3 based on population size estimates in Stempniewicz (2001) and Montevecchi and Stenhouse (2002), and the map of the Norwegian Polar Institute (www.npolar.no).

we used two types of neutral molecular markers (mtDNA and microsatellite loci) to investigate genetic variation within a larger sample of individuals representing the two Little Auk subspecies. mtDNA is useful for examining genetic relationships both at and below the species level because of its high mutation rate and maternal inheritance (Hewitt 2001). Although this rate varies among regions, the control region, which is examined here, does not code for any proteins but is associated with replication and often has a high mutation rate (Avise 2000). Similarly, microsatellites constitute a viable tool to differentiate closely related populations, because tandemly repeated short sequence motifs of 2–6 base pairs (bp) have a high rate of slippage during DNA replication, resulting in high mutation rates (Hedrick 1999, Hewitt 2001). Given the hypothesized long-term isolation (Bédard 1985), we expected genetic divergence between the two subspecies in the examined loci.

METHODS

We collected fresh whole blood or contour body feathers from 75 Little Auks of the two subspecies breeding in northwest Greenland (Paakitsoq Fjord, Thule District, 76°16′N, 68°57′W; *A. a. alle*, $n = 28$), southwest Spitsbergen (Hornsund, 77°00′N, 15°33′E; *A. a. alle*, $n = 31$), and Franz Josef Land (Tikhaya Bay, Hooker Island, 80°18′N, 52°49′E; *A. a. polaris*, $n = 16$). We obtained samples from birds captured in the colonies during the breeding seasons of 2010–2012. We preserved whole blood in 96% ethanol and kept feathers frozen in separate plastic bags until DNA was extracted. All sampled birds were adults (age >3 years, distinguished from subadults by the appearance of wing coverts and flight feathers; Stempniewicz 2001), and presumed not to be related to each other. We measured wing-length (maximum flattened cord) with a stopped wing-ruler (1-mm precision) and head-bill length (from the most distant point of the occiput to the tip of the upper mandible) of each captured individual with dial callipers (0.1-mm precision). These measurements are taken routinely in many bird species and are considered to be representative measurements of Little Auk structural size (Jakubas & Wojczulanis 2007). Birds were measured by different researchers at each site and we could not examine potential interobserver measuring error; however, all researchers were experienced in measuring Little Auks, and the measurements have clear landmarks and should be the least prone to interobserver variability (Goodenough *et al.* 2010). We did not obtain all measurements for a few individuals, and therefore sample sizes differ for biometric and genetic data.

We extracted DNA using commercially available kits and protocols designed for blood and forensic material (Blood Mini and Sherlock AX, respectively; A&A Biotechnology, Gdynia, Poland). For the control region of mtDNA, we designed primers (AlleMCR-H50: 5′-ATGTGGGTGTGtACATTAAAC and AlleMCR-L750: 5′-TTATGCCCAACAAGCATTCAC) based on previously published Little Auk control region sequences and primers (Wojczulanis-Jakubas *et al.* 2014). We added a universal M13 tail (M13F: 5′-GTAAAACGACGGCCAGT) to the heavy strand primer for automated sequencing. We performed polymerase chain reactions (PCRs) in 15-μL final volume with 1× Multiplex Mix (Qiagen, Mississauga, ON, Canada), 0.4 mM of each of the heavy and light strand primers, and approximately 20 ng of template in a TGradient thermocycler (Biometra, Montreal, PQ, Canada), with 50 °C for the annealing temperature and standard temperature cycling. We sequenced the heavy strand of PCR products using BigDye terminator chemistry on an ABI 3730XL analyser (Applied Biosystems, Foster City, CA, USA) at the Genome Quebec Innovation Centre (McGill University, Montreal, QC, Canada). We checked chromatograms and confirmed variable sites manually in FinchTV 1.4.0 (Geospiza Inc., Seattle, WA, USA; http://www.geospiza.com). All sequences obtained have been submitted to GenBank (Accession numbers KM520047–KM520121).

For microsatellite genotyping, we tested 22 loci (with tetranucleotide motifs: BmaAAAC336, BmaACCT555, BmaAGGT503, BmaATTT351, BmaCCAT301, BmaCCAT443, BmaGACA340, BmaGACA456, BmaGATA365, BmaGATA439, BmaGATA464, BmaGATA465, BmaGGAT313, BmaGGAT368, BmaTATC353, BmaTATC356, BmaTATC371, BmaTATC444, BmaTATC453, BmaTGAA523 and BmaCA382; and dinucleotide motif: BmaCA561), all originally developed for the Marbled Murrelet *Brachyramphus marmoratus* (Rew *et al.* 2006). We tested all loci with 5–10 samples from each population. However, only seven loci (Table 1) PCR-amplified consistently and were polymorphic, so we proceed with these loci for all

Table 1. Characteristics of seven microsatellite loci analysed in 75 Little Auks from the two subspecies, the nominative *Alle alle alle* (two populations: northwest Greenland, $n = 28$; and southwest Spitsbergen, $n = 31$) and *Alle alle polaris* (Franz Josef Land, $n = 16$).

Locus	Allele size range (all populations)	All populations	Northwest Greenland					Southwest Spitsbergen					Franz Josef Land				
		N_A	N_A	R	H_O	H_E	*HWE*	N_A	R	H_O	H_E	*HWE*	N_A	R	H_O	H_E	*HWE*
BmaACCT555	112–192	12	10	6.2	0.57	0.60	<0.01	8	5.4	0.50	0.55	ns	3	2.8	0.19	0.28	ns
BmaCCAT301	140–184	12	11	8.9	0.80	0.87	ns	11	8.7	0.72	0.87	ns	9	8.2	0.69	0.84	<0.05*
BmaCCAT443	188–276	19	14	10	0.96	0.89	ns	13	9.6	0.90	0.89	ns	12	9.9	0.75	0.88	<0.05
BmaGATA365	220–320	20	15	10	0.79	0.88	ns*	15	9.8	0.97	0.88	ns	15	12	1.00	0.91	ns
BmaGATA439	216–376	17	14	10	0.77	0.88	ns	12	9.0	0.87	0.87	ns	11	9.2	0.93	0.86	ns
BmaGGAT313	204–348	22	15	11	0.65	0.91	<0.05*	17	12	0.81	0.92	<0.001	12	12.0	0.80	0.91	ns
BmaGGAT368	184–292	22	18	12	0.74	0.91	ns	17	11	0.74	0.91	ns	14	11	0.40	0.89	ns*
All loci (mean)			14	9.8	0.75	0.85	ns	13	9.3	0.79	0.84	ns	11	9.4	0.68	0.80	ns

N_A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; *HWE*, *P*-value of test for Hardy–Weinberg equilibrium; R, allelic richness; ns, not significant. Asterisks at the HWE values indicate that null alleles were suggested for a given locus/population.

samples. We added a universal M13 forward sequence to the 5′ end of the forward primers for subsequent genotyping. We amplified each locus in a separate PCR in 5 μL total volume, with a ready-to-use mix of polymerase, dNTPs and buffer (1× Qiagen Multiplex Mix), 0.15 mM of primers, including the forward M13 primer labelled with D4 dye (Sigma-Aldrich, Oakville, ON, Canada), and approximately 20 ng of DNA template. We conducted PCRs at 59 °C for annealing, except for locus BmaACCT555, for which the annealing temperature was 50 °C. We genotyped PCR products using a Beckman Coulter CEQ 8000 capillary automated sequencer at Queen's University Department of Biology Core Genotyping Facility (Kingston, ON, Canada). We scored alleles visually using the Beckman Coulter CEQ 8000 genetic analysis system (version 9.0).

Statistical analyses

Biometrics

We analysed differences in bird body size among the populations separately for wing and head-bill length (dependent variables) using non-parametric ANOVA (Kruskal–Wallis test) and a *post hoc* Dunn test, using STATISTICA 10.0 (StatSoft Inc., 2010) with alpha = 0.05.

mtDNA

We checked the quality of sequences and trimmed them to the same length in BIOEDIT (Hall 1999). To verify the identity of the PCR-amplified region, we performed BLAST searches (Altschul *et al.* 1990) against the GenBank database. All sequences could be unambiguously aligned without inserting gaps. We performed alignment using MEGA v6 (Tamura *et al.* 2013) and calculated mtDNA polymorphism, quantified as the number of haplotypes (n), haplotype diversity (h) and nucleotide diversity (π) in DNASP v5.10.01 (Librado & Rozas 2009).

For population-level analyses, we defined each sampling location (Greenland, Spitsbergen, Franz Josef Land) as a population. We also grouped the samples according to subspecies. We performed analysis of molecular variance (global AMOVA) with ARLEQUIN v.3.5. (Excoffier & Lischer 2010) using 10 000 permutations to test the null hypothesis of no population structure. We calculated pairwise differentiation between populations or subspecies using Φ_{ST} (with the Tamura–Nei substitution model), a direct analogue of Wright's F_{ST} for

nucleotide sequence divergence. For pairwise comparisons, we tested statistical significance by randomization using 15 000 permutations.

To investigate for phylogeographical structure, we reconstructed relationships among control region haplotypes using the median-joining algorithm in NETWORK v4.5.1.6 (http://www.fluxus-technology.com). The method groups related haplotypes into a tree or network through median vectors. After visual inspection of networks derived from several homoplasy-level parameters (ε), we set ε at 20 for better network solution (default $\varepsilon = 0$). We applied a weight of one for transitions and two for transversions. We resolved ambiguous relationships with a maximum parsimony (MP) heuristic algorithm.

Microsatellites

We tested the microsatellite loci for null alleles and scoring errors using MICROCHECKER v2.2.3 (van Oosterhout *et al.* 2004). We described the genetic diversity of each population by the mean number of alleles per locus, allelic richness standardized to the smallest population sample size (R), and observed and expected heterozygosity (Nei & Roychoudhury 1974) using FSTAT v2.9.3.2 (Goudet 2002). We tested data for deviations from Hardy–Weinberg equilibrium (HWE) using ARLEQUIN, with exact P-values being estimated using the Markov chain Monte Carlo (MCMC) procedure with 100 000 dememorization steps. We also used ARLEQUIN to determine the extent of deviation from independent segregation of loci. Some evidence of deviation from HWE and linkage disequilibrium, and the presence of null alleles was recovered (see Results) but the patterns were not consistent over loci and populations; therefore, all loci were used in further analyses.

We estimated the number and frequency of private alleles (PA) using GENALEX v6 (Peakall & Smouse 2006). To assess private allele richness (PAR), we made use of the software HP-RARE (Kalinowski 2005). We estimated genetic differentiation among populations with the F-statistics of Weir and Cockerham (1984) using pairwise comparisons in FSTAT v2.9.3.2 (Goudet 2002).

To assess the power of our microsatellite data to reject the null hypothesis of genetic homogeneity (Ryman *et al.* 2006), we carried out simulations using POWSIM 4.1 (Ryman & Palm 2006). We tested for true differentiation quantified as $F_{ST} = 0.01$ and 0.005. We retained the default parameters for the number of dememorizations, batches and iterations per batch (1000, 100 and 1000, respectively), modelling over 100 and 200 generations (t) with an effective population size (N_e) of 10 000.

To infer population structure, we performed Bayesian clustering analyses in STRUCTURE 2.3.4 (Pritchard *et al.* 2000) using both the admixed and not admixed models for ancestry. We used sampling locations as prior information to assist clustering. We set the burnin length to 100 000 followed by 1 million iterations of the MCMC estimation procedure. We ran the analyses for values of K from 1 to 3 (number of sampled populations). We used the relative value of the mean log-likelihood of K to interpret the value of K (Pritchard & Wen 2004).

To estimate recurrent gene flow (i.e. past one to three generations) between subspecies we applied BAYESASS v1.3 (Wilson & Rannala 2003) to the microsatellite data. The program employs a Bayesian maximum likelihood analysis to conduct molecular assignments. We ran it with 3 million MCMC iterations with a burnin of 1 million iterations and a sampling frequency of 2000. We set delta to 0.15 (the default value). For the purpose of this analysis we combined samples within each subspecies. The program inferred that the estimated residency (non-migration) rates were high enough for reliable estimation of gene flow (Wilson & Rannala 2003).

RESULTS

Biometrics

We found significant differences in wing-length between birds from Greenland compared with the two other populations (Kruskal–Wallis test, $H_{2,68} = 38.77$, $P < 0.001$, *post hoc* Dunn tests, all $P < 0.001$, Fig. 2). However, all populations differed in head-bill length, with the smallest birds from Greenland, and the largest from Franz Josef Land, with the birds from Spitsbergen being intermediate in size ($H_{2,68} = 40.97$, $P < 0.001$, *post hoc* Dunn tests, all $P < 0.05$; Fig. 2).

mtDNA

mtDNA sequences covered Domain I and Domain II of the avian control region. BLAST searches revealed sequences identical to avian conserved

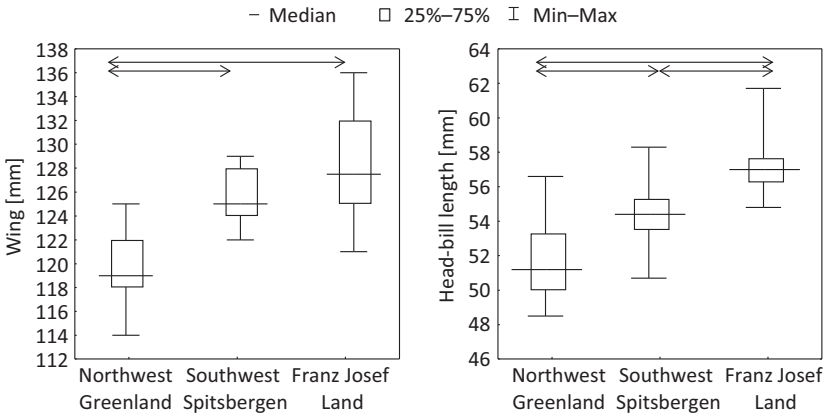

Figure 2. Morphological traits of the two Little Auk subspecies: nominate *Alle alle alle* (two populations: northwest Greenland, $n = 26$; and southwest Spitsbergen, $n = 27$) and *Alle alle polaris* (Franz Josef Land, $n = 16$). Arrows indicate significant differences (Dunn tests, all $P < 0.05$).

blocks (C, D and F Boxes) of other alcids in the expected locations in Domain II (Baker & Marshall 1997, Morris-Pocock *et al.* 2008). The observed variability agreed with the expected pattern of rapid sequence evolution in Domain I (36 variable sites) and with a reduced number of variable sites in the conserved sequence blocks. As expected for true mitochondrial sequences, base pair composition of the light strand was biased against guanine (Baker & Marshall 1997). The nucleotide frequencies were: 23.9% (A), 28.3% (T/U), 30.9% (C) and 16.8% (G). The transition/transversion rate ratios were $k_1 = 68.2$ (purines) and $k_2 = 49.2$ (pyrimidines). The overall transition/transversion bias was $R_t = 29.2$, where $R_t = [A*G*k_1 + T*C*k_2]/[(A + G)*(T + C)]$.

Sequences of all 75 Little Auks included 482 sites, 36 of which were variable and 20 were parsimony informative. Forty-nine haplotypes were recovered ($h = 0.98$, $\pi = 0.008$). All haplotypes occurred at low frequency (one to three individuals) and all were unique to a single population, with the exception of seven haplotypes that were shared between two (Hap1, Hap2, Hap4, Hap22, Hap26) or three populations (Hap3, Hap7; Supporting Information Table S1). Nucleotide diversity within populations was $\pi = 0.005$ for Greenland and Spitsbergen, and $\pi = 0.007$ for Franz Josef Land.

Global AMOVA indicated very low population differentiation ($\Phi_{ST} = -0.018$, $P = 0.87$). Pairwise estimates of population differentiation for mtDNA were low and not significantly different from zero ($\Phi_{ST} = -0.015$ for Franz Josef Land vs. Greenland, $\Phi_{ST} = -0.021$ for Franz Josef Land vs. Spitsbergen, and $\Phi_{ST} = -0.007$ for Spitsbergen vs. Greenland; all $P > 0.05$). Network reconstruction (Fig. 3) revealed a lack of phylogeographical structure. Only in three cases were haplotypes separated by more than one mutational step.

Microsatellites

From 12 to 22 alleles per locus were recovered (Table 1, Supporting Information Table S2). Only two significant deviations from linkage equilibrium were detected (BmaCCAT301 vs. BmaGGAT368, and BmaGATA439 vs. BmaGGAT368; $P < 0.05$), and these were recovered only in the Spitsbergen population. Significant departures from HWE were recovered for three loci (Table 1). MICROCHECKER suggested the presence of null alleles at four loci but the result was not consistent across populations and was not related to the pattern of deviation from HWE (Table 1). Allelic richness was similar across the three populations (Table 1). Private allelic richness was low for all populations (2.07 for Greenland, 1.53 for Spitsbergen and 1.91 for Franz Josef Land). Observed heterozygosity was generally high across all loci and populations, with the exception of two loci (BmaACCT555 and BmaGGAT368) from the Franz Josef Land population (Table 1): no population displayed a significant mean heterozygosity excess ($P > 0.05$, Table 1).

Pairwise estimates of population differentiation for microsatellites were low and not significantly different from zero (0.017 for Franz Josef Land vs.

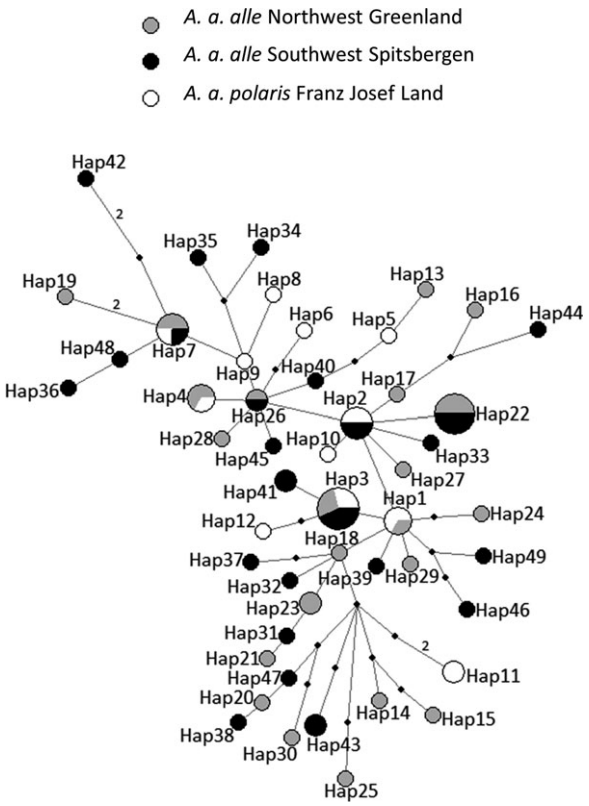

Figure 3. Median-joining network of mtDNA control region haplotypes of the two Little Auk subspecies: nominate *Alle alle alle* (two populations: northwest Greenland, $n = 26$; and southwest Spitsbergen, $n = 27$) and *Alle alle polaris* (Franz Josef Land, $n = 16$). Higher weights were applied to transversions. Circle size is approximately proportional to the number of individuals exhibiting the corresponding haplotype. Connector length is proportional to the number of mutations between haplotypes. Black dots indicate potential intermediate haplotypes that were not sampled.

Greenland, 0.015 for Franz Josef Land vs. Spitsbergen, and –0.004 for Spitsbergen vs. Greenland). POWSIM simulations indicated that our dataset had sufficient power to detect global population differentiation for both $F_{ST} = 0.01$ (100%) and 0.001 (75%). Thus, the lack of genetic differentiation among Little Auk populations is unlikely to be due to the number of polymorphisms recovered or the number of microsatellite loci used in our study.

Bayesian clustering analysis of microsatellite data in STRUCTURE did not reveal any structure: one cluster ($K = 1$) had the highest posterior probability ($P \approx 1$), irrespective of the model used. Analysis of microsatellite data in BAYESASS indicated that 83% of individuals in the *A. a. alle* sample and 82% in the *A. a. polaris* sample derived from their own population, with 18% (90% confidence interval: 15–33%) of individuals representing potential migrants from *A. a. alle* into *A. a. polaris*, and 17% (8–30%) from *A. a. polaris* into *A. a. alle*.

DISCUSSION

We determined morphological differences among the three Little Auk populations, including both subspecies. The results suggest a clinal, eastward increase in body size, with the smallest birds breeding on Greenland, medium-sized birds on Spitsbergen, and the largest individuals breeding on Franz Josef Land attributed to *A. a. polaris*. Although three different researchers measured the birds, we believe the results are robust as both measurements have obvious landmarks, and are the least prone to interobserver variability (Goodenough *et al.* 2010). Additionally, the present results are consistent with a previous study in which the biometric data were collected by a single researcher and that indicated that *A. a. polaris* is significantly larger than *A. a. alle* (Stempniewicz *et al.* 1996).

The larger body size of birds from *A. a. polaris* is consistent with the heat conservation hypothesis. Heat conservation increases via a higher surface area to volume ratio, so larger individuals should prevail in colder areas: Bergman's rule at the interspecific level (Bergmann 1847) and Rensch's/James's rule for intraspecific patterns (Rensch 1938, James 1970, Blackburn *et al.* 1999). Indeed, of all Little Auk populations *A. a. polaris* breeds in the lowest air temperatures (Wojczulanis-Jakubas *et al.* 2011) and probably also winters in similar harsh environmental conditions. A preliminary study of non-breeding movements of the birds from Franz Josef Land shows that they overwinter at higher latitudes than other populations (J. Fort and M. Gavrilo unpubl. data). The smaller size of birds from Greenland is more difficult to explain, as environmental conditions experienced by the birds during the breeding season appear to be similar to those for Spitsbergen populations (Wojczulanis-Jakubas *et al.* 2011). However, the northwest Greenland population in the more southerly wintering grounds may experience less severe conditions compared with the southwestern Spitsbergen population (Fort *et al.* 2013).

Bédard (1985) hypothesized that morphological divergence between the two Little Auk subspecies

resulted from separation of an ancestral population into two glacial refugia during the Pleistocene. If so, we could also propose a similar explanation for the body size variation within the nominate subspecies. However, differentiation at neutral molecular markers was not present among all three populations. Estimates of Φ_{ST} for mtDNA variation did not differ from zero and no phylogeographical structure was recovered. Similarly, the microsatellite data did not support differentiation among populations. Consequently, there is no evidence for more than one genetic cluster among the three populations sampled. This is consistent with a previous investigation of population genetics of the nominate subspecies (Wojczulanis-Jakubas *et al.* 2014).

The virtual absence of geographical variation in neutral markers is probably due at least in part to recurrent gene flow among the populations. Some genetic models predict that even one migrant per generation is sufficient to prevent divergence of populations (Wang 2004). Results from BAYESASS suggest that 18% of individuals from *A. a. alle* migrated into *A. a. polaris* and 17% in the reverse direction. This exchange of individuals is likely to result from natal dispersal (movement between the natal and recruitment site), as adults appear to be faithful to their breeding locations (Wojczulanis-Jakubas *et al.* 2014). There is no information on philopatry or the extent and direction of natal dispersal in the Little Auk, but in other alcids some young may breed away from their natal colony (e.g. Halley & Harris 1993, Harris *et al.* 1996, Olsson *et al.* 1999, Harris & Swann 2002).

Homogeneity at neutral genetic markers could also be due, at least in part, to historical association. The populations occur in areas that were glaciated during the Pleistocene and have probably only been accessible during the past 10 000 years (Jakobsson *et al.* 2014). Even if the populations are completely isolated from each other at present, more time would be needed to generate genetic differences in neutral molecular markers given the large global population size of Little Auks. Slatkin and Maddison (1989) argue that in the absence of gene flow, migration/drift equilibrium is not met until T/N_e approaches 1, where T is the age of the population in generations and N_e is the long-term effective population size. The current census of the Little Auk global population is > 30 million breeding pairs (see Introduction for references), although effective population size is typically 1–2 orders of magnitude smaller than census size (Frankham 1995). Assuming a generation time of at least 5 years (based on existing literature on other auk species of similar body size, De Santo & Nelson 1995, Gaston & Jones 1998, and 13 years of unpublished field observations of the Little Auk) and no gene flow, there has probably not been sufficient time for Little Auks to attain an equilibrium between genetic drift and migration, and to display genetic differences between subspecies.

The apparent lack of genetic structure in the Little Auk is perhaps not surprising. High-latitude seabirds typically have reduced levels of population structure relative to variation among populations sampled from lower latitudes (Adams & Hadley 2012, Friesen 2015). Nevertheless, there can be surprising variation among and within species. For instance, genetic homogeneity in mtDNA of the North Pacific population of Common Guillemot *Uria aalge* contrasts with genetic structuring in the North Atlantic population (Morris-Pocock *et al.* 2008). Similarly, Black-legged Kittiwakes *Rissa tridactyla* are genetically structured in the Atlantic, but not within the Pacific basin (Patirana 2000, McCoy *et al.* 2005). These patterns have been attributed to bottlenecks and/or vicariance experienced by Arctic birds during the Pleistocene glaciations (Hewitt 2000). Relatively recent origin of Arctic habitats in combination with large population sizes may mean that many Arctic species are not in equilibrium with respect to mutation, migration and genetic drift (e.g. Friesen *et al.* 1996, Whitlock & McCauley 1999). As a consequence, little or no genetic structure is observed in many species inhabiting Arctic ecosystems. It has also been suggested that high-latitude species have slower rates of neutral evolution, which additionally restrains genetic structuring of these species (Adams & Hadley 2012).

Lack of correspondence between structure in neutral genetic markers and morphological characters has been reported for many avian species. Such results have been attributed to rapid evolution in morphological characters, creating differences between the subspecies not yet reflected in neutrally evolving DNA (Moen 1991, Zink & Dittmann 1993, Greenberg *et al.* 1998, Questiau *et al.* 1998, Moum & Árnason 2001, Ödeen & Björklund 2003, Johnsen *et al.* 2006, Teplitsky *et al.* 2008). Such rapid morphological evolution may well be occurring in the Little Auk, where larger

body size may offer an adaptive advantage in harsher environmental conditions.

Our results suggest that there is clinal variation in size in this species (see also Wojczulanis-Jakubas *et al.* 2011). According to Moritz's (1994) criteria, none of the three populations, including both subspecies, constitutes an evolutionary significant unit. They are genetically very similar, with no evidence of reciprocal monophyly for mtDNA haplotypes and very weak differentiation in frequencies of nuclear alleles. Different patterns could exist in functional genes. However, mtDNA and microsatellite markers are sufficiently sensitive to detect historical (Pleistocene) vicariance in other high-latitude seabirds (e.g. Morris-Pocock *et al.* 2008). The results of our study do not provide genetic evidence for the two Little Auk subspecies.

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SUPPORTING INFORMATION

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

Table S1. Frequencies of all mtDNA haplotypes in 75 Little Auks from the two subspecies, *A. a. alle* (northwest Greenland, $n = 28$; southwest Spitsbergen, $n = 31$), and *A. a. polaris* (Franz Josef Land, $n = 16$).

Table S2. Microsatellite allele scores for the five microsatellite loci (Loc555(BmaACCT555), Loc301(BmaCCAT301), Loc443(BmaCCAT443), Loc365(BmaGATA365), Loc439(BmaGATA439), Loc313(BmaGGAT313), Loc368(BmaGGAT368)) analysed in 75 Little Auks from the two subspecies, the nominative *A. a. alle* (two colonies: northwest Greenland, NWG; and southwest Spitsbergen, SVA) and *A. a. polaris* (Franz Josef Land, FJL).