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Genetic history, structure and gene flow among populations of *Belgica antarctica*, the only free-living insect in the western Antarctic Peninsula

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ABSTRACT

Changes in climate and environment can impact the sustainability of populations and biodiversity. Understanding population genetic diversity in the past and present can help us better predict species' responses to future environmental change. Antarctica has experienced drastic environmental change which threatens its biodiversity. In this study, we characterized the phylogeography and population genetic structure of *Belgica antarctica*, a wingless midge that is endemic to the western Antarctic Peninsula. This insect has adaptive features to withstand extremes in temperature, salinity, humidity, anoxia and pH. *Belgica antarctica* is widespread on widely dispersed islands of ice-free habitat, but questions remain regarding its genetic history, diversity and gene flow. We created nuclear-based, single nucleotide polymorphism (SNP) markers and genotyped 229 individuals from 11 populations to examine historical and current population genetic patterns. Our results support recent divergence among populations on different islands within the last 1 Mya. Furthermore, despite a lack of wings, *B. antarctica* exhibited frequent migration among islands, perhaps via ocean currents or phoresy with Antarctic vertebrates (*e.g.* seabirds). The close link between the evolutionary history of *B. antarctica* and the region's environment and ecology emphasize the importance of understanding its population dynamics to predict its persistence under environmental change.

1. Introduction

Species inhabiting extreme or fluctuating environments offer valuable insights on how the distribution of genetic variation influences population and species persistence. Since the contributors of biodiversity (*e.g.* genetic diversity, gene flow) inform our predictions on the consequences of climate change (Atkins and Travis, 2010), it is critical to measure genetic variation in the most vulnerable species. Many species have already encountered extirpation or range contraction due to climate change's environmental impacts, especially in polar regions (Hill et al., 2011; Norberg et al., 2012; Robinson 2022). One important region is the Antarctic Peninsula, where extreme environmental change in recent decades, such as rapid warming exceeding 2.5 °C, has had considerable impacts on both its landscape and biodiversity (Vaughan and Doake, 1996; Cook et al., 2005; Turner et al., 2009; Thomas et al., 2009).

In addition to the recent climate change, past geologic and environmental events continue to influence Antarctic biodiversity (Convey et al., 2018). After continental separation and additional volcanic activity, rapid cooling established major ice sheets during the late Miocene and early Pliocene (5-3 Mya). The Pleistocene period (2 Mya – 12,000 ya) included multiple glacial and inter-glacial events which caused cycles of isolation (*e.g.* glacial refugia) and connectedness (Webb and Harwood, 1991; Ingólfsson, 2004; Convey et al., 2009). These geological and temperature changes also altered the oceanic currents that impact local environmental conditions (Böning et al., 2008; Sokolov and

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Rintoul, 2009). The Antarctic Circumpolar Current (ACC), which developed in the late Oligocene (Kennett et al., 1975), connects all global ocean basins, transporting sediments, nutrients and heat. The ACC, which flows from west to east around the continent, may be an effective marine barrier to dispersal between Antarctica and neighboring continents for most species due to its force (Clarke et al., 2005; Fraser et al., 2012). However, transport could occur across the ACC via eddies and regional currents that form within the complex current formations (Clarke et al., 2005). The patterns of currents can also fluctuate under rapid climate change (*e.g.* storms and warming, see Fraser et al., 2018). The most relevant of these is the recently described Antarctic Peninsula Coastal Current (APCC), which flows both north and south from a point located near Trinity Island (close to Spert Island, the northernmost point in our collections, see Fig. 1) along the west coast of the Antarctic Peninsula (Moffat et al., 2008; Moffat and Meredith, 2018).

Vicariance, wind and currents tend to isolate Antarctic terrestrial biota, which display very strong signals of regional differentiation. Molecular phylogenetic and phylogeographic data suggest that regional differentiation and species endemism are the norm rather than the exception (Greenslade, 2018; Collins et al., 2019, 2020; Carapelli et al., 2020a,b). Antarctic species exhibit a high frequency of endemism (Greve et al., 2004; Pugh and Convey, 2008; Griffiths et al., 2009) and ancient species origins (Convey and Stevens, 2007; Convey et al., 2008; Vyverman et al., 2010). At a larger, regional scale there is very limited sharing of mite, springtail or nematode species between the Antarctic Peninsula and the continent across the Gressitt Line at the base of the Peninsula (Maslen and Convey, 2006; Chown and Convey, 2007; Convey et al., 2020). Barriers to migration and gene flow result from conspicuous impediments such as glaciers and the sea, along with others that may be less conspicuous such as the prevailing wind direction or related to extreme environmental events (Chown and Convey, 2007). Many islands in the Western Antarctic Peninsula have specific microhabitats related to their respective abiotic and biotic conditions. Islands differ in temperature, wind exposure, and terrestrial biodiversity which include common vertebrates (seals and sea birds such as penguins and shags) but also small invertebrates, plants and microbes (Convey, 2017). Many of these species have already been impacted by recent and drastic climate change (Ingels et al., 2012). For example, Emperor penguins may be extinct by 2100 (Jenouvrier et al., 2021), and other sea birds have shifted their abundances, distribution and foraging ranges (Croxall et al., 2002; Casanovas et al., 2015; Schrimpf et al., 2018; Korczak-Abshire et al., 2020). Changes in vertebrate community abundance and distribution are likely to cascade to lower trophic levels, which comprise the majority of Antarctic terrestrial biodiversity.

A notable, endemic resident of the western Antarctic Peninsula region is *Belgica antarctica* (Fig. 1A, B), a brachypterous midge (Family: Chironomidae) thought to be of ancient origin within the region (approximately 49 Mya) (Allegrucci et al., 2006). The life cycle of *B. antarctica* extends over two years, during which the insect overwinters in its larval stage (Sugg et al., 1983), overcoming extreme freezing temperatures along with other stressful environmental conditions. *B. antarctica* exhibits a number of physiological traits characteristic of extremophiles, including resilience to wide variations in salinity, pH and water availability, and year-round freeze tolerance (Lopez-Martinez et al., 2008; Elnitsky et al., 2009; Goto et al., 2011; Teets and Denlinger, 2014). Based on the mitochondrial gene *cox1,* populations on different islands show genetic differentiation and recent divergence within the last 1 million years (Allegrucci et al., 2012). However, data from a broader range of the genome is necessary to fully characterize diversity and structure in the western Antarctic Peninsula, which will lead to a better understanding of how genetic differentiation is distributed in *B. antarctica*, and how it may be impacted under current and future climate change.

In this study we characterized the evolutionary history and population genetic structure of *B. antarctica* in the western Antarctic Peninsula and investigated the potential for gene flow in a wingless insect distributed on several islands. Our goal was to use nuclear based molecular markers to confirm the recent divergence suggested by mtDNA, but also determine the current levels gene flow and population structure. We used *B. antarctica* collections from 11 populations and 7 islands (Fig. 1). We included phylogenetic reconstruction with molecular

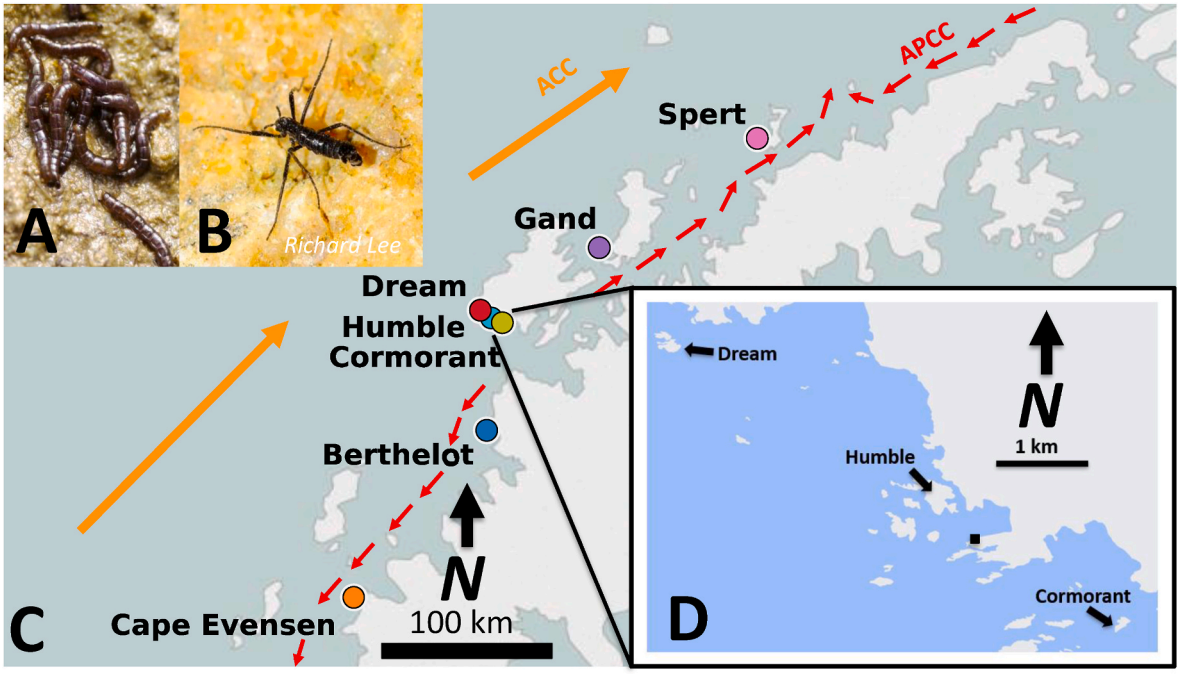

Fig. 1. *Belgica antarctica* (A-larvae; B-adult) and sampling locations. Samples were collected by hand among sites along the western coast of the Antarctic Peninsula (C). Note: Trinity Island is located near Spert Island. Dream, Humble and Cormorant Islands (D) are geographically proximate near the Palmer Research Station (Black square in D) (C, D images taken from GoogleEarth). Colored arrows represent predominant ocean currents (red: Antarctic Peninsula Coastal Current; orange: Antarctic Circumpolar Current based on Moffat and Meredith (2018)).

dating, Approximate Bayesian Computation Random Forests (ABC-RF) and estimates of admixture and migration rates to reconstruct patterns of genetic variation and diversity over both historical and recent time scales. Our data can provide information for possible conservation and persistence of Antarctica's only free-living insect.

2. Materials and methods

2.1. Biological material and DNA extraction

Individual larvae were hand collected from 11 populations distributed on 7 islands in the western Antarctic Peninsula, spanning >350 km, in 2008 and in 2010 field seasons (Fig. 1, Table 1). We collected from 3 islands near the Palmer Research Station (*i.e.* Palmer group: Humble, Cormorant and Dream Islands); from these islands we included multiple collections to estimate genetic structure on a microgeographic scale. Samples were preserved in ethanol or frozen in liquid nitrogen, stored at −80 °C, and transported to the Michel Laboratory at The Ohio State University—Wooster, Ohio. Genomic DNA was extracted using the DNEasy Blood and Tissue kit (Qiagen Inc., Germantown, MA), eluted in 50 μL of TE buffer (10 mM Tris-HCl pH 8.0 and 1 mM de EDTA pH 8.0) and stored at −20 °C.

2.2. SNP marker panel construction

Full details of marker construction can be found in Supporting Information 1. Given that our goal was to produce a core panel of nuclear based polymorphic SNPs for use in population genetic analyses, we first screened whole-genomes for polymorphism using two populations. Briefly, 24 individuals from Humble and Dream Island (12 each island) were whole-genome sequenced with paired-end chemistry on a HiSeq 2500 System (Illumina Inc., San Diego, CA). Each individual had its own sequencing library, indexed with Illumina specific barcodes and pooled prior to sequencing on one lane. We processed Illumina's raw reads with Trimmomatic v0.39 (Bolger et al., 2014) to remove sequencing adapters (with "ILLUMINACLIP:TruSeq3-PE.fa:2:30:10") and to remove low quality reads and bases (we scanned and removed bases in four-nucleotide windows, cutting the base when quality drops below 20 PHRED score, and removed reads with minimum length <50 bases, with SLIDINGWINDOW:4:20 MINLEN:50). Reads that passed quality the quality control were aligned to the available reference genome (Kelley et al., 2014) with Bowtie2 v2.2.6 (Langmead and Salzberg, 2012) using default parameters. SAMtools v1.2 (Li et al., 2009) was used to convert SAM to BAM, to sort the BAMs, and remove PCR duplicates. A total of 225,244,498 reads uniquely mapped to the reference genome (with mapping quality, MAPQ > PHRED 30), yielding a mean read mapping coverage of 6.45 × . After filtering with Vcftools v0.1.15 (Danecek et al 2011), we retained only biallelic SNPs with a genotype quality (GQ) quality higher than 20 PHRED score and mean-site depth between 30 and 80 reads, and present in at least 80% of the individuals. We initially identified 1260 SNPs in total from the Dream and Humble populations with mean-site depth of ~50X, mean GQ ~70, Root-mean-square mapping quality of covering reads (MQ) > 10 (see Supplemental Information 1).

We further refined our SNP list to develop a core set for larger population genetic analyses. Our goal was to find polymorphic SNPs within individual amplicons (~150bp long) that could be analyzed with the Fluidigm Access Array, which sequences 48 unique amplicons simultaneously. In this core set, we included SNPs if they passed the following filtering criteria: 1) no significant deviation of Hardy-Weinberg equilibrium based on Fisher's exact tests (See Supporting Information 1, Section IV); 2) evidence of both homozygote and heterozygote genotypes among all sequenced individuals; and 3) were at least 5 bp apart from another SNP in the scaffold. Based on this approach and from the preliminary sequencing, we developed 48 amplicons that included at least 1 SNP.

Table 1
Information on collection sites for *B. antarctica.* N represents the number of individuals of each population that we retained after initial amplicon sequencing processing and filtering.

Population	Island	Latitude	Longitude	Collection Year	N
B1	Berthelot	65°50.70′S	60°56.90′W	2008	10
C1	Cormorant	64°47.60′S	63°57.87′W	2010	31
C2	Cormorant	64°47.63′S	63°58.06′W	2010	32
CE	Cape Evensen	66°08.70′S	65°43.40′W	2008	8
D1	Dream	64°43.53′S	64°13.40′W	2010	28
D2	Dream	64°43.54′S	64°13.55′W	2010	31
GI	Gand	64°24.40′S	62°48.60′W	2008	7
HD	Humble	64°45.90′S	64°04.10′W	2010	23
HO	Humble	64°45.87′S	64°05.31′W	2010	31
HP	Humble	64°45.89′S	64°05.13′W	2010	25
S1	Spert	63°50.70′S	60°56.90′W	2008	3

2.3. Amplicon sequencing & genotyping of B. antarctica populations

The 48 amplicons (see declaration of supporting data on GitHub) isolated from our initial marker development were sequenced from a total of 235 individuals (see Table 1; this also included the same 21 individuals from Humble and Dream Island from the whole genomic sequencing). Samples were sequenced in two batches, with 5 samples of the first batch repeated in the second batch for quality control, providing a total of 240 libraries. Prior to genotyping, all individual DNA extractions were diluted to a standard 15 ng/μL DNA concentration. Sequencing libraries were prepared with DNA sequencing library preparation reagents and multiplexed PCR was performed following protocols for the Fluidigm® Access Array™ (FAA) System (San Francisco, CA). Amplicon sequencing libraries were then sequenced in two lanes of Illumina's MiSeq ® (one for each batch). We initially sequenced 20% of the samples with the paired-end protocol to estimate sequence coverage and to determine how many samples could be sequenced in the same lane (See Supporting Information 1, Section VII; details of raw read counts and the number of mapped reads per sample and per amplicon are provided in the GitHub, see below).

We automated the raw read processing mapping and variant calling by including all the steps in the PypeAmplicon software (available at zenodo https://doi.org/10.5281/zenodo.1490421; more detail about the pipeline can also be found at Supporting Information 1, Section VIII). Reads were clustered by similarity (>75%) with USEARCH v9.2 (Edgar, 2010). Orphaned reads (from PE data) and clusters with fewer than 25 reads were discarded. Reads that passed the clustering filter were then split into different files with BBsplit v36.64, according to their similarity to each reference amplicon. This served as an additional filter of reads within the cluster that accumulated sequencing errors. Filtered reads were then aligned to their amplicon reference using BBmap v36.64 (both BBsplit and BBmap are part of BBtools, https://sourceforge.net/projects/bbmap/) with default parameters. The BAM file of each amplicon was then merged to produce a multi-sample BAM file. SNPs were called using FreeBayes v1.0.2 (Garrison and Marth 2012). Vcftools v0.1.15 (Danecek et al0.2011) removed SNPs with >5% missing data, individuals with >5% missing data, SNPs with a minor allele frequency <5%, SNPs with greater than 80% linkage disequilibrium with another variant, and all indels and multiallelic SNPs. We also removed any mitochondrial SNPs and retained only the nuclear SNPs. We estimated the observed and expected heterozygosity with Bartlett's test of homogeneity of variances in the R *stats* package (R Core Team, 2013), and tested whether all SNPs exhibited Hardy-Weinberg (HW) equilibrium or any linkage disequilibrium (LD) using the package *hierfstat* (Goudet, 2005). Those not in HW or LD equilibrium were removed, as well as 1 SNP that mapped to mtDNA. After this filtering and quality control among all populations, our population genetic analyses included 64

SNPs distributed across 47 amplicons from the nuclear genome (note a few amplicons had multiple SNPs, analyses using only 1 SNP per amplicon did not change results, data not shown).

2.4. Phylogenetic reconstruction

To investigate the evolutionary history of these 11 populations, we first used the SNAPP plug-in for BEAST2 (Bouckaert et al., 2019) to estimate a species tree based on the nuclear SNPs. For computational tractability we randomly selected five individuals from each population for inclusion in the tree reconstruction and ran SNAPP using the default priors. We ran the analysis for 100,000,000 generations, sampling every 1000 generations. We ran an additional independent analysis for 1 million generations using the same parameters to provide confirmation of convergence. We assessed results for convergence using Tracer (visually assessing the distribution of parameters, and ensuring ESS >200) (Rambaut et al., 2018) and summarized the samples, removing the first 20% as burn-in, using TreeAnnotator (Bouckaert et al., 2019). The resulting tree, based on molecular patterns among individuals, was created using FigTree v1.4.4, and the major consensus trees using Densitree (Bouckaert and Heled, 2014). To convert SNAPP's divergence time estimate from substitution/sites to absolute times among populations, we employed the approach of Harrington et al. (2018) and previous estimates of divergence time between Spert Island, and islands near Humble, Cormorant and Dream (0.870 Mya, see Allegrucci et al., 2012).

We also used an Approximate Bayesian Computation (ABC) with Random Forests (RF) analysis as an independent estimate of divergence times without SNAPP's constraints on previous estimates. To simplify the calculations, we only analyzed the populations that composed the Palmer group, which also reduced the total number polymorphic SNPs to 24. We pooled subpopulations from the same island (*e.g.*, the Cormorant's subpopulations C1 and C2 were pooled together) to increase the sample size and consequently the power of the parameter inference and demographic model choice. Briefly, we first performed an ABC-RF model choice by comparing 11 different scenarios (varying divergence, admixture, bottlenecks, etc.; see Supporting Information 2). This resulted in a reference table used in the model choice step by running 20,000 simulations for each scenario. With this reference table, we grew an independent random forest (RF) with 2000 trees for each model comparison. We then calculated the local (posterior) error rate (defined as one minus the posterior probability of the selected scenario in each model choice analysis, as in Chapuis et al., 2020), and the support each scenario received for each model comparison in order to choose the best trained RF for scenario evaluation. For the best supported scenario(s), we ran an additional 200,000 simulations and for each parameter we estimated, we created 1000 independent RFs. For model choice and parameter estimation, we simulated the data using a wide prior distribution of log-uniform distribution, which allowed a more expanded prior range to capture the signal of ancient events (>1 Mya) if present in the dataset.

2.5. Population genetic analyses

We tested for non-neutral evolutionary processes by first estimating global and population-specific F_{IS}, determining significant deviations from zero using a bootstrap with 1000 replicates (custom R script available upon request). We also used DnaSP v.6 (Rozas et al., 2017) to estimate Tajima's D. Additionally, we used Bayesian inference to assign individuals to clusters representing ancestral variation and studied the distribution of those clusters of ancestral variation among populations using fastSTRUCTURE (Raj et al., 2014). We tested a range of population models (*i.e.* range of potential populations) assigning genetic variation to 2–11 ancestral clusters and then selecting a model that exhibited optimal complexity. This assignment was performed without respect to sample location (*i.e.* no priors were used). We visualized the admixture proportions of this model and the next most complex model using the R package *ggplot2* (Wickham, 2016). We also performed a principal component analysis (PCA) to investigate clustering of genetic variation among populations using the R package *adegenet* (Jombart, 2008). Both the fastSTRUCTURE and PCA analysis were repeated using only the Humble, Cormorant and Dream Island populations as they contained the largest sample sizes and to investigate cryptic population structure. We also used classical population genetics to study differentiation among populations with the *hierfstat's* R package. First, we estimated global F_{ST} (Cockerham and Weir, 1984), and then pairwise F_{ST} between populations. We also used Analysis of Molecular Variance (AMOVA) in the R's package *ade4* (Dray and Dufour, 2007) to test for significant differentiation, first testing a nested model of populations within islands, then a model including island only. We tested for isolation by distance among populations by calculating geographic distance among all populations using the R package *geosphere*, then used a partial Mantel test in the package *ade4* to compare the geographic distance matrix with one of genetic distance (computed using the *adegenet* package using a Euclidean model).

2.6. Migration rate estimate

To more specifically estimate migration among islands, we used the software program Migrate-n (Beerli, 2009). We estimated frequencies using the data, specified a transition-transversion ratio of 2 and an alpha of 1 for rates distribution. Starting parameters were derived from an F_{ST}-like calculation, using a theta model, and we predicted all loci to have a constant mutation rate. During the analysis, the genealogy was updated with a frequency of 0.5, and a Metropolis-Hastings sampler was used together with a uniform prior and no heating scheme. To compare migration with geographic and genetic structure we used a *t*-test in the R *stats* package (R-core team 2013) to test for differences in migration rate between populations located within an island vs. populations among islands which are separated by ocean channels and prevailing currents. We also used a linear model to test for any significant association between migration rates and pairwise F_{ST}.

3. Results

3.1. Amplicon sequencing and genotypic data analysis

A total of 30,302,520 reads were produced with the initial PE sequencing, with an average 631,303 reads per sample and an average raw read coverage of ~13 K reads per sample, per nucleotide. For the second batch, a total of 23,914,076 reads were produced with an average of 124,552 reads per sample, and an average raw read coverage of ~2500 reads per sample per nucleotide (data present on GitHub, see below). All but two PE libraries had raw read depth similar to the average raw read depth of 13,152. For SE libraries, raw read depths were more heterogeneous, with a minimum of 40,291 and maximum of 4,482,416. Despite this variation, the average raw read depth was 2595 per sample/amplicon.

After filtering raw variants and individuals with >5% missing data, we retained the genotypes of 229 out of 235 individuals at 64 SNPs across the nuclear genome. While this core set of SNPs included a few amplicons with multiple SNPs, no linkage disequilibrium as found (Supporting Information 3) Over all samples, observed heterozygosity did not differ from expected heterozygosity ($K^2 = 1.196$, df = 1, p = 0.274). Estimations of the inbreeding coefficient F_{IS} were significantly negative in all populations, with the exceptions of Cormorant-1, Humble-D and Spert (Supporting Information 3) and F_{IS} across populations was −0.225 ($P = 0.01$). Tajima's D was also negative (−0.747) but not significant ($P = -0.233$).

3.2. Phylogenetic evolutionary history

A reconstruction of the evolutionary relationships among these populations suggested that divergence was associated with a north-south cline (Fig. 2A), where Spert Island was found to be ancestral to the other sampled locations, followed by Gand Island. Populations on the same island (Humble, Cormorant and Dream) grouped together with high-support; however, there was ambiguity in relationships among islands (Fig. 2B). For example, populations on Humble and Cormorant showed low posterior values associated with divergence compared to the Dream/Berthelot/Cape Evensen populations; the split between Dream and Berthelot/Cape Evensen also had low posterior values. When we examined alternative relationships using Densitree we observed that Gand Island occasionally grouped with the Humble Island populations, and the Dream Island populations frequently grouped with the Humble and Cormorant Islands populations. We estimated the oldest divergence to have occurred 0.870 Mya (range 0.582 Mya to 1.18 Mya) and divergence among populations from the same island on the scale of less than 100 thousand years ago (Fig. 2C). ABC-RF determined scenario 1 (out of 11 scenarios) to best fit our data. Trained RF rejected non-equilibrium scenarios (scenarios with population size changes), favoring an equilibrium with divergence or an admixture scenario. Based on scenario 1, Cormorant and Humble populations split around 49,000 ya (t_1), and the ancestral population of these two populations split from the Dream population ~0.189 Mya (t_2) (Fig. 3, Supporting Information 2). Divergence times estimates obtained with ABC-RF were more recent

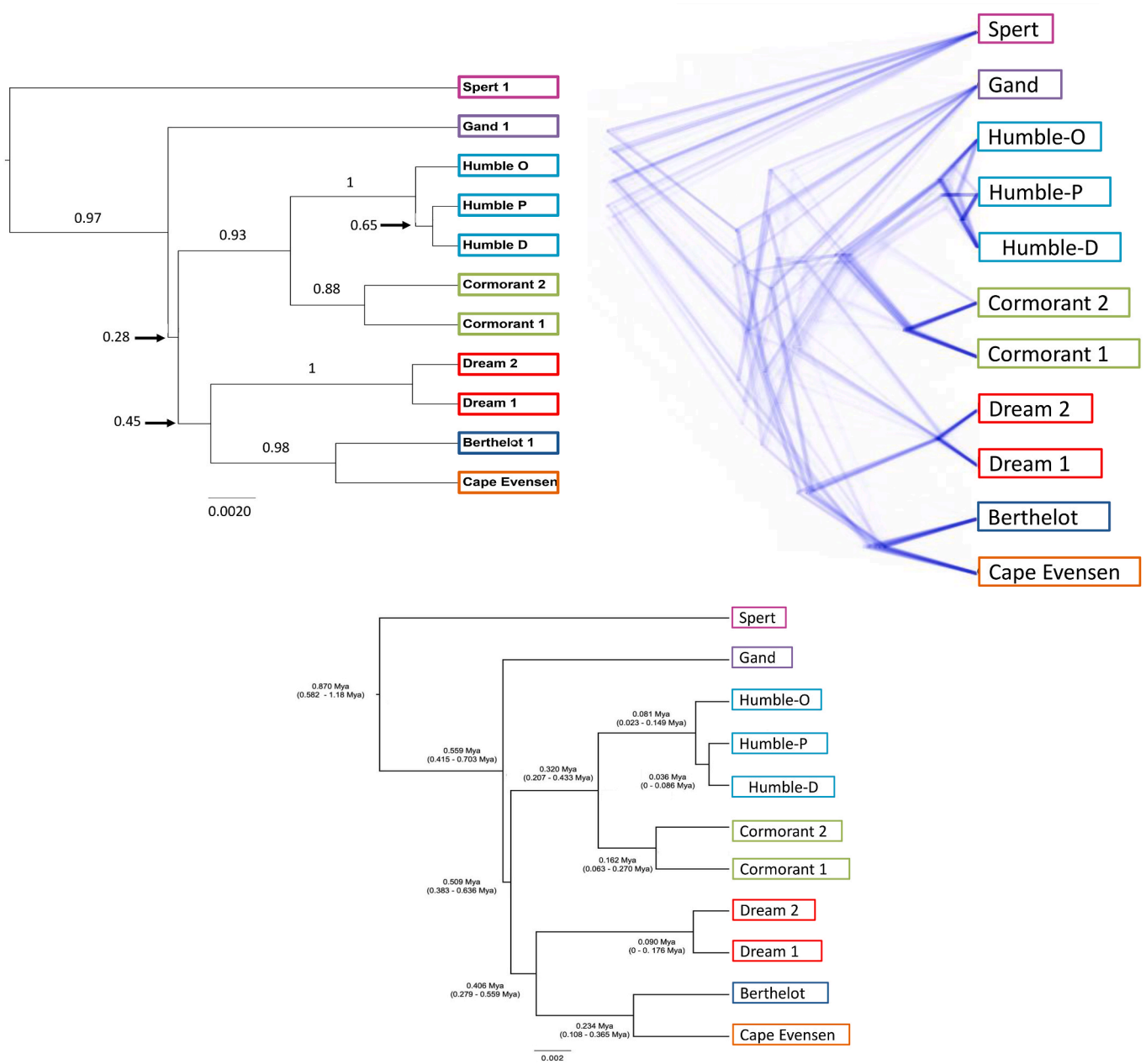

Fig. 2. Phylogenetics of *B. antarctica* in the western Antarctic Peninsula region. (A) Reconstruction of phylogenetic relationships among populations using the Bayesian software package BEAST. Colors correspond with sampling locations (Fig. 1); each population label represents variation from the 5 sampled individuals of that population. Node labels represent posterior probabilities. Branch lengths represent mutations per site. (B) Visualization of the majority consensus trees from the BEAST analysis, showing nodes with ambiguous configurations. (C) Dating of divergences were calibrated assuming the divergence time between Spert and Palmer's populations occurred 0.870 Mya (Allegrucci et al., 2012). Errors represent 95% confidence intervals estimated using the distribution of SNAPP trees (see methods). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

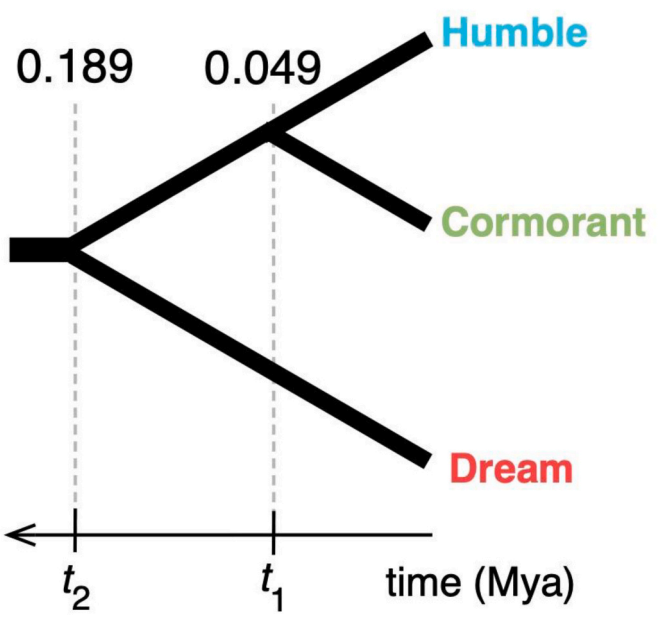

Fig. 3. ABC-RF estimated divergence time among Palmer Group populations. Estimates of t_1 and t_2 in Mya were obtained for scenario 1, see Supporting Information 5.

than SNAPP-BEAST2, but all Palmer group islands diverged within the past ~0.500 Mya.

3.3. Population genetic variation

An examination of genetic variation using PCA revealed substantial similarity among populations, even between different islands (see Supporting Information 3). Principal component 1, which comprised 23.48% of the total variance, revealed a separation between the Humble/Cormorant/Spert populations and the Dream/Gand/Berthelot populations, although clusters were not completely discrete. We also observed some separation between Humble and Gand/Cape Evensen populations along the PC2 (12.71%), PC3 (11.40%), and PC4 (7.92%) axes, but, in general, variation was widely shared among populations. When examined using a reduced dataset of only one SNP per amplicon, these same patterns were replicated (Supporting Information 6, Section IV).

Results of fastSTRUCTURE indicated that the model with the highest marginal likelihood of explaining variation in these populations included two clusters (marginal likelihood = −0.236; Fig. 4A). Visualization of these results suggested that populations fell into two groups, but none of the populations had >50% average identity with a secondary cluster (Fig. 4B). Humble populations appeared similar to Cormorant populations in their degree of admixture, while Berthelot, Dream, Cape Evensen and Spert shared a small but similar proportion of the secondary cluster. We also visualized results of the model with the second highest marginal likelihood (K = 3, marginal likelihood = −0.273; Fig. 4C), and observed individuals with representation of a third cluster from Berthelot, Dream and Gand Islands with smaller representation from Cape Evensen, Humble-D and Humble-O. Spert, Cormorant, and Humble-P populations lacked individuals with a discernible representation from this third cluster. A separate analysis using only Humble, Cormorant, and Dream island populations provided the same patterns, with Dream islands showing evidence of a separate cluster (Supporting Information 3, Section VI). The membership proportion represented in orange in Fig. 4b is consistent with the separation of Dream population from Humble and Cormorant populations on the PCA and on the phylogenetic trees. []

Measures of genetic structure indicated slight to moderate genetic differentiation among populations (global F_{ST} = 0.133, P = 0.01), despite their distribution among islands and the fact that adults are wingless. Pairwise FST between populations ranged from 0.007 (C1–C2) to 0.160 (S1-HP, Fig. 5C; Supporting Information 3). Among islands, the lowest pairwise F_{ST} occurred between HP and C2 (FST = 0.0153). Similarly, an AMOVA revealed significant structure among islands and among populations (among islands: 28% variance, P = 0.01; among populations: 4% variance, P = 0.01; within populations: 68% variance, P = 0.01; Supporting Information 6).

3.4. Estimating rates of migration

Differentiation can be biased by geographic distance between populations (shown in Fig. 5A), and a permuted Mantel test revealed significant isolation by distance among these populations (R^2 = 0.578, P = 0.022). We included isolation by distance as a prior to estimate migration among *B. antarctica* populations. Rates of migration varied between 13.67 migrants per generation (median posterior) to 471.67 migrants per generation (Fig. 5B, Supplemental Information 3). Four populations exhibited consistently high immigration rates: Berthelot, Cape Evensen, Gand and Spert, while patterns of emigration were more variable across all populations. An exception to this was Berthelot, which consistently contributed large numbers of migrants to almost all populations. Different populations on the same island varied in the amount of immigration and emigration; for example, migration from Humble-O to Dream-2 was much higher than from Humble-O to Dream-1. Similarly, the estimated migration from both Dream populations was much higher to Cormorant-1 than Cormorant-2, but the alternative was much less likely to occur (*i.e.* from Cormorant to Dream). An examination of migration among just Humble, Cormorant, and Dream islands replicated these patterns (Supporting Information 6, Section IX). A comparison of estimated migration rates among and within islands suggested that migration was much more frequent via marine (*e.g.* sea and air) environments than land (t = −22.94, df = 93.88, $P < 0.0001$; see Supporting Information 3). Contrary to our expectations, rates of migration were slightly but significantly positively correlated with F_{ST} (β = 0.0001, $F_{(1,108)}$ = 22.91, R^2 = 0.17, $P < 0.0001$; see Supporting Information 3). This unexpected result was not explained by the contribution of unequal migration rates to the increased estimated F_{ST}, as suggested by Wilkinson-Herbots and Ettridge (2004), since populations exhibiting a greater migration inequality were not more or less differentiated ($\beta = 5.039 \times 10^{-5}$, $F_{(1,53)}$ = 0.938, R^2 = −0.0011, P = 0.337).

4. Discussion

An organism's past and current population history can help predict its adaptability and persistence under rapid environmental change. Characterizing the history of Antarctic species, which have endured some of the most drastic long-term and recent environmental change, provides inferences for how other organisms may fare under similar climatic and environmental shifts. This study represents a phylogenetic and population genetic characterization of *B. antarctica*, one of the few extant, endemic, terrestrial animal species to inhabit Antarctica. Our results provide new insights into this extremophile, in particular that divergence among islands generally occurred within the past 1Mya, and that populations exhibit complex patterns of asymmetric migration despite being wingless as adults.

4.1. Phylogeographic divergence of B. antarctica populations

Using SNPs amplified from across the nuclear genome in different phylogenetic analyses, we dated population divergence among islands to be between 1.18 Mya – 49,000 ya, with the more geographic proximate islands of Humble, Dream, and Cormorant populations having diverged within the last 509,000 to 49,000 ya. The north to south divergence-cline we observed in our phylogenetic reconstruction

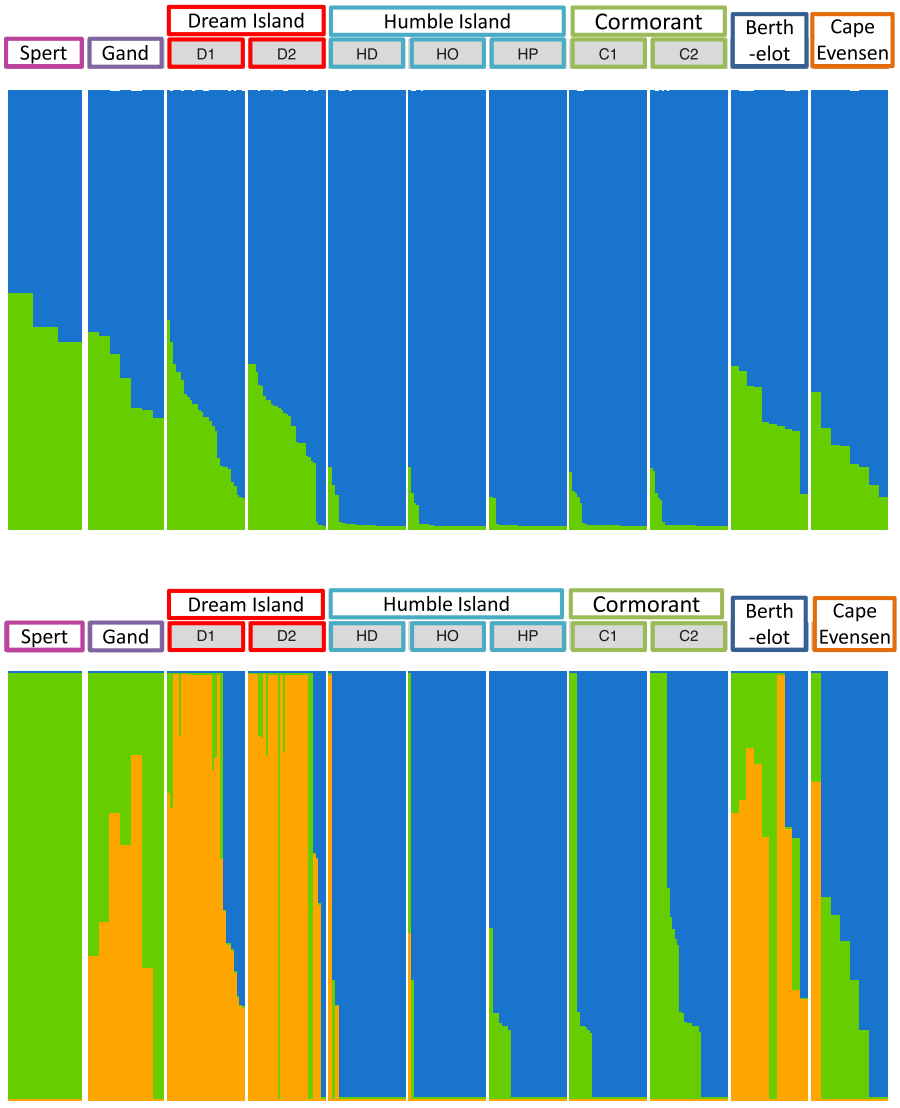

Fig. 4. Population genetic structure of *B. antarctica*. Examination of the marginal likelihood of models with assignment to 2–11 clusters (A) indicates that genetic variation falls in two clusters. (B) FastSTRUCTURE results of the most likely, 2-cluster model indicates populations fall in two groups based on high or low amounts of admixture. (C) FastSTRUCTURE results of the 3-cluster model.

supports an initial southwards establishment from the northern Antarctic Peninsula (or the South Shetland Islands, not sampled in this study) similar to north-south clines observed in other Antarctic Peninsula invertebrates (McGaughran et al., 2019).

Our divergence estimates concur with previous studies using the mitochondrial gene *cox1*, which suggested divergence from populations on Livingston Island (a northern island) to have taken place 1.26 Mya, and divergence from Spert Island between 0.75 and 1 Mya (Allegrucci et al., 2012). Divergence among Berthelet, Cape Evensen and Gand was estimated to have occurred less than 0.25 Mya (Allegrucci et al., 2012). Other sympatric Antarctic invertebrates exhibit similar patterns of divergence: for example, population divergence and expansion in the springtail *Cryptopygus antarcticus antarcticus* occurred within the past million years (McGaughran et al., 2010, 2019; van Vuuren et al., 2018). Evidence from our analyses and others suggest that population genetic patterns in *Belgica antarctica* reflect more recent activity than older geological and climatic shifts. We date population divergence here to the Pleistocene, a time of dramatic fluctuations in climate and ice coverage of Antarctica. Advances and retreats of ice sheets during that time led to isolation among species globally, and Antarctic populations were no exception. Periods of extensive glaciation resulted in retreat of many species to glacial refugia, followed by subsequent expansion within the last couple million years ago (Convey and Smith, 2005; Convey and Stevens, 2007; Fraser et al., 2012, 2014). The constant advance and retreat of ice sheets, both in the past and more recently, have also impacted Antarctic ocean currents and biodiversity, which may influence migration and gene flow in *B. antarctica* (Fraser et al., 2018).

4.2. *Many by marine, few by land—Migration of B. antarctica among Antarctic islands*

Despite observing signatures of historical population divergence, we detected recent migration in *B. antarctica* on the order of hundreds of individuals per generation. This migration is both variable and asymmetrical among populations, even within the same island. Evidence of substantial migration could explain the significantly negative estimates of F_{IS} we observed among populations if this migration somehow leads to preferential outbreeding. Greater migration rates among populations connected by marine environments than by land indicate the most likely mode of dispersal for *B. antarctica* is by the sea or air, which has been observed in other terrestrial Antarctic and Arctic arthropods (Coulson et al., 2002; Gressitt, 1965). Larvae of the closely related species and

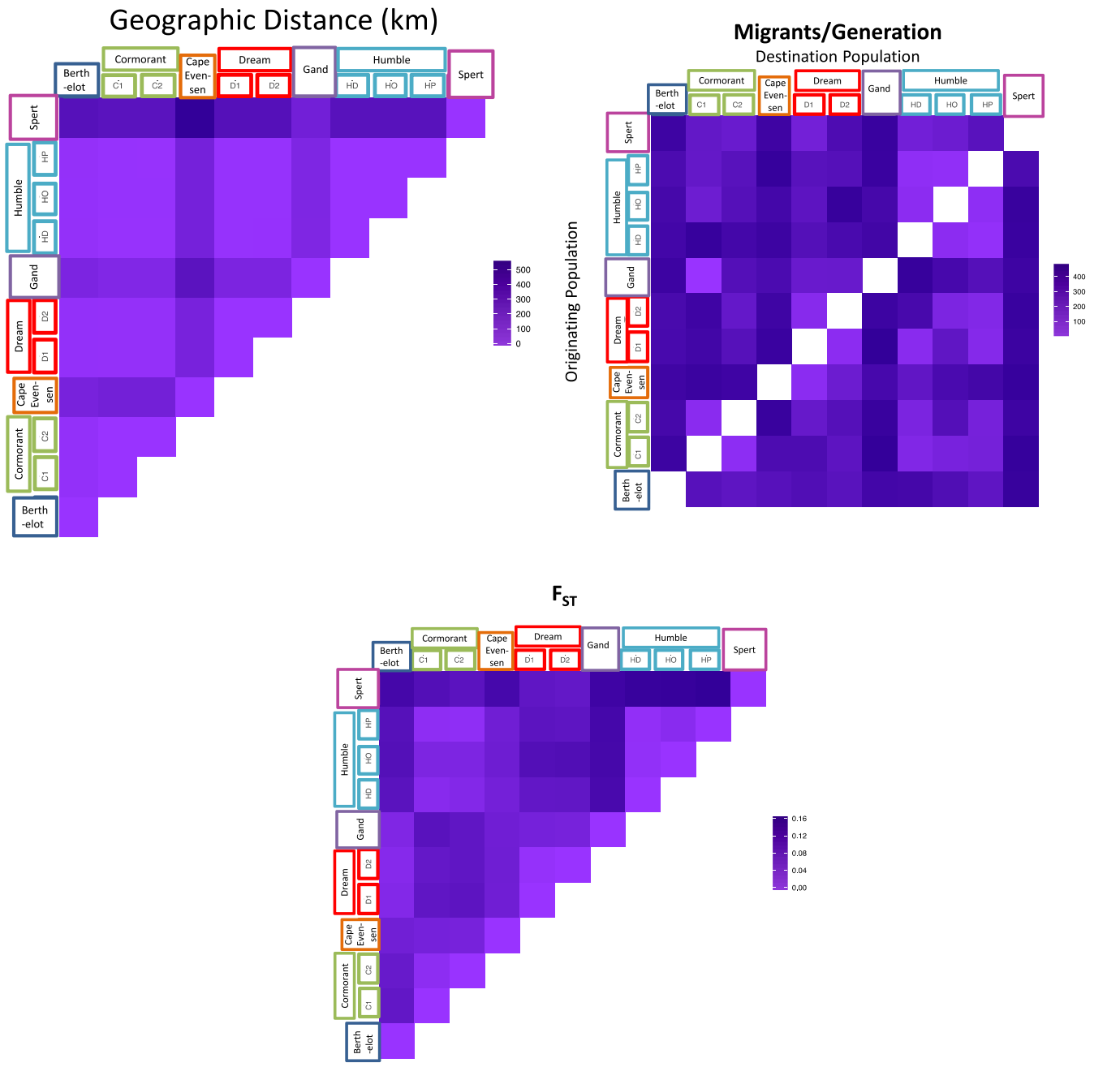

Fig. 5. Migration and genetic isolation by distance. Different visualizations of distances between populations are colored with reference to the sampling locations (Fig. 1). (A) Pairwise geographic distance was compared with genetic variation and revealed significant isolation by distance ($p = 0.003$). (B) Rates of migration between populations were estimated using the Bayesian program Migrate-n. Migration rates are shown from the populations on the y-axis to the populations on the x-axis. (C) Pairwise F_{ST} between populations reveals structure of genetic variation reflective of the phylogenetic relationships and *a priori* clustering.

sub-Antarctic native *Eretmoptera murphyi* are tolerant to salinity and would likely survive a marine-based invasion of the western Antarctic Peninsula (Bartlett et al., 2021). As the two species share similar stress tolerance characteristics relating to salinity and submergence (Elnitsky et al., 2009), this highlights that such dispersal in *B. antarctica* is highly plausible. Our estimated migration patterns do not strictly follow the direction of the APCC at a broad scale in this region (northward and southward from the central populations of Humble, Dream and Cormorant) in contrast to other species in this region (Muñoz-Ramírez et al., 2020). However, contemporary current patterns at a fine scale among these islands exhibit great complexity (Gille et al., 2016; Bartlett et al., 2021) and tend to fluctuate throughout the seasons (Heil and Allison, 1999). Larvae can also adeptly respond to different levels of salinity and osmotic stress (Lopez-Martinez et al., 2008). Many other chironomid larvae are aquatic and adults use surface tension to remain on water surfaces (as do springtails – Coulson et al., 2002), and we have observed *B. antarctica* adults on the surface of seawater. Migration could also be achieved through phoresy on various vertebrates inhabiting the region, with entrapment in seal fur perhaps being plausible (Bartlett et al., 2021), or within clumps of vegetation transferred into the sea by meltwater or island to island by marine vertebrates (*i.e.* birds, Parnikoza et al., 2012, 2018). *Belgica antarctica* is frequently common around penguin and seal rookeries and larvae could become attached to feathers or fur during transit. We have also observed birds such as the blue-eyed

shag flying with large clumps of grass and moss, which could conceivably contain *B. antarctica*.

An alternative hypothesis to explain migration patterns in *B. antarctica* may be wind-mediated, as observed or proposed in other Antarctic terrestrial arthropods (Hawes et al., 2007; Vega et al., 2020) that navigate cyclonic circulation patterns. Aerial trapping on Lagoon Island (Marguerite Bay) suggests that large numbers of springtails (~150,000 in one month) are transported at least on a local scale by air. The greatest impediment to such dispersal in arthropods arises from desiccation during transfer (Biersma et al., 2018). As *B. antarctica* is extremely tolerant of desiccation (Elnitsky et al., 2009; Teets and Denlinger, 2014), their movement via wind may be plausible. However, wind-mediated dispersal would likely show connections among populations that are closer on the same island, and not connected by more disparate marine environments as our data suggest. Regardless of the mechanism, our data indicate substantial migration among islands, therefore, any alterations in either the marine currents or regional vertebrate biodiversity could secondarily alter the evolutionary history and potential persistence of *B. antarctica*.

Despite migration estimate, we observed slight to moderate population structure both among populations on the same island and among islands. F_{ST} among populations within islands, though significant, was low. Previous work using mitochondrial gene data also estimated high regional structure (F_{SC} = 0.608; Allegrucci et al., 2012). Other invertebrates in this region exhibit similarly high structure among island populations, reflecting isolation during glaciation events (McGaughran et al., 2010, 2019; van Vuuren et al., 2018), though these studies found no evidence of contemporary gene flow. Population structure using nuclear SNP data in sympatric *Cryptopygus* springtails revealed a very similar pattern; pairwise F_{ST} ranged from 0.08 to 0.296 within the Antarctic Peninsula (McGaughran et al., 2019).

Dream, Cormorant and Humble are the geographically closest locations (found near Palmer Station), yet they show a pattern of genetic relationships that does not entirely correlate with their geographical proximity. Dream was more similar to populations outside the Palmer group. The admixture analysis assuming k = 3 groups indicated that a third group is the source of genetic variation found on Dream but not Humble and Cormorant populations. The same group membership (in orange) was also shared with Gand (on the North of the Palmer group) and Berthelot (on the South of the Palmer group). The source population of the alleles that made the third admixture group is unknown, and additional samples might help identify the source population(s).

Local adaptation is one potential explanation for the discrepancy between our estimations of migration rates and population structure. This hypothesis is consistent with microhabitats within and among island populations (Contador et al., 2020; Spacht et al., 2021). Local adaptation can inflate genetic differentiation (Charlesworth et al., 2017), as does recurrent extinction and colonization (Whitlock and McCauley, 1990; Giles and Goudet, 1997). It is possible that the many climatic and glacial fluctuations over the past several million years caused dynamic changes in the connections among populations which have each left conflicting signatures on genetic variation, as has been the case in other Antarctic species (Barnes et al., 2006). Lastly, it is also worth considering that migrants may not always contribute to the gene pool, particularly if local adaptation acts to optimize phenotypes for each locality, as has been suggested for the freshwater crustacean *Boeckella poppei* (Maturana et al., 2020). Since *B. antarctica* spend 2 years as larvae before transitioning to the adult stage, any immigrant larvae must survive on its new location for at least 1 winter. Biotic interactions such as algal abundance appear to be more important for the abundance of *B. antarctica* and may further facilitate local adaptation based on the resident community and biodiversity (Potts et al., 2020). In this case, the asymmetries in migration rates may reflect the potentially low likelihood of larval immigrants to survive and reproduce due to island-specific selection pressures and decreased fitness after migration.

4.3. Conclusions

Belgica antarctica exhibits signatures of past divergence, with more recent dispersion and admixture. Our population genetic data indicate that recent admixture most likely via ocean currents, phoresy, or other inter-island migration may facilitate its further persistence in this current period of rapid climate change. Our data also suggest that the distribution of these populations and the structure of their genetic variation is very likely to be impacted by major shifts in climate or geology, particularly if they influence larger vertebrates or ocean currents. Continuing to understand the impact of environmental history on the evolution of this species will contextualize its unique physiological adaptations to the Antarctic environmental extremes, and better predict its adaptability and evolutionary trajectory.

Data availability

All sequences generated with the amplicon sequencing were deposited at the National Center for Biotechnology Information (NCBI) and are available under the following BioProject accession number: PRJNA565153. Additional data can be found at the GitHub repository: https://github.com/vitorpavinato/Belgica_ampliconseq_phylogeography).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.polar.2023.100945.

References

Allegrucci, G., Carchini, G., Convey, P., Sbordoni, V., 2012. Evolutionary geographic relationships among orthocladine chironomid midges from maritime Antarctic and sub-Antarctic islands. Biol. J. Linn. Soc. 106, 258–274.

Allegrucci, G., Carchini, G., Todisco, V., 2006. A molecular phylogeny of Antarctic Chironomidae and its implications for biogeographical history. Polar Biol. 29, 320–326.

Atkins, K.E., Travis, J.M.J., 2010. Local adaptation and the evolution of species' ranges under climate change. J. Theor. Biol. 266, 449–457.

Barnes, D.K.A., Hodgson, D.A., Convey, P., Allen, C.S., Clarke, A., 2006. Incursion and excursion of Antarctic biota: past, present and future. Global Ecol. Biogeogr. 15, 121–142.

Bartlett, J.C., Convey, P., Hughes, K.A., Thorpe, S.E., Hayward, S.A.L., 2021. Ocean currents as a potential dispersal pathway for Antarctica's most persistent non-native terrestrial insect. Polar Biol. 44, 209–216.

Beerli, P., 2009. How to use MIGRATE or why are Markov chain Monte Carlo programs difficult to use. In: Bertorelle, G., Bruford, M., Hauffe, H., Rizzoli, A., Vernesi, C. (Eds.), Population Genetics for Animal Conservation. Cambridge University Press, Cambridge, UK, pp. 42–79.

Biersma, E.M., Jackson, J.A., Bracegirdle, T.J., Griffiths, H., Linse, K., Convey, P., 2018. Low genetic variation between South American and Antarctic populations of the bank-forming moss *Chorisodontium aciphyllum* (Dicranaceae). Polar Biol. 41, 599–610.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
Böning, C.W., Dispert, A., Visbeck, M., Rintoul, S.R., Schwarzkopf, F.U., 2008. The response of the Antarctic Circumpolar Current to recent climate change. Nat. Geosci. 1, 864–869.
Bouckaert, R.R., Heled, J., 2014. DensiTree 2: Seeing Trees through the Forest. bioRxiv, 012401.
Bouckaert, R., Vaughan, T.G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., et al., 2019. Beast 2.5: an advanced software platform for Bayesian evolutionary analysis. In: Pertea, M. (Ed.), PLOS Comput Biol, vol. 15, e1006650.
Carapelli, A., Cucini, C., Fanciulli, P.P., Frati, F., Convey, P., Nardi, F., 2020a. Molecular comparison among three Antarctic endemic springtail species and description of the mitochondrial genome of *Friesea gretae* (Hexapoda, Collembola). Diversity 12, 450.
Carapelli, A., Greenslade, P., Nardi, F., Leo, C., Convey, P., Frati, F., et al., 2020b. Evidence for cryptic diversity in the Pan-Antarctic; Springtail *Friesea antarctica* and the description of two new species. Insects 11.
Casanovas, P., Naveen, R., Forrest, S., Poncet, J., Lynch, H.J., 2015. A comprehensive coastal seabird survey maps out the front lines of ecological change on the western Antarctic Peninsula. Polar Biol. 38, 927–940.
Charlesworth, D., Barton, N.H., Charlesworth, B., 2017. The sources of adaptive variation. Proc R Soc B Biol Sci 284, 20162864.
Chown, S.L., Convey, P., 2007. Spatial and temporal variability across life's hierarchies in the terrestrial Antarctic. Philos Trans R Soc B Biol Sci 362, 2307–2331.
Chapuis, M.P., Raynal, L., Plantamp, C., Meynard, C.N., Blondin, L., Marin, J.-M., Estoup, A., 2020. A young age of subspecific divergence in the desert locust inferred by ABC random forest. Mol. Ecol. 29, 4542–4558.
Clarke, A., Barnes, D.K.A., Hodgson, D.A., 2005. How isolated is Antarctica? Trends Ecol. Evol. 20, 1–3.
Cockerham, C.C., Weir, B.S., 1984. Covariances of relatives stemming from a population undergoing mixed self and random mating. Biometrics 40, 157–164.
Collins, G.E., Hogg, I.D., Convey, P., Barnes, A.D., McDonald, I.R., 2019. Spatial and temporal scales matter when assessing the species and genetic diversity of springtails (Collembola) in Antarctica. Front Ecol Evol 7, 76.
Collins, G.E., Hogg, I.D., Convey, P., Sancho, L.G., Cowan, D.A., Lyons, W.B., et al., 2020. Genetic diversity of soil invertebrates corroborates timing estimates for past collapses of the West Antarctic Ice Sheet. Proc. Natl. Acad. Sci. U. S. A. 117, 22293–22302.
Contador, T., Gañan, M., Bizama, G., Fuentes-Jaque, G., Morales, L., Rendoll, J., et al., 2020. Assessing distribution shifts and ecophysiological characteristics of the only Antarctic winged midge under climate change scenarios. Sci. Rep. 10, 9087.
Convey, P., 2017. Antarctic ecosystems. Encycl Biodivers 1, 179–187.
Convey, P., Biersma, E.M., Casanova-Katny, A., Maturana, C.S., 2020. Refuges Of Antarctic Diversity. Past Antarct, pp. 181–200.
Convey, P., Bowman, V., Chown, S.L., Francis, J.E., Fraser, C., Smellie, J., et al., 2018. Ice bound Antarctica: biotic consequences of the shift from a temperate to a polar climate. In: Hoorn, C., Perrigo, A., Antonelli, A. (Eds.), Mountains, Climate, and Biodiversity. Wiley, Oxford, UK, p. 544.
Convey, P., Gibson, J.A.E., Hillenbrand, C.-D., Hodgson, D.A., Pugh, P.J.A., Smellie, J.L., et al., 2008. Antarctic terrestrial life – challenging the history of the frozen continent? Biol. Rev. 83, 103–117.
Convey, P., Smith, R.I.L., 2005. Responses of terrestrial Antarctic ecosystems to climate change. In: Rozema, J., Aerts, R., Cornelissen, H. (Eds.), Plants and Climate Change. Tasks for vegetation science, 41. Springer, Dordrecht.
Convey, P., Stevens, M.I., 2007. Antarctic biodiversity. Science 317, 1877–1878.
Convey, P., Stevens, M.I., Hodgson, D.A., Smellie, J.L., Hillenbrand, C.-D., Barnes, D.K. A., et al., 2009. Exploring biological constraints on the glacial history of Antarctica. Quat. Sci. Rev. 28, 3035–3048.
Cook, A.J., Fox, A.J., Vaughan, D.G., Ferrigno, J.G., 2005. Retreating glacier fronts on the Antarctic Peninsula over the past half-century. Science 308, 541–544.
Coulson, S., Hodkinson, I., Webb, N., Harrison, J.A., 2002. Survival of terrestrial soil-dwelling arthropods on and in seawater: implications for trans-oceanic dispersal. Funct. Ecol. 16, 353–356.
Croxall, J.P., Trathan, P.N., Murphy, E.J., 2002. Environmental change and Antarctic seabird populations. Science 297, 1510–1514.
Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S., McVean, G., Durbin, R., 1000 Genomes Project Analysis Group, 2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158.
Dray, S., Dufour, A.-B., 2007. The **ade4** Package: implementing the duality diagram for ecologists. J. Stat. Software 22.
Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461.
Elnitsky, M.A., Benoit, J.B., Lopez-Martinez, G., Denlinger, D.L., Lee, R.E., 2009. Osmoregulation and salinity tolerance in the Antarctic midge, Belgicaantarctica: seawater exposure confers enhanced tolerance to freezing anddehydration. J. Exp. Biol. 212, 2864–2871.
Fraser, A.D., Massom, R.A., Michael, K.J., Galton-Fenzi, B.K., Lieser, J.L., 2012. East Antarctic landfast sea ice distribution and variability, 2000–08. J. Clim. 25, 1137–1156.
Fraser, C.I., Terauds, A., Smellie, J., Convey, P., Chown, S.L., 2014. Geothermal activity helps life survive glacial cycles. Proc. Natl. Acad. Sci. U. S. A. 111, 5634–5639.
Fraser, C.I., Morrison, A., Hogg, A., et al., 2018. Antarctica's ecological isolation will be broken by storm-driven dispersal and warming. Nat. Clim. Change 8, 704–718.
Garrison, E., Marth, G., 2012. Haplotype-based Variant Detection from Short-Read Sequencing. https://doi.org/10.48550/arXiv.1207.3907 arXiv:1207.3907.
Giles, B.E., Goudet, J., 1997. Genetic Differentiation in *Silene dioica* metapopulations: estimation of spatiotemporal effects in a successional plant species. Am. Nat. 149, 507–526.
Gille, S., McKee, D., Martinson, D., 2016. Temporal changes in the antarctic circumpolar current: implications for the antarctic continental shelves. Oceanography 29, 96–105.
Goto, S.G., Philip, B.N., Teets, N.M., Kawarasaki, Y., Lee, R.E., Denlinger, D.L., 2011. Functional characterization of an aquaporin in the Antarctic midge *Belgica antarctica*. J. Insect Physiol. 57, 1106–1114.
Goudet, J., 2005. hierfstat, a package for r to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5, 184–186.
Greenslade, P., 2018. An antarctic biogeographical anomaly resolved: the true identity of a widespread species of Collembola. Polar Biol. 41, 969–981.
Gressitt, J., 1965. Biogeography and ecology of land arthropods of Antarctica. In: Mieghem, J van, Oye, P van (Eds.), Biogeography and Ecology in Antarctica. Springer Netherlands, Dordrecht, p. 762.
Greve, M., Gremmen, N.J.M., Gaston, K.J., Chown, S.L., 2004. Nestedness of Southern Ocean island biotas: ecological perspectives on a biogeographical conundrum. J. Biogeogr. 32, 155–168.
Griffiths, H.J., Barnes, D.K.A., Linse, K., 2009. Towards a generalized biogeography of the Southern Ocean benthos. J. Biogeogr. 36, 162–177.
Hawes, T.C., Worland, M.R., Bale, J.S., Convey, P., 2007. Rafting in antarctic collembola. J Zool 274, 44–50.
Harrington, S.M., Hollingsworth, B.D., Higham, T.E., Reeder, T.W., 2018. Pleistocene climatic fluctuations drive isolation and secondary contact in the red diamond rattlesnake (*Crotalus ruber*) in Baja California. J. Biogeogr. 45, 64–75.
Heil, P., Allison, I., 1999. The pattern and variability of Antarctic sea-ice drift in the Indian Ocean and western Pacific sectors. J Geophys Res Ocean 104, 15789–15802.
Hill, J.K., Griffiths, H.M., Thomas, C.D., 2011. Climate change and evolutionary adaptations at species' range margins. Annu. Rev. Entomol. 56, 143–159.
Ingels, J., Vanreusel, A., Brandt, A., Catarino, A.I., Bruno, D., De Ridder, C., Dubois, R., Gooday, A.J., Martin, P., Pasotti, F., Robert, H., 2012. Possible effects of global environmental changes on Antarctic benthos: a synthesis across five major taxa. Ecol. Evol. 2, 453–485.
Ingólfsson, Ó., 2004. Quaternary glacial and climate history of Antarctica. Dev. Quat. Sci. 2, 3–43.
Jenouvrier, S., Che-Castaldo, J., Wolf, S., Holland, M., Labrousse, S., LaRue, M., Wienecke, B., Fretwell, P., Barbraud, C., Greenwald, N., Stroeve, J., Trathan, P.N., 2021. The call of the emperor penguin: legal responses to species threatened by climate change. Global Change Biol. 27, 5008–5029.
Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403–1405.
Kelley, J.L., Peyton, J.T., Fiston-Lavier, A.-S., Teets, N.M., Yee, M.-C., Johnston, J.S., et al., 2014. Compact genome of the Antarctic midge is likely an adaptation to an extreme environment. Nat. Commun. 5, 4611.
Kennett, J.P., Houtz, R.E., Andrews, P.B., Edwards, A.R., Gostin, V.A., Hajos, M., et al., 1975. Cenozoic paleoceanography in the southwest Pacific Ocean, Antarctic glaciation, and the development of the circumantarctic current. In: Initial Reports of the Deep Sea Drilling Project, vol. 29. U.S. Government Printing Office, pp. 1155–1169.
Korczak-Abshire, M., Hinke, J.T., Milinevsky, G., Juáres, M.A., Watters, G.M., 2020. Coastal regions of the northern Antarctic Peninsula are key for gentoo populations. Bio. Lett. 17, 20200708.
Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359.
Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al., 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079.
Lopez-Martinez, G., Elnitsky, M.A., Benoit, J.B., Lee, R.E., Denlinger, D.L., 2008. High resistance to oxidative damage in the Antarctic midge *Belgica antarctica*, and developmentally linked expression of genes encoding superoxide dismutase, catalase and heat shock proteins. Insect Biochem. Mol. Biol. 38, 796–804.
Maslen, N.R., Convey, P., 2006. Nematode diversity and distribution in the southern maritime Antarctic—clues to history? Soil Biol. Biochem. 38, 3141–3151.
Maturana, C.S., Segovia, N.I., González-Wevar, C.A., Díaz, A., Rosenfeld, S., Poulin, E., et al., 2020. Evidence of strong small-scale population structure in the Antarctic freshwater copepod *Boeckella poppei* in lakes on Signy Island, South Orkney Islands. Limnol. Oceanogr. 65, 2024–2040.
McGaughran, A., Stevens, M.I., Holland, B.R., 2010. Biogeography of circum-Antarctic springtails. Mol. Phylogenet. Evol. 57, 48–58.
McGaughran, A., Terauds, A., Convey, P., Fraser, C.I., 2019. Genome-wide SNP data reveal improved evidence for Antarctic glacial refugia and dispersal of terrestrial invertebrates. Mol. Ecol. 28, 4941–4957.
Moffat, C., Beardsley, R.C., Owens, B., van Lipzig, N., 2008. A first description of the antarctic Peninsula coastal current. Deep Sea Res. Part II Top. Stud. Oceanogr. 55, 277–293.
Moffat, C., Meredith, M., 2018. Shelf–ocean exchange and hydrography west of the Antarctic Peninsula: a review. Philos Trans R Soc A Math Phys Eng Sci 376, 20170164.
Muñoz-Ramírez, C.P., Barnes, D.K.A., Cárdenas, L., Meridith, M.P., Morley, S.A., Roman-Gonzalez, A., Sands, C.J., Scourse, J., Brante, A., 2020. Gene flow in the antarctic bivalve *Aequiyoldia eightsii* (jay, 1839) suggests a role for the antarctic Peninsula coastal current in larval dispersal. R. Soc. Open Sci. 7, 200603.
Norberg, J., Urban, M.C., Velland, M., Klausmeier, C.A., Loeuille, N., 2012. Eco-evolutionary responses of biodiversity to climate change. Nat. Clim. Change 2, 747–751.

Parnikoza, I., Dykyy, I., Ivanets, V., Kozeretska, I., Kunakh, V., Rozok, A., Ochyra, R., Convey, P., 2012. Transfer of Antarctic herb tundra formation fragments by the kelp gull in the Argentine Islands area. Polar Biol. 35, 1753–1758.
Parnikoza, I., Rozok, A., Convey, P., Veselski, M., Esefeld, J., Ochyra, R., Mustafa, O., Braun, C., Peter, H.-U., Smykla, J., Kunakh, V., Kozeretska, I., 2018. Spread of Antarctic vegetation by the kelp gull: comparison of two maritime Antarctic regions. Polar Biol. 41, 1143–1155.
Potts, L.J., Gantz, J.D., Kawarasaki, Y., Philip, B.N., Gonthier, D.J., Law, A.D., et al., 2020. Environmental factors influencing fine-scale distribution of Antarctica's only endemic insect. Oecologia 194, 529–539.
Pugh, P.J.A., Convey, P., 2008. Surviving out in the cold: antarctic endemic invertebrates and their refugia. J. Biogeogr. 35, 2176–2186.
Raj, A., Stephens, M., Pritchard, J.K., 2014. fastSTRUCTURE: variational inference of population structure in large SNP data sets. Genetics 197, 573–589.
Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using tracer 1.7. Syst. Biol. 67, 901.
Robinson, S.A., 2022. Climate change and extreme events are changing the biology of Polar Regions. Global Change Biol. 28, 5861–5864.
Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large datasets. Mol. Biol. Evol. 34, 3299–3302.
Schrimpf, M., Naveen, R., Lynch, H., 2018. Population status of the Antarctic shag *Phalacrocorax (atriceps) bransfieldensis*. Antarct. Sci. 30, 151–159.
Sokolov, S., Rintoul, S.R., 2009. Circumpolar structure and distribution of the Antarctic Circumpolar Current fronts: 1. Mean circumpolar paths. J. Geophys. Res. 114, C11018.
Spacht, D.E., Gantz, J.D., Devlin, J.J., McCabe, E.A., Lee, R.E., Denlinger, D.L., Teets, N. M., 2021. Fine-scale variation in microhabitat conditions influences physiology and metabolism in an Antarctic insect. Oceologia 197, 373–385.
Sugg, P., Edwards, J.S., Baust, J., 1983. Phenology and life history of *Belgica antarctica*, an Antarctic midge (Diptera: Chironomidae). Ecol. Entomol. 8, 105–113.
Teets, N., Denlinger, D., 2014. Surviving in a frozen desert: environmental stress physiology of terrestrial Antarctic arthropods. J. Exp. Biol. 217.
Thomas, E.R., Dennis, P.F., Bracegirdle, T.J., Franzke, C., 2009. Ice core evidence for significant 100-year regional warming on the Antarctic Peninsula. Geophys. Res. Lett. 36, L20704.
Turner, J., Bindschadler, R., Convey, P., di Prisco, G., Fahrbach, E., Gutt, J., et al., 2009. Antarctic Climate Change and the Environment. Scientific committee on Antarctic research, Cambridge, UK.
Vaughan, D.G., Doake, C.S.M., 1996. Recent atmospheric warming and retreat of ice shelves on the Antarctic Peninsula. Nature 379, 328–331.
Vega, G.C., Convey, P., Hughes, K.A., Olalla-Tárraga, M.Á., 2020. Humans and wind, shaping Antarctic soil arthropod biodiversity. Insect Conserv Divers 13, 63–76.
van Vuuren, B.J., Lee, J.E., Convey, P., Chown, S.L., 2018. Conservation implications of spatial genetic structure in two species of oribatid mites from the Antarctic Peninsula and the Scotia Arc. Antarct. Sci. 30, 105–114.
Vyverman, W., Verleyen, E., Wilmotte, A., Hodgson, D.A., Willems, A., Peeters, K., et al., 2010. Evidence for widespread endemism among Antarctic micro-organisms. Pol. Sci. 4, 103–113.
Webb, P.-N., Harwood, D.M., 1991. Late Cenozoic glacial history of the Ross embayment, Antarctica. Quat. Sci. Rev. 10, 215–223.
Whitlock, M.C., McCauley, D.E., 1990. Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution 44, 1717–1724.
Wickham, H., 2016. No Ggplot2 – Elegant Graphics for Data Analysis, second ed. Springer International Publishing. Title, 2nd edn.
Wilkinson-Herbots, H.M., Ettridge, R., 2004. The effect of unequal migrates rates on FST. Theor. Popul. Biol. 66, 185–197.