Short Communication

Sympatry of genetically distinct Atlantic Puffins (*Fratercula arctica*) in the High Arctic

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Across its range, the Atlantic Puffin *Fratercula arctica* is divided into four separate genetic clusters that correspond with geography and/or size differences. However, in the Western Atlantic High Arctic, there is a Puffin colony (Thule) that comprises two discrete size phenotypes. Using whole genome sequencing data of six Thule individuals from these two phenotypes, we found that Thule consists of three distinct genetic clusters, with no signs of recent interbreeding. Our results suggest the beginnings of a potential northward shift of boreal Atlantic Puffins in the West Atlantic, consistent with responses to a warming High Arctic climate.

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The Arctic is undergoing an accelerated pace of warming and dramatic increases in human disturbance (Huntington *et al.* 2007, Serreze & Barry 2011). Ongoing northward range shifts of boreal species are increasing the likelihood of hybridization or lineage replacement of endemic Arctic populations (Kelly *et al.* 2010, Garcia-Elfring *et al.* 2017, Gallant *et al.* 2020). Although the logistical challenges intrinsic to the Arctic limit sample access, it is essential to expand genomic studies into the Arctic to help understand ongoing biotic change and taxonomic baselines, and to conserve Arctic biodiversity (Colella *et al.* 2020).

The Atlantic Puffin *Fratercula arctica* (hereafter 'Puffin', see Fig. S1) is an iconic seabird and of conservation concern (globally vulnerable, BirdLife International 2017). It is distributed across the North Atlantic from Spitsbergen and northern Greenland, to France and Maine (Harris & Wanless 2011; Fig. 1a). Whole genome resequencing has identified four separate Puffin genetic clusters that are partially consistent with subspecies delineations and latitudinal variation in body size (Harris & Wanless 2011). The smallest Puffins (*F. a. grabae*) form a single genetic cluster found in the UK and France. Mid-sized Puffins (*F. a. arctica*) are represented by two boreal genetic clusters along the North American Atlantic coast and in Iceland/Norway/Faroes, respectively. The largest Puffins (*F. a. naumanni*) form the most distinct genetic cluster and inhabit the High Arctic (e.g. Spitsbergen; Salomonsen 1944, Burnham *et al.* 2020a, Kersten *et al.* 2021). Finally, there is an *F. a. arctica*/*F. a. naumanni* hybrid population on the island of Bjørnøya (Fig. 1; Harris & Wanless 2011, Kersten *et al.* 2021).

On Dalrymple Rock Island (Igánaq: 76°28′21.65″N, 70°13′12.40″W; Greenland) near Thule Air Base, there is a small Puffin colony (hereafter 'Thule') that falls well within the expected High Arctic distribution of *F. a. naumanni* (66–79°) (Harris & Wanless 2011, Gaston & Provencher 2012, Burnham *et al.* 2020a). Unlike previously studied colonies, Thule consists of discrete large and mid-sized Puffin phenotypes. Large-sized Puffins are most common, with mid-sized Puffins representing fewer than 9% of individuals (the total Thule population size is 15–35 pairs) (Burnham *et al.* 2020a). Mid-sized individuals are similar in size to *F. a. arctica* and have been observed for multiple breeding seasons (Burnham *et al.* 2020a). Migratory monitoring data previously collected from both size phenotypes show an equally diverse non-breeding season distribution, with Thule Puffins using locations thousands of kilometres apart (Burnham *et al.* 2021). It is unclear if the size differentiation in Thule is the result of extreme size
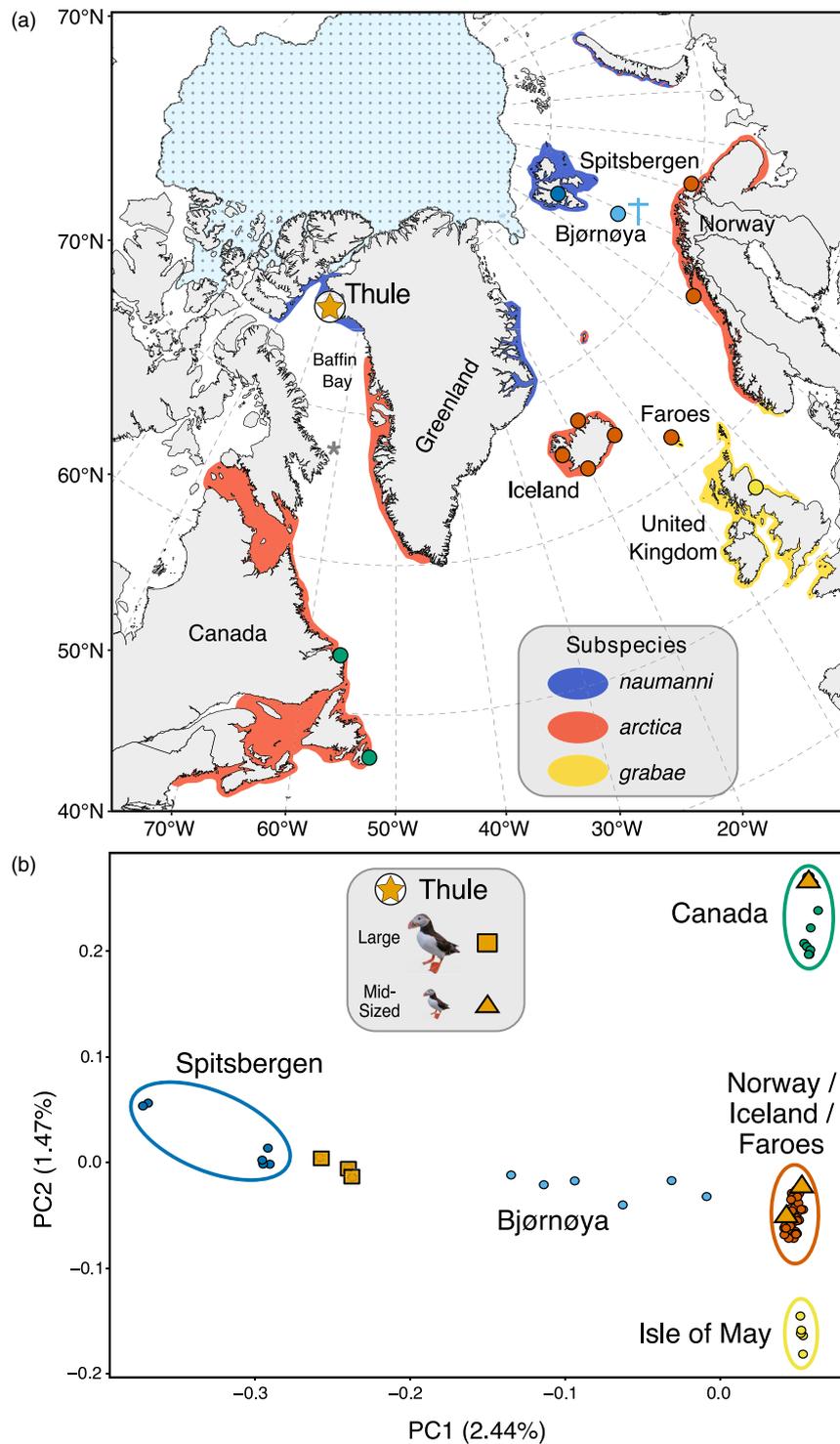
Figure 1. (a) Map presenting the 13 sites included in this study. Sites are coloured according to the genetic Atlantic Puffin clusters identified previously (Kersten *et al.* 2021) and shading highlights the range of the recognized subspecies. The cross depicts a confirmed hybrid zone. The asterisk indicates a large-bodied Puffin that was collected offshore at the Minarets during the breeding season. (b) Genetic structure (principal components analysis; PCA) based on genome-wide variation (*n* = 1 116 341 single nucleotide polymorphisms) for 77 individuals. Each circle represents a sample and colours indicate membership to a genetic cluster.

variation in Western Arctic *F. a. naumanni*, or if these mid-sized individuals are dispersed members of a different genetic cluster. Here we used whole genome sequencing to clarify the genomic relationship of Puffins in Thule. We discuss our results in light of ongoing boreal species shifts in response to climate change in the rapidly warming High Arctic.

METHODS

Blood from six adult Puffins from Thule (three mid-sized and three large-sized birds, see Fig. S1 and Data S1 and S2) was collected between 2012 and 2015 during the colony egg incubation period (July–August). Sampling was conducted following the guidelines established in Fair *et al.* (2010) and with permissions from the Greenland authorities. Size differences were visibly noticeable in the field, but to ensure a systematic classification, individuals were assigned a phenotype based on their wing length/beak size ratio cluster. The individuals sequenced were observed at Thule for two to three breeding seasons between 2012 and 2015, except for one large-sized male that was only observed during the last field season. Breeding status of sampled individuals was unknown because of site access constraints.

DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) or with a 5 M salt solution (Miller *et al.* 1988). Genomic libraries were built and sequenced (Illumina Hiseq4000; Illumina, San Diego, CA, USA) at the Norwegian Sequencing Centre (sequencing data have been deposited at the European Nucleotide Archive; Kersten 2022). Sequencing reads were mapped to the Atlantic Puffin assembly (European Nucleotide Archive Accession: CAJHIB020000000.2) using PALEOMIX v1.2.14 (Schubert *et al.* 2014), and analysed together with previously published genome data from 71 individuals representing 12 breeding colonies that acted as an integral reference for this study (Fig. 1a; Kersten *et al.* 2021, Kersten 2022). Further details on the methods and analyses can be found in Appendix S1.

Population structure was assessed using a genotype likelihood panel of 1 116 341 variant sites using ANGSD v.0.931 (Korneliussen *et al.* 2014; detailed in Appendix S1). A principal components analysis (PCA) was conducted with PCAngsd v0.982 (Meisner & Albrechtsen 2018) and individual ancestry proportions were estimated using ngsAdmix v32 (Skotte *et al.* 2013) and CLUMPAK (Kopelman *et al.* 2015), including a hierarchical approach, i.e. individuals from one cluster identified at $K = 2$ were removed followed by rerunning the analysis. Population and individual-based maximum likelihood phylogenetic trees with and without migration edges were built with Treemix v1.13 (Pickrell & Pritchard 2012) using the Razorbill *Alca torda* genome (GCA_008658365.1) as an outgroup. Individual

pairwise genetic distance (p-distance) matrices were calculated with ngsDist v1.0.8 (Vieira *et al.* 2015).

Puffin colonies were divided into seven groups that included the four previously identified (Kersten *et al.* 2021) genetic clusters (Spitsbergen ($n = 6$), Iceland/Norway/Faroes ($n = 42$), Isle of May ($n = 5$), Canada ($n = 12$)) and the hybrid population on Bjørnøya ($n = 6$), as well as the two Thule size classes. These previous analyses by Kersten *et al.* (2021) detected varying levels of genetic diversity in the different clusters. To investigate genetic diversity of the Thule Puffins compared with their respective clusters, we analysed heterozygosity, runs of homozygosity (RoH) and individual inbreeding coefficients (F_{RoH}) using one- and two-dimensional site-frequency spectra (see Appendix S1).

Recent admixture was assessed by calculating *f*3-statistics in Treemix for each unique combination of ((A,B),C) of the seven Puffin groups. In addition, gene flow and admixture were investigated with a genome-wide ABBA–BABA *D*-statistics calculated in ANGSD comparing all possible triplets of the Puffin genetic groups and Thule morphologies with the Razorbill as outgroup and a significance threshold corrected for multiple testing (see Appendix S1).

RESULTS

The PCA revealed that the two size classes of Puffins at Thule were genetically distinct. We also observed genetic differentiation within the three mid-sized Thule Puffins (Fig. 1b). One individual grouped with the Western Atlantic cluster whereas the other two fell within the Iceland/Norway/Faroes cluster. In contrast, the three large individuals were all genetically similar to each other and most closely related to *F. a. naumanni* in Spitsbergen (Fig. 1b). Ancestry components estimated from the model-based clustering using $K = 2$–4 (Fig. 2), as supported by delta *K* (Evanno *et al.* 2005) and biological expectations (Fig. S2), as well as individual-based maximum likelihood phylogenetic trees (Fig. S3), confirmed these assignments of the Puffins from Thule. The best supported *K* value was 4 based on hierarchical analyses (Figs S4 and S5) and known biological and geographical differences between genetic clusters. Individual pairwise genetic distances mirrored the results visualized in the PCA and Admixture plot (Fig. S6).

Heterozygosity ($\chi^2 = 38.49$, $P = 8.99 \times 10^{-7}$, df = 6), inbreeding coefficients ($\chi^2 = 50.32$, $P = 4.06 \times 10^{-9}$, df = 6) and RoH lengths ($\chi^2 = 119.71$, $P = 1.90 \times 10^{-23}$, df = 6) were significantly different among the seven Puffin groups, and, for the Thule birds, values were largely consistent with their genetic clusters (Figs S7 and S8). The large Thule birds and *F. a. naumanni* had similar F_{RoH} that were significantly
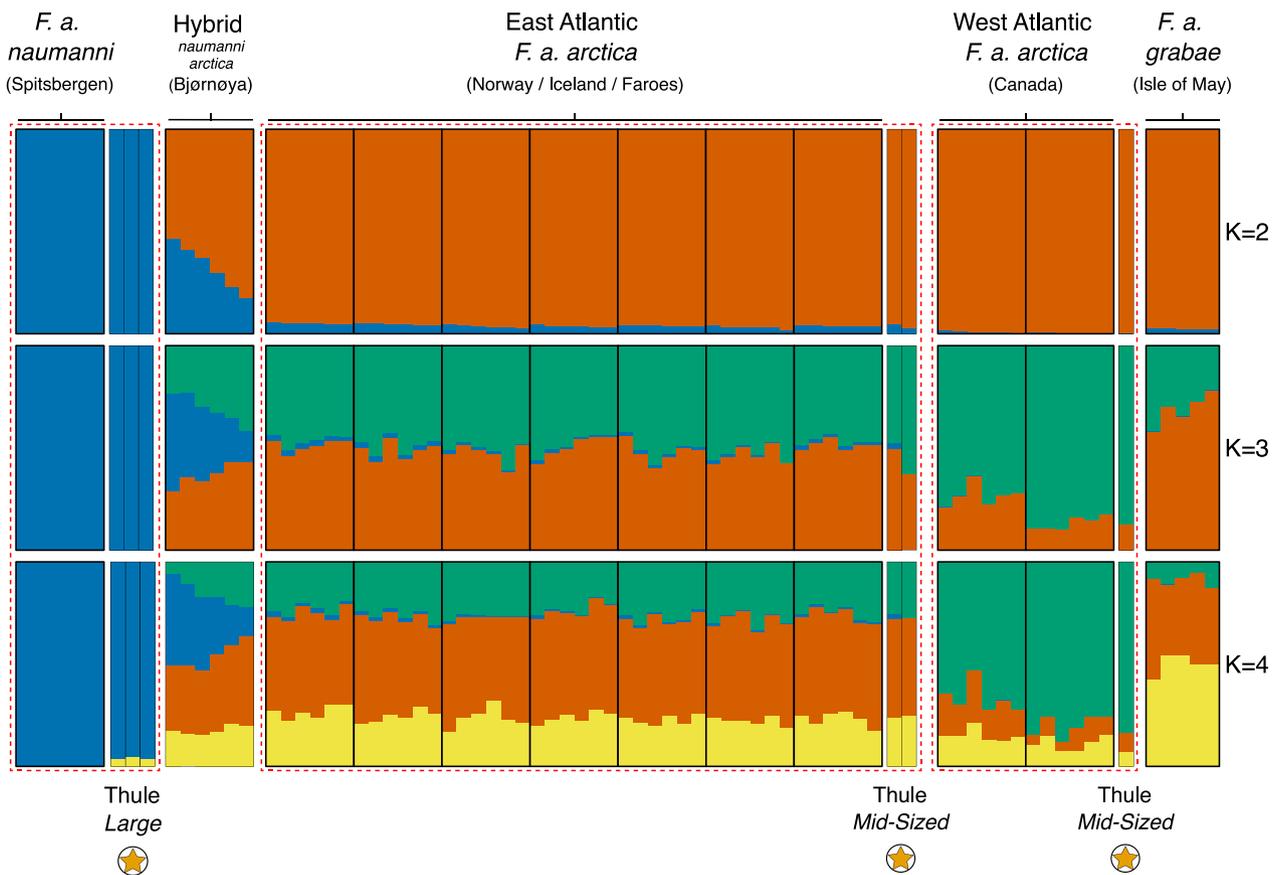
Figure 2. Genomic structure of 77 Atlantic Puffins across 13 colonies based on CLUMPAK-averaged admixture plots of the best *K* values. Colours indicate ancestry fraction to the different ancestral populations. Thule Puffins (labelled with a star symbol) have distinct ancestry components that are similar to those of three major genetic clusters (highlighted by dashed red line).

higher than the Norway/Iceland/Faroes genetic unit (*P* < 0.05 and *P* < 0.0001, respectively; Fig. S8). RoHs were significantly longer (*P* < 0.05) across the genome in large Thule Puffins relative to all other genetic units except *F. a. naumanni* (Fig. S7c), and the maximum size of homozygous tracts was similar in large Thule Puffins and *F. a. naumanni*.

No evidence for interbreeding was visible between the two Thule size phenotypes and genetic groups. Population-based maximum likelihood phylogenetic trees using up to two migration edges as determined by different threshold models (Figs S9 and S10) and *f*3-statistics (Table S1) did not show significant evidence for gene flow between any populations except from Spitsbergen into Bjørnøya, a known hybrid Puffin population (Kersten *et al.* 2021). The ABBA–BABA analysis showed significant *D*-statistics, and *Z* values were highest between large-sized Thule individuals and Spitsbergen (Table 1). Mid-sized Thule individuals showed weaker, albeit significant, signs of introgression

with the Canadian genetic cluster. There was also an expected sign of introgression between Spitsbergen and Bjørnøya (Table 1). The admixture analysis showed a small (3.5–4.4%) admixed portion of the genome in the large Thule birds visible at *K* = 4, but not at *K* = 2 or 3 (Fig. 2).

DISCUSSION

Whole genome sequencing data analyses revealed that the Thule Puffin colony consists of individuals from multiple distinct genetic units, with no detectable recent interbreeding between size phenotypes. Remarkably, the mid-sized individuals found at Thule represented the two distinct West and East Atlantic *F. a. arctica* genetic clusters.

The large Thule Puffins were closely related to Puffins on Spitsbergen (*F. a. naumanni*) and showed similar levels of heterozygosity, inbreeding and RoH lengths. However, the populations at Thule and Spitsbergen

Table 1. ABBA–BABA analyses between Thule phenotypes and previously identified Atlantic Puffin genetic clusters (Kersten *et al.* 2021).

D	Z	P (adj)	nABBA	nBABA	nBlocks	H1	H2	H3	H4
Spitsbergen & Bjørnøya									
−0.0417	−17.32	0.000	3686.47	4007.01	19 152	Bjørnøya	Nor/Ice/Far	Spitsbergen	Razorbill
−0.0393	−15.20	0.000	3704.27	4007.01	19 446	Spitsbergen	Nor/Ice/Far	Bjørnøya	Razorbill
−0.0396	−14.25	0.000	3696.96	4001.84	18 526	Bjørnøya	Canada	Spitsbergen	Razorbill
−0.0386	−13.21	0.000	3704.27	4001.84	18 744	Spitsbergen	Canada	Bjørnøya	Razorbill
−0.0373	−11.32	0.000	3711.59	3999.58	17 995	Bjørnøya	Isle of May	Spitsbergen	Razorbill
−0.0373	−10.97	0.000	3711.77	3999.58	18 168	Spitsbergen	Isle of May	Bjørnøya	Razorbill
−0.0398	−10.61	0.000	3687.53	3993.49	17 690	Bjørnøya	Thule (mid-sized)	Spitsbergen	Razorbill
−0.0364	−9.49	0.000	3713.30	3993.49	17 781	Spitsbergen	Thule (mid-sized)	Bjørnøya	Razorbill
Spitsbergen & Thule (large)									
−0.1018	−35.17	0.000	3568.19	4377.16	17 759	Spitsbergen	Nor/Ice/Far	Thule (large)	Razorbill
−0.1014	−31.02	0.000	3566.21	4371.33	17 028	Spitsbergen	Canada	Thule (large)	Razorbill
0.1000	30.81	0.000	4377.16	3581.44	19 145	Nor/Ice/Far	Thule (large)	Spitsbergen	Razorbill
0.0980	27.88	0.000	4371.33	3591.38	18 368	Canada	Thule (large)	Spitsbergen	Razorbill
−0.1031	−27.15	0.000	3560.93	4379.88	16 415	Spitsbergen	Isle of May	Thule (large)	Razorbill
0.0954	24.12	0.000	4379.88	3616.92	17 534	Isle of May	Thule (large)	Spitsbergen	Razorbill
−0.0998	−23.51	0.000	3578.09	4371.15	16 093	Spitsbergen	Thule (mid-sized)	Thule (large)	Razorbill
−0.0981	−22.67	0.000	3590.41	4371.15	16 957	Thule (large)	Thule (mid-sized)	Spitsbergen	Razorbill
−0.0657	−18.79	0.000	3703.18	4224.30	16 622	Spitsbergen	Bjørnøya	Thule (large)	Razorbill
0.0596	15.98	0.000	4224.30	3748.99	17 816	Bjørnøya	Thule (large)	Spitsbergen	Razorbill
Bjørnøya & Thule (large)									
−0.0374	−14.18	0.000	3703.39	3991.44	17 732	Bjørnøya	Nor/Ice/Far	Thule (large)	Razorbill
−0.0370	−12.11	0.000	3704.24	3988.68	16 969	Bjørnøya	Canada	Thule (large)	Razorbill
−0.0387	−10.57	0.000	3698.75	3996.18	16 336	Bjørnøya	Isle of May	Thule (large)	Razorbill
0.0333	10.45	0.000	3991.44	3734.28	19 418	Nor/Ice/Far	Thule (large)	Bjørnøya	Razorbill
0.0326	9.42	0.000	3988.68	3736.52	18 572	Canada	Thule (large)	Bjørnøya	Razorbill
−0.0355	−8.52	0.000	3705.81	3978.37	16 036	Bjørnøya	Thule (mid-sized)	Thule (large)	Razorbill
0.0312	7.98	0.000	3996.18	3754.56	17 685	Isle of May	Thule (large)	Bjørnøya	Razorbill
−0.0304	−7.06	0.000	3743.59	3978.37	17 033	Thule (large)	Thule (mid-sized)	Bjørnøya	Razorbill
Canada & Thule (mid-sized)									
−0.0061	−3.01	0.006	3775.56	3822.24	17 920	Canada	Nor/Ice/Far	Thule (mid-sized)	Razorbill
0.0081	2.80	0.012	3822.24	3760.67	20 347	Nor/Ice/Far	Thule (mid-sized)	Canada	Razorbill
0.0112	2.77	0.013	3839.05	3753.65	18 024	Thule (large)	Thule (mid-sized)	Canada	Razorbill
−0.0097	−2.48	0.029	3765.61	3839.05	16 784	Canada	Thule (large)	Thule (mid-sized)	Razorbill
0.0081	2.46	0.029	3819.62	3758.02	16 979	Spitsbergen	Canada	Thule (mid-sized)	Razorbill
Others									
−0.0089	−2.47	0.029	3764.52	3832.06	18 081	Canada	Thule (large)	Isle of May	Razorbill
0.0105	2.34	0.039	3837.38	3757.49	16 402	Thule (large)	Thule (mid-sized)	Isle of May	Razorbill

Significant pairwise genome-wide comparisons are shown. Negative values signal introgression between H1 and H3, positive between H2 and H3.

were not panmictic, showing greater genetic differentiation than that between West and East Atlantic *F. a. arctica* (Fig. S6). Though our limited sample size probably impacts differentiation estimate accuracy, Thule and Spitsbergen are c. 5300 km apart (over water) and the observed patterns align with previous findings of isolation by distance within Puffin Evolutionary Significant Units (ESUs; Kersten *et al.* 2021). Moreover, available tracking data indicate no non-breeding season distribution overlap between birds from these High Arctic colonies (Fayet *et al.* 2017, Burnham *et al.* 2021, Kersten *et al.* 2021). Large Thule Puffins

overwinter south of Iceland close to eastern Greenland whereas Spitsbergen Puffins overwinter north of Iceland (Burnham *et al.* 2021, Kersten *et al.* 2021). Non-overlapping overwintering grounds are recognized as a leading cause of population structure among seabirds (Puffins, Kersten *et al.* 2021; Black-browed Albatross *Thalassarche melanophris*, Burg & Croxall 2001; Thick-billed Murre *Uria lomvia*, Tigano *et al.* 2017). The western High Arctic (including Thule) has previously been speculated to represent a small, isolated, unique and vulnerable Puffin population (Gaston & Provencher 2012). Our results indicate that large Puffins at Thule are

genetically most similar to the Spitsbergen *F. a. naumanni*, but the two populations should be managed separately given the lack of non-breeding distribution overlap and observed genetic differentiation (Moritz 1994).

Mid-sized Thule Puffins clustered closely to either *F. a. arctica* genetic cluster. One female was closely related to Puffins from the boreal West Atlantic, whereas the other two Puffins (a male and a female) clustered with Puffins from Iceland/Norway/Faroes. The high genetic similarity to these genetic clusters and lack of detectable admixture with the large Thule birds suggests that these are dispersed individuals from southern natal colonies. Their overwintering areas support a southern origin (Burnham *et al.* 2021), corresponding to their respective genetic clusters and not the regions used by the larger Thule individuals (Fayet *et al.* 2017, Burnham *et al.* 2021, Kersten *et al.* 2021). Specifically, the female from the West Atlantic ESU (ID: 8408 in Burnham *et al.* 2021) overwintered in the Labrador Sea and North Atlantic, corresponding with colonies from Canada (Fayet *et al.* 2017). The two Puffins from the East Atlantic ESU overwintered near West Iceland (ID: 7363, male) and the Azores (ID: 8406, female; see Burnham *et al.* 2021), overlapping with Puffins from the Iceland/Norway/Faroes (Fayet *et al.* 2017).

Despite observing both phenotypes during the breeding season, no recent interbreeding was identified at Thule. Though detection of gene flow may be hampered by lack of genetic differentiation between closely related subspecies, contemporary introgression has previously been detected in Puffins at Bjørnøya (Kersten *et al.* 2021). Recent gene flow is expected to generate significant ABBA–BABA statistics (Barlow *et al.* 2018, Westbury *et al.* 2021). In Thule, no significant comparisons supported introgression between the size classes. Comparisons only supported our findings of genetic similarity between large Thule Puffins and Spitsbergen, as well as between mid-sized individuals and their boreal genetic clusters. Hence, there is no evidence that recent interbreeding has occurred between the morphologies at Thule. The potential cohabitation of distinct subspecies at Thule is a deviation from previously detected patterns of clear geographical boundaries and hybridization upon contact (Harris & Wanless 2011, Kersten *et al.* 2021). Historical records of phenotype variation at currently unsampled colonies in the East Arctic (Novaya Zemlya and Jan Mayen; Salomonsen 1944, Harris & Wanless 2011) suggest other Puffin colonies could also contain multiple subspecies; however, unlike Thule, this is probably accompanied by hybridization because there are also records of intermediate morphotypes (Salomonsen 1944, Harris & Wanless 2011). It can be speculated that a barrier to interbreeding at Thule may arise from sub-species' behavioural differences; different overwintering areas may lead to asynchronous colony arrival and mis-matched timing of pair bonding (Ketterson *et al.* 2015).

Sympatric distinct subspecies are unusual, especially in seabirds where new contact zones typically result in hybridization (Scopoli's Shearwater *Calonectris diomedea*, Munilla *et al.* 2016; gadfly petrels *Pterodroma* spp., Brown *et al.* 2010). The absence of evidence for hybridization at Thule is also unusual for Arctic species, where hybridization with low-latitude taxa upon contact is common and a key potential pathway for adaptation to climate change (Colella *et al.* 2020, Charles & Stehlik 2021). Importantly, hybridization expectations are clearly visible in the contact zone on Bjørnøya (this study, Kersten *et al.* 2021). Collectively, this suggests cohabitation at Thule may also be recent and interbreeding could arise in the future. Although we do not know the driving mechanisms, we hypothesize that climate warming may be pushing a northern range expansion of *F. a. arctica*, similar to those observed during the Little Ice Age (1620–1770 CE, Walker & Meijer 2021). Under this hypothesis, the mid-sized individuals at Thule may represent the very early stages of a range shift in boreal Puffins. Similar range shifts have already been detected in Western Atlantic Arctic populations of Thickbilled Murres *U. lomvia*- and Razorbills *A. torda* due to the extended habitable period in the Arctic (Patterson *et al.* 2021) and the northern shift of fish stocks (Gaston & Woo 2008). Additionally, an increase of boreal seabird species has been recorded in the East Atlantic Arctic (Descamps & Strøm 2021) and the first Atlantic records of Pacific species of *Fratercula* have also occurred at Thule, probably facilitated by recent Arctic sea-ice loss (Burnham *et al.* 2020b). The hypothesized northern range shifts of Puffins must now be confirmed with temporal samples and additional colonies (i.e. Arctic Canada). Nonetheless, it is clear that valuable insights about Arctic biodiversity can be gained even from a small number of individuals. Further studies are urgently needed across the Arctic to better understand the biodiversity present and the rapidly evolving responses to climate change.

AUTHOR CONTRIBUTIONS

Deborah M. Leigh: Conceptualization (lead); data curation (supporting); formal analysis (supporting); funding acquisition (lead); investigation (equal); methodology (equal); resources (equal); validation (supporting); visualization (supporting); writing – original draft (lead); writing – review and editing (lead). **Oliver Kersten:** Data

curation (lead); formal analysis (lead); investigation (equal); methodology (lead); software (lead); validation (lead); visualization (lead); writing – original draft (equal); writing – review and editing (equal). **Bastiaan Star:** Data curation (supporting); formal analysis (supporting); funding acquisition (supporting); investigation (supporting); methodology (supporting); project administration (supporting); resources (supporting); supervision (supporting); validation (supporting); visualization (supporting); writing – original draft (equal); writing – review and editing (equal). **Tycho Anker-Nilssen:** Resources (supporting); writing – review and editing (supporting). **Kurt Burnham:** Investigation (lead); resources (supporting); writing – review and editing (supporting). **Jeff Johnson:** Resources (supporting); writing – review and editing (supporting). **Jennifer Provencher:** Conceptualization (lead); resources (supporting); writing – review and editing (supporting). **Sanne Boessenkool:** Formal analysis (equal); funding acquisition (lead); investigation (equal); methodology (equal); project administration (lead); resources (equal); supervision (lead); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICAL NOTE

All research in Greenland was conducted after ethical approval and issuance of permits by the Government of Greenland, Department of Fisheries, Hunting and Agriculture (High Arctic Institute permit numbers: Sags nr. 2012–065141, Dok. nr. 888887, Sags nr. 2013–083369, Dok. nr. 1204884, Sags nr. 2014–099682, Dok nr. 1594176, Sags nr. 2015–115204, Dok. nr. 1975643). Capture, handling and blood collection followed the Ornithological Council's *Guidelines to the use of wild birds in research* (Fair *et al.* 2010).

Data Availability Statement

Raw read data have been deposited in the European Nucleotide Archive (ENA, www.ebi.ac.uk/ena) under study accession number PRJEB40631 (see Data S1

for individual sample accession numbers) found at https://www.ebi.ac.uk/ena/browser/view/PRJEB40631. Full code used for the population genomic analyses is available on Zenodo under the DOI https://doi.org/10.5281/zenodo.5950720. This includes versions of any software used, if relevant, and any specific variables or parameters used to generate, test and process the dataset of this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Size comparison using wing length and beak size between adult Atlantic Puffins measured at the Thule colony during the breeding season in 2010–16.

Figure S2. Delta *K* as a function of the number of ancestral clusters (*K*) as calculated by the method of Evanno for *K* = 1–9.

Figure S3. Individual-based Treemix analysis of 77 Atlantic Puffins.

Figure S4. Delta *K* as a function of the number of ancestral clusters (*K*) as calculated by the method of Evanno for *K* = 1–9 after removing Spitsbergen, the large Thule morphs and Bjørnøya individuals.

Figure S5. Hierarchical genomic structure of 62 Puffins based on CLUMPAK-averaged admixture plots of the best *K* values.

Figure S6. Heatmap of genetic distances between 77 Atlantic Puffin individuals.

Figure S7. Estimates of individual genome-wide heterozygosity, individual inbreeding coefficients and length distribution of runs of homozygosity tracts longer than 500 kb for Puffins from each genomic cluster.

Figure S8. Genome-wide heterozygosity and inbreeding compared between Puffins of the Thule colony and colonies of the previously identified population genomic clusters.

Figure S9. Estimation of the optimal number of migration edges (m) for a Treemix-generated population-based maximum likelihood tree using optM.

Figure S10. Population-based Treemix analyses of 13 Atlantic Puffin colonies applying up to two migrations.

Table S1. Significant recent admixture signal between genomic Atlantic Puffin clusters as revealed by *f*3-statistics.

Appendix S1. Detailed methodological description of the whole genome analysis of six Atlantic Puffin individuals from a Western Atlantic High Arctic colony.

Data S1. Summary information on all analysed samples.

Data S2. Biometrics of all analysed specimens.