Receding ice drove parallel expansions in Southern Ocean penguins.

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Abstract
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 hypothesise 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 analysing population genomic datasets spanning three 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 post-glacial 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/declining demographic histories inferred for four 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 post-glacial colonisation of sub-Antarctic 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.

Keywords
Sphenisciformes, Climate Change, Last Glacial Maximum, Refugia, Genomics.
Significance statement

We analyse population genomic datasets across three 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 post-glacial warming, contrasting with stable/declining demographic histories inferred for four 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.

Introduction

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 –25,000 years 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 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 (10-11).
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 (6-7, 12).
Recent genetic data, however, hint at major ecosystem-wide change following reductions in
southern winter sea-ice (7, 13-14). Importantly, past expansions can be reconstructed via
genetic analysis of modern populations (2, 15). While several studies of Southern Ocean
species have detected limited population genetic structure, consistent with recent
demographic shifts and/or gene flow (9, 13-14, 16-19), a comprehensive genome-wide
assessment of Southern Ocean wildlife is lacking. Moreover, as responses to climate change
can potentially vary among species (14, 20-21), distinguishing between concerted (multi-
species) versus idiosyncratic (single species) shifts may be crucial to forecasting responses to
future climate change (22).

Penguins (Sphenisciformes) are iconic marine birds that inhabit all major southern
landmasses, with their greatest species diversity in Antarctica and the sub-Antarctic (Fig. 1;
SI Appendix, Fig. S1). Although most penguins are natally philopatric (23), some can
disperse vast distances traversing major Southern Ocean fronts (24-25), and represent
important components of both coastal and marine ecosystems (26). Here we analyse several
thousand single nucleotide polymorphisms (SNPs) across 11 Antarctic, sub-Antarctic 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 re-colonisation of Antarctic and sub-
Antarctic coasts during post-LGM warming. In contrast, demographic histories inferred for
four temperate penguin species are relatively stable/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 (28), SNAPP (29), Tajima’s D (30) and Multi-dice (31) were based on 3,000-13,000 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/$F_{ST}$ analyses (see also (19)), whereas Snares-crested [E. robustus] and the northern rockopper [E. moseleyi] penguin were excluded from some analyses due to their small sample sizes (Fig. 1, SI Appendix, Tables S4-S5). These analyses revealed comparable postglacial $N_e$ expansions for six southern species (macaroni/royal, eastern rockhopper [E. filholi], Adélie [Pygoscelis adeliae], gentoo [P. papua], king [Aptenodytes patagonicus] and emperor [A. forsteri] penguins) (Fig. 2, Fig. 3a; SI Appendix, Table S1, Figs. S2-S3), with the emperor penguin expanding slightly earlier. Additionally, two of three demographic analyses supported recent expansion in a seventh species (chinstap [Pygoscelis antarctica]) (Fig. 3a). Notably, these seven species all predominantly occur south of the LGM sea-ice limit (Fig. 1; see [6-8, 23]). By contrast, four species inferred to have relatively stable/declining recent demographic histories (Fig. 3a, Figs. S2-S3) are all predominantly found north of the LGM sea-ice zone (Figs. 1-2): the northern rockhopper (Eudyptes moseleyi; Gough and Amsterdam Islands), western rockhopper (E. chrysocome; predominantly the Falkland Islands and southern South America), Fiordland-crested (E. pachyrhynchus, southern New Zealand) and Snares-crested (E. robustus, The Snares and Western Chain) penguins.
The expansion timeframes inferred for most southern lineages (20,000 – 15,000 years ago) correspond to a period of rapid post-LGM warming (27) (Fig. 2a; 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 [16, 32]). The magnitude of inferred postglacial N_{e} expansions is on average a 2.7-fold increase (ranging from 1.19 – 4.4 fold increase) (Fig. 2a; 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 Multi-dice to test for synchronous versus asynchronous expansions across the seven ‘expanded’ species identified based on our demographic analyses (Fig. 3a). To this end, we modelled a single expansion event within the last 50,000 years in which up to seven species co-expanded. The synchronous expansion event was inferred to have occurred 20,779 – 24,804 years ago, depending on the summary statistics chosen (Fig. 3b; SI Appendix, Table S3). While only two or three 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 Multi-dice 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 [13, 14, 33-34]) consistently revealed shallow genetic structure within species (Fig 1; SI Appendix, Figs. S4-S7; SI Appendix, Tables S6-S7). In all cases apart from gentoo penguins, we found that panmixia ($K=1$) was supported, but that using location priors found evidence for additional fine-scale structure, as previously reported [9, 13, 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, $F_{st}$, PCoA, Structure, DAPC, SNAPP and phylogenetic analyses for *Eudyptes, Pygoscelis* and *Aptenodytes* all revealed relatively shallow within-species genomic structure among southern populations (Fig 1; SI Appendix, Figs. S4-S7; SI Appendix, Tables S6-S7; [11, 16, 34-35]). In contrast to the recent genetic exchange inferred within most species, and between macaroni and royal penguins (18-19, 35), these analyses detected little or no admixture among species (Figs. S4-S7; SI Appendix).

**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, four species currently breeding north of the LGM sea-ice zone exhibited genetic signatures of relatively stable/declining demographies (Fig. 3a). Although estimates of precise LGM breeding ranges for penguins remain elusive (but see [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, Falklands islands, southern South America, and New Zealand’s southern islands (Fig. 2b; see [6-7]). Indeed, several recent studies have suggested that post-LGM reductions in sea-ice were accompanied by rapid re-
colonisation of high-latitude shores (7, 11, 14) (Fig. 2c). Recent demographic studies of
penguins (Adélie, emperor and king) (16-17, 32) and the southern elephant seal (37), for
example, have inferred rapid postglacial recolonisation events. By contrast, recent snow-
petrel analyses provide only limited evidence for such postglacial shifts (12). 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 (see Fig. 2b), some potential LGM refugia may
be suggested on the basis of current distributions (e.g. 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 (10, 11), including penguins (9, 38). However,
several penguin species can clearly traverse such boundaries (24-25), and this exceptional
dispersal ability may help to explain their apparently rapid biogeographic shifts in response to
changing climate (see also [37]).

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 (16-17) (i.e. $K = 1; F_{st} < 0.02$; Fig. 1 and 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 analysed 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 use of location priors at higher values of $K$ reveals additional, fine-scale population differentiation (Fig. 1 and Fig. S4), see also (9, 33-34), such structure can potentially evolve rapidly (e.g. 41). Interestingly, the relatively shallow differentiation observed within and among some colonies (e.g. emperor (9, 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 (9, 13, 17-18), and may have considerable relevance over ecological timeframes (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 (13, 42). As global temperatures continue to increase, mid-latitude biota will continue to shift towards the poles (11) or alternatively may
face extinction (6, 11). 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

DArT-Seq™ library preparation and filtering: DNA was extracted from 428 Eudyptes
penguin samples spanning six species (Fig. 1; SI Appendix, Fig. S1; SI Appendix, Table S4;
macaroni/royal penguins were combined; see [18-19]) using a modified Qiagen DNeasy
Blood and Tissue kit. Library preparation and SNP discovery was 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 three lanes on an Illumina Hiseq 2500. Sequences were processed using in-house
proprietary DArT analytical pipelines. We used DartR v1.1.6 (52) in R v.3.5.1 (R Core Team,
2018) to filter the DArT-seq™ data for ten separate Eudyptes datasets (based on previous
systematic discussions [18, 35], 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 five
penguin species generated and examined by (9, 33-34), comprising Adélie (Pygoscelis
adeliae; n=87), gentoo (P. papua; n=36), chinstrap (P. antarctica; n=44), king (Aptenodytes patagonicus; n=64) and emperor (A. forsteri; 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 newly sequenced in this study, we created a maximum likelihood phylogeny using RAxML-HPC v.8.2.1 (53) (SI Appendix, Fig. S5). We undertook similar population structure analyses for Eudyptes as previously implemented for Pygoscelis and Aptenodytes in (9), 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, Tables S5-S7). Genetic clusters were visualised using three methods: principal coordinates analyses (PCoA) using adegenet (54) (SI Appendix, Fig. S4); the Evanno method (55) in Structure v.2.3.4 (56), to estimate the most likely K (Fig. 1; SI Appendix, Fig. S4); and discriminant analysis of principal components (DAPC) using adegenet (SI Appendix, Fig. S4). We used the SNAPP tree set analyser in BEAST v.2.4.7 (30, 57) to investigate gene flow between closely related Eudyptes species (SI Appendix, Fig. S6), based on our results and systematic discussions of (18, 35). While SNAPP analyses have been previously generated for the emperor, king and gentoo datasets (9, 33-34), we also undertook SNAPP analyses for the chinstrap and Adélie penguin datasets obtained from (9) (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 years, by estimating the time and magnitude of demographic changes using four different approaches (northern rockhopper and Snares crested penguin were excluded from some analyses due to low sample size).
Specifically, we reconstructed the demographic histories using CubSFS (Fig. 2A; SI Appendix, Figs. S2-S3; SI Appendix, 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-S9); and tested for synchronous expansion using Multi-dice (Fig. 3b; 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 have reported shallow population genetic structure within most *Pygoscelis* and *Aptenodytes* species (9, 13, 16–17, 33–34) (Fig. 1), given the relatively shallow $F_{ST}$ values involved, and reported $K = 1$ ([9, 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 (9)). To account for the deeper genetic structure observed in gentoo populations (e.g. four distinct lineages [9]; Fig. 1), we limited most subsequent analyses of this species to one lineage (Fig. 1; see [9]). For each *Pygoscelis*, *Eudyptes* and *Aptenodytes* vcf file, we projected the folded allele frequency spectrum down to increase the number of segregating sites using EasySFS. We then adjusted the number of monomorphic sites in our allele 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 – 14 years (from [42]). For all models, we used a mutation rate of $2.6 \times 10^{-7}$ per locus per generation (14, 16).

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**Data availability**

Raw fastq reads are available from the Short Read Archive (DOIXXX). Additional DArT-Seq files, Structure and SNAPP input and output files, and the original and amended SFS are also available on FigShare (DOI:10.6084/m9.figshare.c.4475300).

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**Figure Legends**

**Fig. 1.** Sampling locations and genetic Structure plots for 11 penguin species (royal/macaroni are considered one species). The map (adapted from [6]) 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 (mid blue shading), Last Glacial Maximum (LGM) winter sea-ice (dark blue shading) (see [6-7, 8]), LGM land extent (green) and glaciation during the LGM (white). Four species (indicated by squares) breed north of the LGM sea-ice limit, whereas seven species (indicated by circles) breed in southern regions affected by LGM sea-ice. The top Structure plot for each species (top two for gentoo) represents the most likely number of genetic clusters 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 (9). Structure plots for Adélie, emperor, gentoo, king and chinstrap penguins are adapted from (9). 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 [9]). Numerical codes for sampling locations (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 Harbour (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); Point Géologie (POI); Pétrels island (PÉT); Cape Roget (ROG);
Cape Washington (WAS). The asterix on Marion Island indicates the “white-faced”
phenotype of macaroni/royal penguin. Coloured symbols (squares/circles) are consistent with
Figs. 2 - 3.

**Fig. 2.** Population expansions/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). 95% confidence intervals are given by solid colour
intervals. Median for bootstrap replicates is given by the dotted line, and the solid line gives
the demographic reconstruction for the amended SFS. A 50 thousand year record of Antarctic
temperature change (grey line in each plot) as estimated from the EPICA Dome C Ice Core
[27] is shown in each plot. The grey bar in each plot shows the LGM. B) shows the winter
sea-ice and sea-level during the LGM, with putative refugia shown (orange ellipses for sub-
Antarctic penguins; grey 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 sub-Antarctic 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 sub-Antarctic penguins,
while site names in white indicate possible refugia regions for Antarctic penguins. C) shows
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, while sites names marked in grey indicate locations where penguins may have bred during the LGM (as shown in B]). Note, these LGM breeding ranges in both B and C are uncertain. The maps have been adapted from (6). As the Snares crested penguin was included in other demographic analyses (see Fig. 3), the species is shown in both B and C. Coloured symbols (squares/circles) are consistent with Figs. 1 and 3.

**Fig. 3.** Summary of demographic results for 11 penguin species. A) shows 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 penguin [all south of the LGM sea-ice, represented by circles]) or ‘declining/stable’ (blue: northern rockhopper, western rockhopper, Fiordland crested, Snares crested penguin [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) Multi-dice results, suggesting a LGM expansion, with the mean of the co-expansion time parameter inferred at 24,065 years (mode: 20,778; median: 24,065). Coloured symbols (squares/circles) are consistent with Figs. 1 - 2.