



# Receding ice drove parallel expansions in Southern Ocean penguins

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**Climate shifts are key drivers of ecosystem change. Despite the critical importance of Antarctica and the Southern Ocean for global climate, the extent of climate-driven ecological change in this region remains controversial. In particular, the biological effects of changing sea ice conditions are poorly understood. We hypothesize that rapid postglacial reductions in sea ice drove biological shifts across multiple widespread Southern Ocean species. We test for demographic shifts driven by climate events over recent millennia by analyzing population genomic datasets spanning 3 penguin genera (*Eudyptes*, *Pygoscelis*, and *Aptenodytes*). Demographic analyses for multiple species (macaroni/royal, eastern rockhopper, Adélie, gentoo, king, and emperor) currently inhabiting southern coastlines affected by heavy sea ice conditions during the Last Glacial Maximum (LGM) yielded genetic signatures of near-simultaneous population expansions associated with postglacial warming. Populations of the ice-adapted emperor penguin are inferred to have expanded slightly earlier than those of species requiring ice-free terrain. These concerted high-latitude expansion events contrast with relatively stable or declining demographic histories inferred for 4 penguin species (northern rockhopper, western rockhopper, Fiordland crested, and Snares crested) that apparently persisted throughout the LGM in ice-free habitats. Limited genetic structure detected in all ice-affected species across the vast Southern Ocean may reflect both rapid postglacial colonization of subantarctic and Antarctic shores, in addition to recent genetic exchange among populations. Together, these analyses highlight dramatic, ecosystem-wide responses to past Southern Ocean climate change and suggest potential for further shifts as warming continues.**

Sphenisciformes | climate change | Last Glacial Maximum | refugia | genomics

Climate change is substantially impacting the abundance and distribution of wildlife, with many species' ranges shifting poleward as a result of climate warming (1). Similar shifts occurred after the Last Glacial Maximum (LGM; 18,000 to 25,000 y ago) (2, 3), as temperate refugial populations of many species expanded into high latitudes. While such range shifts may be readily achieved on continents [where terrestrial habitats are essentially continuous

(4)], the challenges are more pronounced for isolated or fragmented populations that rely on long-distance dispersal (5, 6). For instance, many high-latitude coastal and terrestrial ecosystems of

## Significance

**We analyze population genomic datasets across 3 penguin genera to test for demographic shifts driven by historical climate events. Numerous species inhabiting coastlines affected by heavy sea ice during the Last Glacial Maximum show genomic signatures of near-simultaneous population expansions associated with postglacial warming, contrasting with stable or declining demographic histories inferred for species occupying consistently ice free habitats. Shallow population genomic structure detected within species distributed across the vast Southern Ocean likely provides further evidence for recent demographic shifts and recent genetic exchange among populations. Our results demonstrate dramatic, ecosystem-wide responses to climate change and highlight the potential for future biological shifts in the Southern Ocean as global warming continues.**

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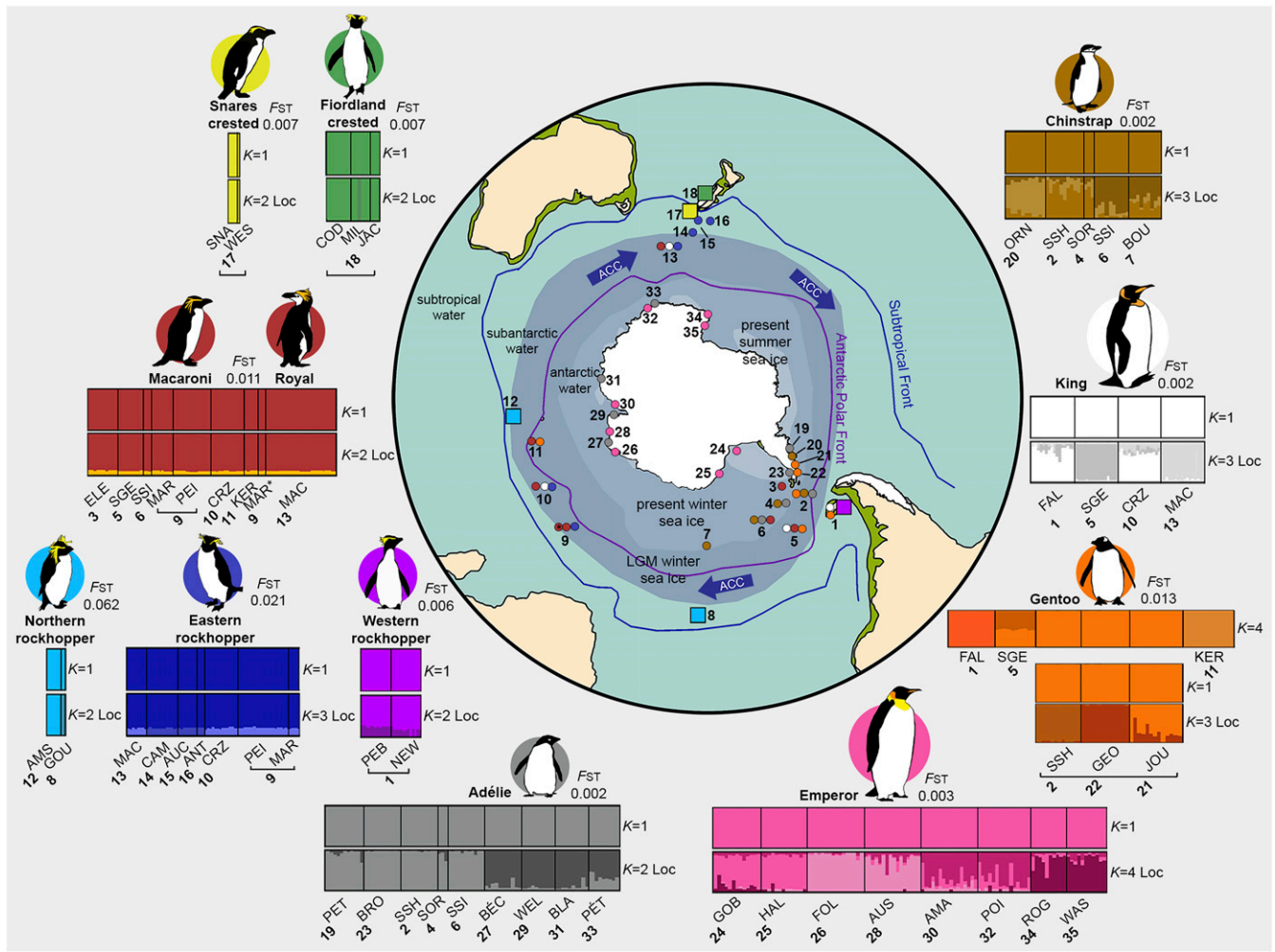
Data deposition: The raw data reported in this paper has been deposited in the NCBI Short Read Archive BioProject PRJNA589336. Additional files are available on figshare (DOI: 10.6084/m9.figshare.c.4475300).

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**Fig. 1.** Sampling locations and Structure plots for 11 penguin species (royal/macaroni are considered one species). The map (adapted from ref. 6; copyright (2012) with permission from Elsevier) shows the Antarctic Circumpolar Current (ACC), the Subtropical Front (blue line), the Antarctic Polar Front (purple line), present summer (light blue shading) and winter sea ice (medium blue shading), LGM winter sea ice (dark blue shading) (see refs. 6, 9, and 30), LGM land extent (green), and glaciation during the LGM (white). Four species (indicated by squares) breed north of the LGM sea ice limit, whereas 7 species (indicated by circles) breed in southern regions affected by LGM sea ice. The top Structure plot for each species (top 2 for gentoo) represents the most likely number of genetic clusters ( $K$ ) as determined via the Evanno method. The bottom Structure plot for each species shows a higher value of  $K$  to illustrate recently evolved fine-scale genetic structure that can only be detected using location priors (Loc), as demonstrated by ref. 14. Structure plots for Adélie, emperor, gentoo, king, and chinstrap penguins adapted from ref. 14, which is licensed under CC BY 4.0. With the exception of the gentoo penguin, all analyses demonstrated a most likely  $K$  of 1, with relatively shallow  $F_{ST}$  values (global  $F_{ST}$  is shown beside each species) (see also ref. 14). Numerical codes for sampling localities (details in *SI Appendix, Fig. S1*) are indicated on the map and underneath Structure plots. Sampling localities: Falkland Islands (FAL, PEB, NEW), South Shetland Islands (SSH), Elephant Island (ELE), South Orkney Islands (SOR), South Georgia (SGE), South Sandwich Islands (SSI), Bouvet (BOU), Gough Island and Tristan da Cunha (GOU), Marion Island (MAR), Prince Edward Islands (PEI), Crozet (CRZ), Kerguelen (KER), Amsterdam Island (AMS), Macquarie Island (MAC), Campbell Island (CAM), Auckland Islands (AUC), Antipodes Islands (ANT), The Snares (SNA), Western Chain (WES), Codfish Island (COD), Milford Sound (MIL), Jackson Head (JAC), Peterman Island (PET), Orne Harbor (ORN), Jougla Point (JOU), George's Point (GEO), Brown Bluff (BRO), Gould Bay (GOB), Halley Bay (HAL), Fold Island (FOL), Béchervaise Island (BÉC), Auster (AUS), Welch Island (WEL), Amanda Bay (AMA), Blakeney Point (BLA), Pointe Géologie (POI), Pétrels Island (PÉT), Cape Roget (ROG), Cape Washington (WAS). The asterisk on Marion Island indicates the "white-faced" phenotype of macaroni/royal penguin. Colored symbols (squares and circles) are consistent with Figs. 2 and 3.

the Southern Hemisphere are isolated by vast ocean gaps (Fig. 1). Southern Ocean circumpolar fronts (including the Subtropical Front and the Antarctic Polar Front) may present additional physical and thermal barriers to southward range expansion of isolated southern coastal populations (7, 8).

Understanding past shifts in species distributions is crucial for forecasting responses to contemporary and future climate change. Currently, there is considerable uncertainty surrounding the extent to which high-latitude wildlife populations might have persisted in the Southern Ocean throughout the LGM versus the extent of post-LGM expansion (6, 9, 10). Recent genetic data, however, hint at major ecosystem-wide change following reductions in southern winter sea ice (9, 11, 12). Importantly, past

expansions can be reconstructed via genetic analysis of modern populations (2, 13). While several studies of Southern Ocean species have detected limited population genetic structure, consistent with recent demographic shifts and/or gene flow (11, 12, 14–18), a comprehensive genome-wide assessment of Southern Ocean wildlife is lacking. Moreover, as responses to climate change can potentially vary among species (12, 19, 20), distinguishing between concerted (multispecies) versus idiosyncratic (single species) shifts may be crucial to forecasting responses to future climate change (21).

Penguins (Sphenisciformes) are iconic marine birds that inhabit all major southern landmasses, with their greatest species diversity in Antarctica and the subantarctic (Fig. 1 and *SI Appendix, Fig. S1*). Although most penguins are natally philopatric

(22), some can disperse vast distances traversing major Southern Ocean fronts (23, 24) and represent important components of both coastal and marine ecosystems (25). Here we analyze several thousand single nucleotide polymorphisms (SNPs) across 11 Antarctic, subantarctic, and temperate penguin species to test for concerted responses to climate change. We detect genomic signatures of population expansion in multiple species currently distributed largely within the LGM sea ice zone, consistent with concerted recolonization of Antarctic and subantarctic coasts during post-LGM warming. In contrast, demographic histories inferred for 4 temperate penguin species are relatively stable or declining. Our results suggest consistent population dynamics across a species-rich high-latitude assemblage in response to postglacial ice reduction and demonstrate the potential for rapid change to Southern Ocean ecosystems under future warming.

## Results

Demographic reconstructions of effective population sizes ( $N_e$ ) for 11 penguin species using CubSFS (26), SNAPP (SNP and AFLP package for phylogenetic analysis) (27), Tajima's D (28), and Multidice (29) were based on 3,000 to 13,000 SNPs per species (SI Appendix, Tables S1–S3). Macaroni and royal (*Eudyptes chrysolophus chrysolophus*/*E. c. schlegeli*) penguins were considered a single species based on structure/ $F_{ST}$  (fixation index) analyses (see also ref. 18), whereas Snares crested (*E. robustus*) and the northern rockhopper (*E. moseleyi*) penguins were excluded from some analyses due to their small sample sizes (Fig. 1 and SI Appendix, Tables S4–S5). These analyses revealed comparable postglacial  $N_e$  expansions for 6 southern species (macaroni/royal, eastern rockhopper [*E. filholi*], Adélie [*Pygoscelis adeliae*], gentoo [*P. papua*], king [*Aptenodytes patagonicus*], and emperor [*A. forsteri*] penguins) (Figs. 2 and 3A and SI Appendix, Table S1 and Figs. S2 and S3), with the emperor penguin expanding slightly earlier. Additionally, 2 of 3 demographic analyses supported recent expansion in a seventh species (chinstrap [*Pygoscelis antarctica*]) (Fig. 3A). Notably, these 7 species all predominantly occur south of the LGM sea ice limit (Fig. 1) (see refs. 6, 9, 22, and 30). By contrast, 4 species inferred to have relatively stable or declining recent demographic histories (Fig. 3A and SI Appendix, Figs. S2 and S3) are all predominantly found north of the LGM sea ice zone (Figs. 1 and 2): the northern rockhopper (Gough and Amsterdam Islands), western rockhopper (*E. chrysolophus*; predominantly the Falkland Islands and southern South America), Fiordland crested (*E. pachyrhynchus*, southern New Zealand), and Snares crested (The Snares and Western Chain) penguins.

The expansion time frames inferred for most southern lineages (20,000 to 15,000 y ago) correspond to a period of rapid post-LGM warming (31) (Fig. 2A and SI Appendix, Table S1). These reconstructions suggest populations of the ice-adapted emperor penguin expanded earlier than those of most other southern penguin lineages which require ice-free terrain (see also refs. 15 and 32). The magnitude of inferred postglacial  $N_e$  expansions is, on average, a 2.7-fold increase (ranging from 1.19- to 4.4-fold increase) (Fig. 2A and SI Appendix, Table S1). We detected some variation in the outcomes of different demographic analyses for particular species, perhaps a reflection of varying sensitivity of different model-based approaches and/or biological signal. For example, the CubSFS analysis contrasted with other approaches in suggesting chinstrap penguin populations expanded prior to the LGM and declined following the LGM. Overall, however, there is broad support for “stable/declining” demographic trajectories for species inhabiting LGM ice-free regions versus predominantly “expanding” trajectories for LGM ice-affected species (Fig. 3A).

We used Multidice to test for synchronous versus asynchronous expansions across the 7 expanded species identified based on our demographic analyses (Fig. 3A). To this end, we modeled a single expansion event within the last 50,000 y in which up to 7

species coexpanded. The synchronous expansion event was inferred to have occurred 20,779 to 24,804 y ago, depending on the summary statistics chosen (Fig. 3B and SI Appendix, Table S3). While only 2 or 3 of these southern species were inferred to have expanded simultaneously (SI Appendix, Table S3), minor differences between inferred expansion timings (Fig. 2A) likely hindered the ability for Multidice to detect a single expansion event corresponding to all expanding species.

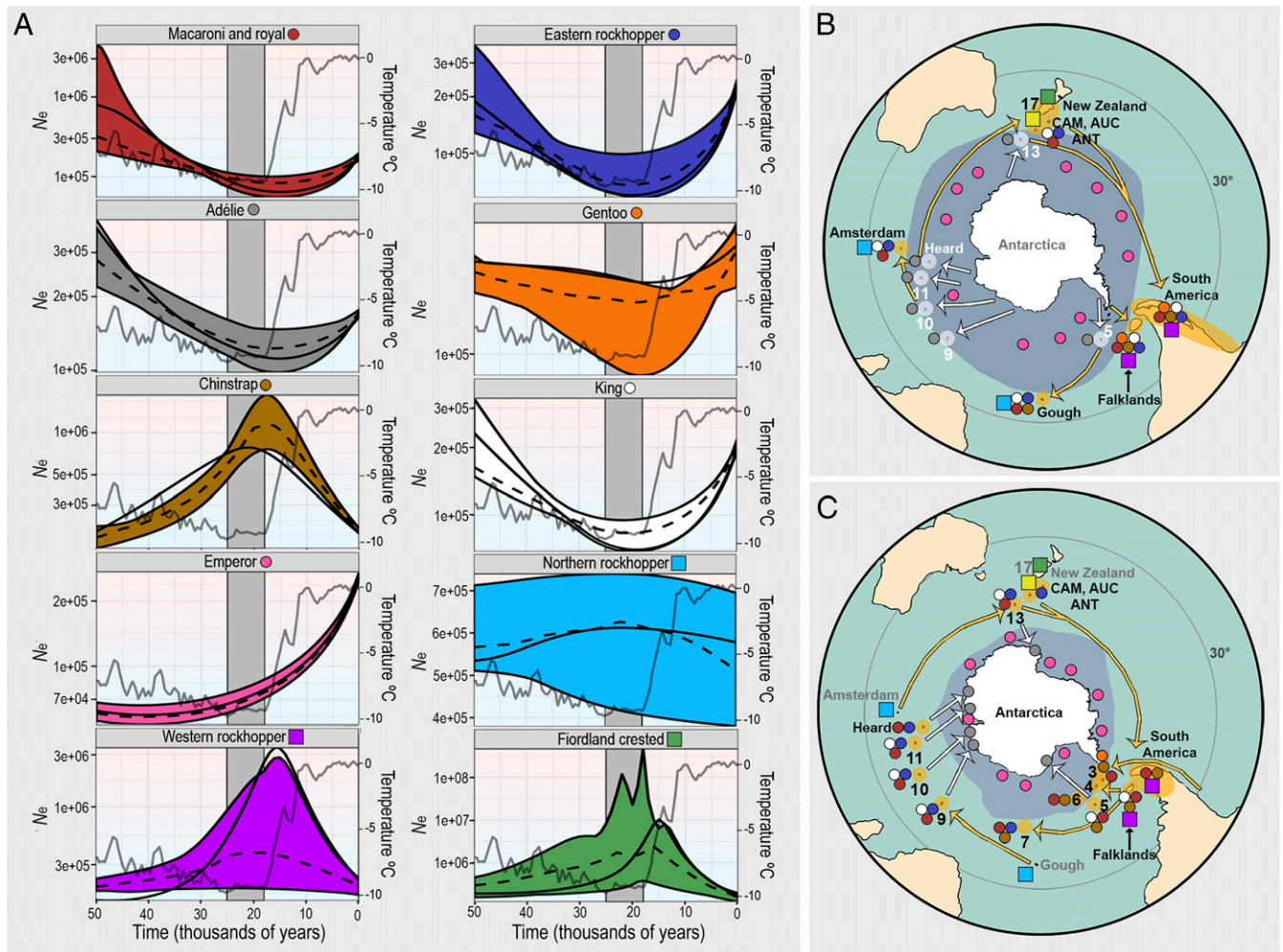
Tests for intraspecific genomic divergence across the ranges of individual species (including previous analyses of *Pygoscelis* and *Aptenodytes* species; see refs. 11, 12, 33, and 34) consistently revealed shallow genetic structure within species (Fig. 1 and SI Appendix, Figs. S4–S7 and Tables S6 and S7). In all cases apart from gentoo penguins, we found that panmixia ( $K$  [number of genetic clusters] = 1) was supported but that using location priors found evidence for additional fine-scale structure, as previously reported (11, 12, 14, 33, 34). Such patterns are consistent with post-LGM demographic and biogeographic expansions (for southern LGM sea ice species) and recent genetic exchange among populations. Specifically,  $F_{ST}$ , principal coordinates analyses (PCoA), Structure, discriminant analysis of principal components (DAPC), SNAPP, and phylogenetic analyses for *Eudyptes*, *Pygoscelis*, and *Aptenodytes* all revealed relatively shallow within-species genomic structure among southern populations (Fig. 1 and SI Appendix, Figs. S4–S7 and Tables S6 and S7) (14, 15, 34, 35). In contrast to the recent genetic exchange inferred within most species and between macaroni and royal penguins (17, 18, 35), these analyses detected little or no admixture among species (SI Appendix, Figs. S4–S7).

## Discussion

Our study detected broadly consistent genome-wide signatures of post-LGM expansion across penguin species that currently breed south of the LGM sea ice zone (Fig. 3A). By contrast, 4 species currently breeding north of the LGM sea ice zone exhibited genetic signatures of relatively stable or declining demographies (Fig. 3A). Although estimates of precise LGM breeding ranges for penguins remain elusive (but see ref. 36), our findings are consistent with the hypothesis of (6) that during the LGM, many Southern Ocean species retreated to ice-free refugia (e.g., Gough, Amsterdam, and Falklands islands; southern South America; and New Zealand's southern islands) (Fig. 2B; see refs. 6 and 9). Indeed, several recent studies have suggested that post-LGM reductions in sea ice were accompanied by rapid recolonization of high-latitude shores (8, 9, 12) (Fig. 2C). Recent demographic studies of penguins (Adélie, emperor, and king) (15, 16, 32) and the southern elephant seal (37), for example, inferred rapid postglacial recolonization events. By contrast, recent snow petrel analyses provide only limited evidence for such postglacial shifts (10). The choice of mutation rate, and possibly time dependency issues, might play some part in these apparently conflicting patterns among taxa. Some contrasting responses among species may also stem from interspecific ecological differences (e.g., variation in feeding ecology, philopatry, habitat preferences). Shifting oceanographic and coastal environmental features associated with postglacial warming may also have impacted local species.

While most LGM coasts are now inundated (Fig. 2B), some potential LGM refugia may be suggested on the basis of current distributions (e.g., the eastern rockhopper penguin likely expanded south from the Auckland, Campbell, and Antipodes islands; Fig. 2C). Previous studies have concluded that the Southern Ocean's circumpolar fronts can represent important barriers to dispersal for many marine species (7, 8), including penguins (14, 38). However, several penguin species can clearly traverse such boundaries (23, 24), and this exceptional dispersal ability may help to explain their apparently rapid biogeographic shifts in response to changing climate (see also ref. 37).



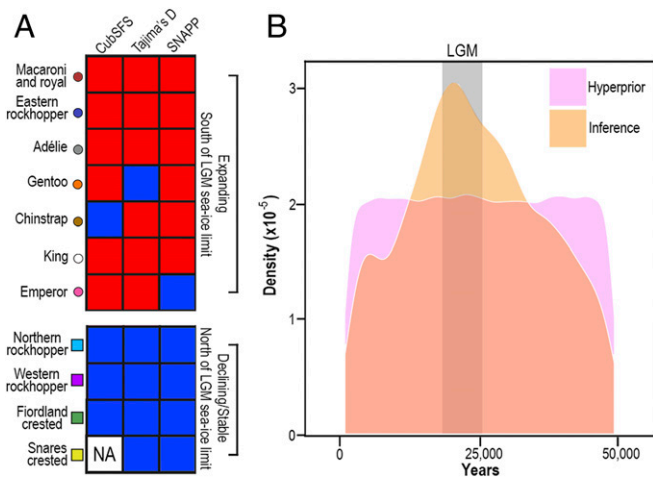


**Fig. 2.** Population expansions and contractions of penguin species in relation to the LGM. Species breeding south of the LGM sea ice limit are represented by circles in A, B, and C, and species breeding north of the LGM sea ice limit are represented by squares in A, B, and C. (A) CubSFS demographic reconstructions for 10 penguin species (Snares crested penguin is excluded due to low sample size). The 95% CIs are given by solid color intervals. The median for bootstrap replicates is given by the dotted line, and the solid line gives the demographic reconstruction for the amended SFS. A 50,000 y record of Antarctic temperature change (gray line in each plot) as estimated from the EPICA (European Project for Ice Coring in Antarctica) Dome C ice core (27) is shown in each plot. The gray bar in each plot shows the LGM. (B) The winter sea ice and sea level during the LGM, with putative refugia shown (orange ellipses for subantarctic penguins; gray points outlined in opaque white for all Antarctic penguins except the emperor penguin). Arrows indicate likely glacial retractions of southern species in response to LGM sea ice (white arrows indicate retractions of Antarctic penguins to the fringes of the summer sea ice during the LGM [except the emperor penguin]; orange arrows indicate retraction of subantarctic penguins to refugial islands north of LGM sea ice). The emperor penguin presumably bred on the fringes of the summer sea ice during the LGM (indicated by pink points). Site names in black indicate possible refugia regions for subantarctic penguins, while site names in white indicate possible refugia regions for Antarctic penguins. (B) Numerical codes for Antarctic penguin refugial regions are: 5 (South Georgia), 9 (Marion and Prince Edward Islands), 10 (Crozet), 11 (Kerguelen), 13 (Macquarie Island), and Heard (Heard Island). Numerical codes/abbreviations for subantarctic penguin refugial regions are: Falklands (Falkland Islands); Gough (Gough Island); Amsterdam (Amsterdam Island); 17 (Snares and Western Chain); New Zealand, CAM, AUC, ANT (Campbell, Auckland, and Antipodes Islands); and South America. (C) The present sea level and winter sea ice extent, with possible post-LGM routes of recolonization back to Antarctic and southern island habitats (white arrows for penguins breeding in Antarctica [except the emperor penguin]; yellow arrows for penguins breeding on southern islands). Regions where penguins likely persisted are shown with orange ellipses. The emperor penguin breeds on the fringes of the summer sea ice, which is marked with pink points. Site names in black indicate where each penguin species currently breeds, where site names marked in gray indicate locations where additional penguin species than present may have bred during the LGM (as shown in B). (C) Numerical codes/abbreviations are: Falklands (Falkland Islands); 3 (Elephant Island); 4 (South Orkney Island); 5 (South Georgia); 6 (South Sandwich Islands); Gough (Gough Island); 7 (Bouvet); 9 (Marion and Prince Edward Islands); 10 (Crozet); 11 (Kerguelen); Heard (Heard Island); Amsterdam (Amsterdam Island); 13 (Macquarie Island); 17 (Snares and Western Chain); New Zealand, CAM, AUC, ANT (Campbell, Auckland, and Antipodes Islands); and South America. Note that the LGM breeding ranges in both B and C are uncertain. Maps adapted from ref. 6. Copyright (2012) with permission from Elsevier. As the Snares crested penguin was included in other demographic analyses (Fig. 3), the species is shown in both B and C. Colored symbols (squares and circles) are consistent with Figs. 1 and 3.

While CubSFS suggested the chinstrap penguin may have declined following the LGM, Tajima's D and SNAPP supported population expansion for this species, comparable to results for other southern species (Fig. 3A). This anomaly may perhaps reflect issues with the mutation rate and/or generation time used or may indicate an idiosyncratic ecological response for this southern species (e.g., variation in feeding ecology, philopatry, habitat

preferences, sensitivity to oceanographic fronts). Based on evidence from combined demographic analyses (Fig. 3A), the suggestion that chinstrap penguins have declined since the LGM should be treated with some caution.

A consistent finding of our study is the lack of major genome-wide differentiation across the ranges of most penguin species, including several species showing circumpolar near homogeneity



**Fig. 3.** Summary of demographic results for 11 penguin species. (A) The combined results of CubSFS, Tajima's D, and SNAPP theta values. Species are broadly classified as "expanding" (red: macaroni/royal, eastern rockhopper, Adélie, gentoo, chinstrap, king, and emperor penguins [all south of the LGM sea ice, represented by circles]) or "declining/stable" (blue: northern rockhopper, western rockhopper, Fiordland crested, and Snares crested penguins [all north of the LGM sea ice, represented by squares]) on the basis of a majority of these analytical outputs. NA indicates when a species was excluded from an analysis due to limited sample size. All analyses specifically address post-LGM demographic change, with the exception of Tajima's D, which may also be influenced by earlier demographic events. (B) Multidice results, suggesting a LGM expansion, with the mean of the coexpansion time parameter inferred at 24,065 y (mode: 20,778; median: 24,065). Colored symbols (squares and circles) are consistent with Figs. 1 and 2.

(15, 16) (i.e.,  $K = 1$ ;  $F_{ST} < 0.02$ ; Fig. 1 and *SI Appendix, Table S6*). These relatively shallow  $F_{ST}$  values contrast with more substantial structure and evidence for multiple Southern Ocean refugia in white-chinned petrels [ $K = 3$ ;  $F_{ST} > 0.10$  (39)]. While biallelic markers such as the SNPs analyzed here are theoretically capable of yielding  $F_{ST}$  as high as 1 (i.e., fixed differences at all loci), we note that the upper range of this parameter can be limited by allele frequency distribution (40), and thus, these values should be treated with some caution. While the use of location priors at higher values of  $K$  reveals additional, fine-scale population differentiation (Fig. 1 and *SI Appendix, Fig. S4*) (see also refs. 14, 33, and 34), such structure can potentially evolve rapidly (e.g., ref. 41). Interestingly, the relatively shallow differentiation observed within and among some colonies [e.g., emperor (14, 34)] may also provide additional evidence of recent or ongoing gene flow and admixture, sometimes over vast distances (Fig. 1). Subtle population differentiation detectable with location priors might reflect the influence of contemporary oceanographic fronts and/or changes in local sea ice conditions, as previously suggested by (11, 14, 16, 17), and may have considerable relevance over ecological time frames (e.g., conservation management, studies of migration).

Understanding how biota responded to past climate change is essential for predicting species distributions and population sizes under future climate projections and for developing appropriate conservation management strategies (11, 42). As global temperatures continue to increase, midlatitude biota will continue to shift toward the poles (8) or, alternatively, may face extinction (6, 8). Many penguin populations are currently declining or are predicted to decline as warming continues (43–45). Some of the northernmost colonies of Adélie and emperor penguins have already disappeared (43, 46), and in the case of emperor penguins, these changes have been linked directly to reductions in sea ice (47). By contrast, populations of gentoo penguin are apparently expanding their ranges southward as the climate warms (48). Our study broadly demonstrates the demographic sensitivity of Southern Ocean

wildlife to the effects of past climate change (49), highlighting the potential for future shifts under anthropogenic climate change.

## Materials and Methods

The research was approved by the Otago University Animal Ethics Committee (AEC) 61/2016, Oxford University Local AEC, University of Western Australia AEC, Woods Hole Oceanographic Institution Animal Care and Use Committee, Institut Polaire Français Paul Emile Victor ethics committee and The Zoological Society of London. Consultation was undertaken with the University of Otago's Ngāi Tahu Research Consultation Committee.

**DART-Seq Library Preparation and Filtering.** DNA was extracted from 428 *Eudyptes* penguin samples spanning 6 species (Fig. 1 and *SI Appendix, Fig. S1* and *Table S4*; macaroni/royal penguins were considered a single species; see refs. 17, 18 and 35) using a modified Qiagen DNeasy Blood and Tissue kit. Library preparation and SNP discovery were performed on the 282 highest-quality DNA extracts using Diversity Arrays Technology Pty. Ltd. (DART-seq) in Canberra, Australia (50). Each sample was processed following (51) and was sequenced across 3 lanes on an Illumina Hi-Seq 2500. Sequences were processed using in-house proprietary DART analytical pipelines. We used DART version 1.1.6 (52) in R version 3.5.1 (R Core Team, 2018) (53) to filter the DART-seq data for 10 separate *Eudyptes* datasets [based on previous systematic discussions (17, 35) and *SI Appendix, Table S5*]. For these *Eudyptes* datasets, we filtered on reproducibility ( $t = 1$ ) and filtered out monomorphic loci, loci with call rates  $< 0.95\%$ , all individuals with call rates  $< 0.90\%$ , all loci with trimmed sequence tags, and all loci that departed from Hardy-Weinberg equilibrium in any colony ( $P = 0.05$  following Bonferroni correction). We also obtained filtered RAD-seq datasets from an additional 5 penguin species generated and examined by refs. 14, 33, and 34, comprising Adélie ( $n = 87$ ), gentoo ( $n = 36$ ), chinstrap ( $n = 44$ ), king ( $n = 64$ ), and emperor ( $n = 110$ ) penguins (*SI Appendix, Table S8*). See *SI Appendix* for details.

**Phylogenomic Analysis and Population Structure.** To clarify the evolutionary relationships among our *Eudyptes* samples sequenced in this study, we created a maximum likelihood phylogeny using RAxML-HPC version 8.2.1 (54) (*SI Appendix, Fig. S5*). We undertook similar population structure analyses for *Eudyptes* as previously implemented for *Pygoscelis* and *Aptenodytes* in ref. 14 as follows: We calculated population summary statistics, including the number of private alleles, observed and expected heterozygosity, the inbreeding coefficient, and global and pairwise  $F_{ST}$  (Fig. 1 and *SI Appendix, Tables S5–S7*). Genetic clusters were visualized using 3 methods: PCoA using adegenet (55) (*SI Appendix, Fig. S4*), the Evanno method (56) in Structure version 2.3.4 (57) to estimate the most likely  $K$  (Fig. 1 and *SI Appendix, Fig. S4*), and DAPC using adegenet (*SI Appendix, Fig. S4*). We used the SNAPP tree set analyzer in BEAST version 2.4.7 (27, 58) to investigate gene flow between closely related *Eudyptes* species (*SI Appendix, Fig. S6*) based on our results and systematic discussions of refs. 17 and 35. While SNAPP analyses were previously generated for the emperor, king, and gentoo datasets (14, 33, 34), we also undertook SNAPP analyses for the chinstrap and Adélie penguin datasets obtained from (14) (*SI Appendix, Fig. S7*). See *SI Appendix* for details.

**Testing for Demographic Expansions.** We reconstructed population histories for 11 *Eudyptes*, *Pygoscelis*, and *Aptenodytes* species over the last 1,000,000 y by estimating the time and magnitude of demographic changes using 4 different approaches (northern rockhopper and Snares crested penguins were excluded from some analyses due to low sample size). Specifically, we reconstructed the demographic histories using CubSFS (Fig. 2A and *SI Appendix, Figs. S2 and S3 and Table S1*), obtained Tajima's D (*SI Appendix, Table S2*), identified the change in theta values as inferred by our previous SNAPP analyses (*SI Appendix, Figs. S8 and S9*), and tested for synchronous expansion using Multidice (Fig. 3B and *SI Appendix, Table S3*). As the *Eudyptes* and *Pygoscelis/Aptenodytes* datasets were obtained using different pipelines (DART-seq versus RAD-seq), we applied further stringent filtering to ensure consistency between the datasets (*SI Appendix*). While previous studies reported shallow population genetic structure within most *Pygoscelis* and *Aptenodytes* species (11, 14–16, 33, 34) (Fig. 1), given the relatively shallow  $F_{ST}$  values involved and reported  $K = 1$  (14, 33, 34), we consider this fine-scale structure likely to have evolved recently and broadly consistent with a scenario of high gene flow suitable for combined demographic analysis [with the exception of the gentoo penguin (14)]. To account for the deeper genetic structure observed in gentoo populations [e.g., 4 distinct lineages (14)] (Fig. 1), we limited most subsequent analyses of this species to one lineage (Fig. 1) (see ref. 14). For each *Pygoscelis*, *Eudyptes*, and *Aptenodytes* VCF file, we projected



the folded site frequency spectrum down to increase the number of segregating sites using EasySFS. We then adjusted the number of monomorphic sites in our site frequency spectrum to reflect the total number of monomorphic loci within each species following down projection. For all analyses, we assumed a generation time of 8 to 14 y (from ref. 42). For all models, we used a mutation rate of  $2.6 \times 10^{-7}$  per locus per generation (12, 15).

**Data Availability.** The raw data reported in this paper has been deposited in the NCBI Short Read Archive BioProject PRJNA589336 (<https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA589336>). Additional DArT-Seq files, Structure and SNAPP input and output files, and the site frequency spectrum (SFS) are also available on figshare (DOI: 10.6084/m9.figshare.c.4475300) (59).

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