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Genomic Structure and Demographic History of the Brown Fur Seal: From the Ice Age to Current Isolation

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ABSTRACT

Aim: *Arctocephalus pusillus* (the brown fur seal) has historically experienced population fluctuations associated with glacial cycles and anthropogenic exploitation. Previous mitochondrial DNA (mtDNA) and microsatellite analyses suggested a post-glacial population expansion in *A. pusillus*, followed by allopatric divergence that resulted in two extant subspecies: the Australian (AFS, *A. p. doriferus*) and Cape (CFS, *A. p. pusillus*) fur seals. A fine-scale analysis documenting the subspecies' evolutionary history, stochasticity and patterns of gene flow remains largely unexplored. Here, using high-resolution genome-wide markers, we investigate the demographic history of the species, gene flow among *A. pusillus* colonies and drivers of genomic variation across fine and broad spatial scales.

Location: Southern Africa, Australia.

Taxon: *Arctocephalus pusillus*.

Methods: Analyse putative outlier and neutral Single Nucleotide Polymorphisms (SNPs) from genotype-by-sequencing (GBS) data of 92 individuals across five CFS and three AFS colonies.

Results: The seal subspecies show clear genomic differentiation that appears to reflect genome-wide processes. Putative outlier loci show elevated divergence between subspecies, some of which link to functional annotations at orthologous genes in the *A. gazella* reference genome (arcGaz4_h1). We detect some genomic differentiation among AFS colonies despite inferring substantial gene flow between most colonies within each subspecies (proportion of first generation migrants = 0.087–0.258). Demographic inference supports a recent vicariant lineage divergence (~12,000–23,000 years ago) in *A. pusillus* and post-glacial population declines in the CFS and AFS, but it does not provide evidence of recent population bottlenecks.

Main Conclusions: This study advances our understanding of evolutionary forces driving differentiation across mobile subspecific pinniped lineages and underscores the utility of genome-wide data in resolving patterns of population structure across global and regional scales. Offering novel insights into *A. pusillus* evolution, our work contributes a Southern Hemisphere perspective towards global knowledge of marine mammal demographic responses to glacial cycles.

1 | Introduction

Many species share a history of large-scale population reductions, whether environmentally- or anthropogenically-induced (McCauley et al. 2015; Stoffel et al. 2018; Peart et al. 2020). Substantial declines in population sizes can reduce genomic diversity and compromise the adaptive potential of a species, despite recoveries in numbers (Frankham et al. 1999; Allendorf et al. 2008). However, the long-term losses in genomic diversity incurred from population declines are complex and species-specific (Stoffel et al. 2018; Frankham et al. 1999; Allendorf et al. 2008); and, in many species, these genomic losses have not been well quantified (Leigh et al. 2019).

The brown fur seal (BFS; *Arctocephalus pusillus* Schreber, 1775) can act as a case study to investigate the effects of large-scale population reductions on genomic diversity. The species experienced extensive population size bottlenecks over the past four centuries (David 1987; Warneke and Shaughnessy 1985) as well as likely population size fluctuations associated with glacial cycles (Matthee et al. 2006; Malan et al. 2022). Currently, two allopatric subspecies of *A. pusillus* are recognised. The Cape fur seal (CFS, *A. p. pusillus*) inhabits the coastlines of South Africa, Namibia and southern Angola, and the Australian fur seal (AFS, *A. p. doriferus*) occurs along the southern and south-eastern coasts of Australia (Kirkman and Arnould 2018). Since these two subspecies have been exposed to different demographic histories, comparisons of genomic diversity among these taxa are particularly interesting. In addition, contrasting ecological regimes have been reported for the species that may be implicated in morphological change among them (Kirkman et al. 2019; Repenning et al. 1971 in Hofmeyr 2015; Lutjeharms 2006; Ridgway and Dunn 2003; Pearce and Phillips 1988; Shannon and Nelson 1996; Knox et al. 2017; Arnould and Hindell 2001; Kirkwood and McIntosh 2021). The Cape fur seal occurs in the highly productive Benguela Current and predominantly undertakes shallower, pelagic foraging dives whereas the AFS comparatively exploits low-productivity waters of the Bass Strait and exhibits deeper, benthic foraging dives. These observations can provide a strong a priori basis for testing adaptive divergence between these subspecies.

In the past 400 years, intensive harvesting of both *A. pusillus* subspecies for oil, skins and meat led to considerable population size declines (David 1987; Warneke and Shaughnessy 1985). Exploitation of the CFS contributed to the extirpation of 23 colonies by the late 1800's (Shaughnessy 1984 in David and van Sittert 2008). At its most reduced state, the population size of the CFS may have fallen below 100,000 individuals (Shaughnessy and Butterworth 1981 in Kirkman et al. 2007; David and van Sittert 2008), representing ~5%–7% of the current estimated population size of ~1.5–2 million seals which breed across more than 40 colonies (Butterworth et al. 1995; Kirkman et al. 2013; Hofmeyr 2015). Harvesting of the AFS contributed to the extirpation of all but 10 breeding colonies by the early 1920's (Warneke and Shaughnessy 1985 in McIntosh et al. 2018), and pup estimates converted to population size suggest that AFS population numbers may have dropped below 10,000 individuals by the mid 1800's (McIntosh et al. 2022; Gibbens and Arnould 2009; Kirkwood and McIntosh 2021). A bottleneck population of this size would represent ~11% of the contemporary AFS population, which is estimated at a minimum of 90,000 individuals across 26 breeding colonies (McIntosh et al. 2022). The implementation of increasingly restrictive legal protection during the 19th and 20th centuries allowed both subspecies to make population recoveries, although the AFS to a lesser extent than the CFS (Kirkman et al. 2013 and Kirkwood et al. 2005 in Hofmeyr 2015). Contemporary AFS numbers are estimated at ~28%–47% of pre-exploitation levels (McIntosh et al. 2018 in Geeson et al. 2023). Legal harvesting continues only among the CFS in Namibia under regulated quotas of 80,000 pups and 6000 bulls per annum, although these are not usually met (Republic of Namibia 2000, s. 27; Ministry of Environment, Forestry and Tourism 2022).

Like other pinnipeds (Harington 2008; Liu et al. 2022; Younger et al. 2016), the prehistoric population size and distribution of *A. pusillus* likely fluctuated in response to glacial cycles (Malan et al. 2022; Matthee et al. 2006), but the impacts of these cycles on the diversification and dispersal of Southern Hemisphere marine mammals are still poorly understood (Fraser et al. 2012; Faria et al. 2021). Evidence from microsatellite and mitochondrial DNA (mtDNA) suggests that *A. pusillus* experienced a population size expansion ~18 thousand years ago (kya), coinciding with the end of the Last Glacial Maximum (LGM), and that the AFS lineage subsequently diverged from the CFS lineage ~13 kya following a founder event to Australia (Malan et al. 2022). Older mtDNA analyses suggest a population size expansion and lineage divergence in *A. pusillus* ~37–18 kya (Matthee et al. 2006). It is possible that these two subspecies may show adaptive divergence based on their ecology.

Given the power of genomic analyses (Cabrera and Palsbøll 2017; Bradbury et al. 2015; Allendorf et al. 2010), it seems reasonable to suggest that our current understanding of the timing and magnitude of historical demographic events in the BFS, as well as potential gene flow within and between the CFS and AFS (Malan et al. 2022; Stoffel et al. 2018), can be regarded as coarse estimates. Specifically, estimates of historic effective population size from mtDNA are accompanied by confidence intervals that are too broad to permit meaningful inference (Malan et al. 2022), and two separate investigations using microsatellite DNA have failed to detect signals of known anthropogenically induced population bottlenecks in the BFS (Malan et al. 2022; Stoffel et al. 2018). The failure to detect these signals may reflect genomic resilience to these short-term bottlenecks, as has been observed in other pinnipeds (Stoffel et al. 2018), or may reflect insufficient marker resolution (Hoban et al. 2013). Although microsatellite and mtDNA markers show clear genetic differentiation between the CFS and AFS, population genetic substructure has not been detected within the AFS nor resolved in the CFS (Matthee et al. 2006; Lancaster et al. 2010; Malan et al. 2022; Robbertse et al. 2025). Fine-scale population substructure is predicted based on harem-style reproduction, philopatric tendencies of *A. pusillus* and the geographical distances between some colony pairs (Schliemann 1990; Warneke and Shaughnessy 1985; Rand 1967 in Kirkman 2010).

For this study we predict that genome-wide screening of polymorphisms will improve estimates of the magnitude and/or timing of historic demographic events (Shafer et al. 2015; Cammen et al. 2018; Cleary et al. 2019; Liu et al. 2022; Peralta

et al. 2024), as well as improve statistical power to detect superficial population genomic structure (Jeffries et al. 2016). Moreover, by allowing candidate adaptive Single Nucleotide Polymorphisms (SNPs) to be identified, high-throughput sequencing (HTS) approaches offer the opportunity to investigate putative drivers of differentiation between the parallel, allopatric subspecies of *A. pusillus* (Hohenlohe et al. 2010; Allendorf et al. 2010; Supple and Shapiro 2018; Nadeau et al. 2014).

Using genome-wide markers across 92 *A. pusillus* individuals, we investigate genomic structure, demographic history and connectivity across the species range. We examine the timing and direction of post-glacial population size changes and assess the timing and mode of divergence between the CFS and AFS. We estimate genomic differentiation between the CFS and AFS across putative neutral and outlier loci and investigate whether anthropogenically induced population reductions left detectable signatures in genomic diversity. Together, our findings provide insights into the effects of climatic oscillations and large-scale population reductions on the evolution of a mobile marine mammal occurring in the southern Hemisphere.

2 | Methods

2.1 | Sampling, DNA Extraction and Library Preparation

Flipper clippings were collected from 52 CFS and 42 AFS pups between 1998 and 2019 using non-destructive methods. Five CFS colonies were sampled: Cape Cross ($n=16$), Pelican Point ($n=17$), Sinclair's Island ($n=2$), Kleinzee ($n=15$) and Mossel Bay ($n=2$). Three AFS colonies were sampled: Lady Julia Percy ($n=18$), Seal Rocks ($n=13$) and the Skerries ($n=11$; Figure 1a). Samples were collected from pups younger than 6 months of age to allow birth colonies to be identified (Oosthuizen 1991) and stored in ethanol until DNA extraction. DNA was extracted using a NucleoSpinTM Tissue Kit (Macherey-Nagel) and DNA quantity and quality assessed with Qubit fluorometer analysis and gel electrophoresis. DNA extractions with concentrations >20 nanograms per millilitre (ng/ml) were sent to CD Genomics in New York, USA, for genotype-by-sequencing (GBS) library preparation using the *PstI* and *MspI* enzymes, followed by sequencing on an Illumina NovaSeq 6000 PE150 lane. Two laboratory blanks (negative controls) were included during DNA extraction and sequencing.

2.2 | Data Cleaning

The quality of sequencing reads was assessed using FastQC v0.11.5 (Andrews 2010) and MultiQC v1.14 (Ewels et al. 2016). Then, Illumina sequences were demultiplexed and cleaned using the process_radtags program in Stacks v2.59 (Rochette et al. 2019). Reads with low quality scores, uncalled bases, and Phred scores <20 were discarded (*−q*, *−c*, *−s* 20), and barcodes and RAD-Tag cut sites were rescued (*−r*). The quality of cleaned reads was checked using MultiQC v1.14. The number and proportion of reads retained across samples was visually assessed for colony-level differences using the process_radtags_stats.R script (Catchen Lab 2025; Bitbucket repository). To minimise missing data at the level of individual genotypes, individuals with <1 million reads were removed from the dataset (Rivera-Colón and Catchen 2021).

2.3 | Reference Alignment

In order to conduct reference-based genotype inference, cleaned reads were aligned to the Antarctic fur seal (*Arctocephalus gazella*) reference genome (arcGaz4_h1, GenBankvGCA_040869175.1; Hench et al. 2024). The *A. gazella* genome represents the closest available reference genome for *A. pusillus* (Nagel et al. 2019). To facilitate read alignment, the arcGaz4_h1 genome was indexed using the index() command in BWA v0.7.13 (Li 2013), and reads were aligned with default parameters using the mem() command in BWA. SAM output files were converted to BAM files using the sort() command in SAMtools v1.19.1 (Li, Handsaker, et al. 2009). In order to choose an appropriate downstream genotype inference approach, average sequencing depth was calculated for each sample across covered regions using the depth() command in SAMtools and then averaged across samples. Based on sequencing depth, genotype inference was performed using ANGSD v0.938 (Korneliussen et al. 2014), which incorporates genotype uncertainty into downstream analyses and is widely applied to low- to moderate-coverage genomic data (Lou et al. 2021).

2.4 | Genotype Likelihood Calculation and Genotype Calling

Genotype likelihoods (GLs) were calculated in ANGSD using the SOAPsnp model (Li, Li, et al. 2009) for sites that were biallelic at a *p*-value $\leq 1\times10^{-6}$, had a minor allele frequency (MAF) ≥ 0.01 and were genotyped in $\geq 60\%$ of individuals (see Note S1 for detailed methods). BEAGLE and BCF files were generated as outputs, and the BCF file was converted to a VCF file in BCFtools v1.19 (Danecek and Bonfield 2023). All sites represented in the two negative controls, which might represent library contamination, were removed from the BEAGLE and VCF files using BCFtools. Mean read depth (per-individual per-site) was assessed across the VCF file using the query() command in BCFtools. Because many downstream programs still require hard genotype (HG) input formats, HGs were also called in ANGSD using the SOAPsnp model for sites that were biallelic at a *p*-value $\leq 1\times10^{-6}$, were genotyped in $\geq 60\%$ of individuals and had a posterior probability ≥ 0.95, a MAF ≥ 0.01, a minimum read depth ≥ 4 and a maximum read depth ≤ 58 (see Note S1).

2.5 | Data Filtering and Subsetting

The output HG BCF file was converted to a VCF file using BCFtools (negative controls and putatively contaminated sites were removed). The HG VCF file was filtered for linkage disequilibrium (LD) in PLINK v2.0 (Chang et al. 2015) using a sliding window of 50 SNPs, step size of 5 SNPs and an r2 threshold of 0.2 (see Paris et al. 2022). As a standard component of data cleaning, the HG dataset was screened for duplicate samples: pairwise relatedness was estimated for all sample

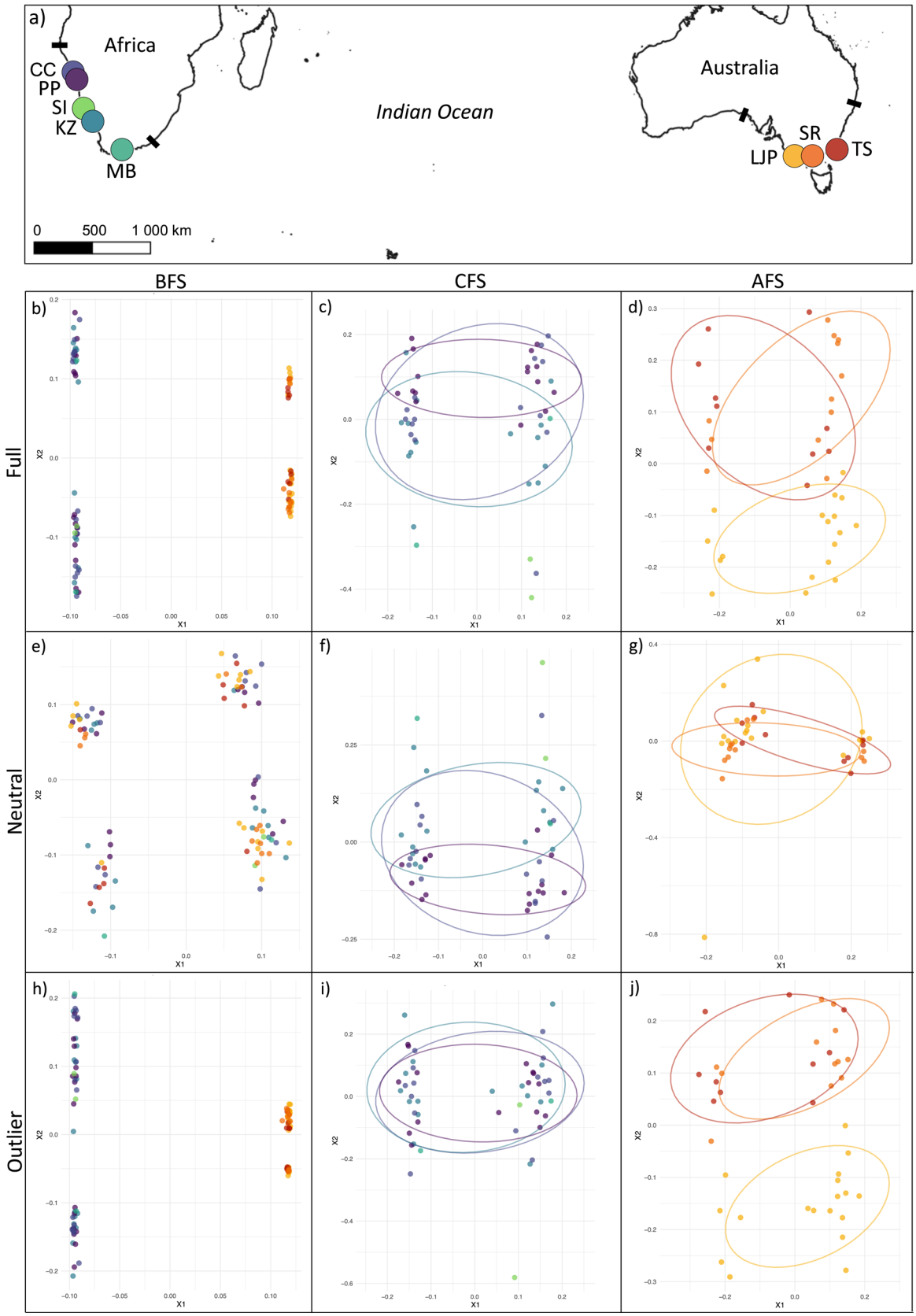

FIGURE 1 | (a) A map of brown fur seal (BFS, *Arctocephalus pusillus*) sampling localities at Cape Cross (CC), Pelican Point (PP), Sinclair's Island (SI), Kleinzee (KZ), Mossel Bay (MB), Lady Julia Percy (LJP), Seal Rocks (SR) and The Skerries (TS) colonies. The core distributions of the Cape (CFS, *A. p. pusillus*) and Australian (AFS, *A. p. doriferus*) fur seals are shown between tick marks along the southern African and Australian coasts, respectively (Hofmeyr 2015). Principal component analyses (PCA) are shown for the following genotype likelihood (GL) datasets: (b) full BFS, (c) full CFS, (d) full AFS, (e) neutral BFS, (f) neutral CFS, (g) neutral AFS, (h) outlier BFS, (i) outlier CFS and (j) outlier AFS. PCAs were generated in PCANGSD (Meisner and Albrechtsen 2018) using colony colours assignments shown in Figure 1a.

pairs using the -relatedness2 method (Manichaikul et al. 2010) implemented in VCFTOOLS (Danecek et al. 2011). Estimates of genetic similarity were interpreted using the kinship coefficient (ϕ) thresholds proposed by Manichaikul et al. (2010), where values of $\phi > 0.354$ are considered indicative of duplicate or identical samples. In order to minimise missing data at the individual genotype level, the distribution of missing data was assessed across individuals using the missing() command in PLINK so that individuals with disproportionately high levels of missing data could be removed from the GL and HG datasets. Based on this distribution of missing data, the threshold of missing data permitted in individuals was set to $\leq 40\%$ of sites, which is a threshold applied in other population genomics studies (Vargas-Fonseca et al. 2021). Finally, to ensure HG sequencing depth was sufficient for downstream inference, read depth was calculated per-individual per-site across the VCF file using the query() command in BCFTOOLS.

Subspecies-specific data subsets were created from HG and GL datasets using samples collected in Africa (CFS) and Australia (AFS); datasets with both subspecies are referred to as 'BFS' datasets. All HG VCFs were filtered in BCFTOOLS to retain sites with a MAF ≥ 0.01 in order to account for any changed allele frequencies during upstream filtering.

2.6 | Detection of Putative Outliers

The 'pcadapt' model in PCANGSD identifies candidate outlier SNPs by detecting loci with strong correlations to principal components representing population structure (Meisner and Albrechtsen 2018; Rosemeis 2025). Using this approach, putative outlier SNPs were identified across the GL BFS BEAGLE file in PCANGSD v1.10 to partition SNPs into putatively adaptive and neutral sets for downstream population genomic analyses. Candidate outlier SNPs were identified at a raw p-value threshold of < 0.05 (see Note S2 for detailed methods). These candidate outlier SNPs were extracted from the GL BFS, CFS, and AFS BEAGLE files to generate corresponding GL outlier datasets. In order to create neutral GL and HG datasets, candidate outlier SNPs were removed from GL BFS, CFS, and AFS BEAGLE files as well as from the HG BFS VCF file. The original GL and HG BFS, CFS, and AFS datasets containing both neutral and outlier SNPs are hereafter referred to as the 'full' SNP datasets.

2.7 | Summary Statistics

Colony-level estimates of mean observed heterozygosity (H_O), expected heterozygosity (H_E) and nucleotide diversity (π) were calculated in ARLEQUIN v3.5.2.2 (Excoffier and Lischer 2010) from the neutral HG dataset. Summary statistics were calculated for all colonies except Mossel Bay and Sinclair's Island, which have a sample size of $n = 2$. The inbreeding coefficient (F_{IS}) was calculated as $F_{IS} = 1\text{-}H_O/H_E$ (Weir and Cockerham 1984), and π was based on pairwise differences. Random subsets of 5000 SNPs were selected from the neutral HG VCF in BCFTOOLS, and after Arlequin internal filtering excluded loci with more than 0.5% missing data, the number of loci retained for analyses (1873–2893 loci per population) was adequate (> 1000 loci per population; Aguirre-Liguori et al. 2020). The input file for ARLEQUIN (.arp) was generated using PGDSPIDER v2.1.1.5 (Lischer and Excoffier 2011).

2.8 | Differentiation and Structure Between CFS and AFS

Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was conducted and colony-level pairwise fixation index (F_{ST}) calculated in ARLEQUIN from the full, neutral and outlier BFS HG datasets. Mossel Bay and Sinclair's Island colonies were again excluded and subsets of 5000 SNPs were randomly selected in BCFTOOLS in order to ensure the input file was small enough to manually edit in Nano. Both AMOVA and F_{ST} analyses utilised a distance matrix based on pairwise differences, and statistical significance was assessed via the default number of 10,000 permutations. For AMOVA analyses, genomic variation was partitioned hierarchically across subspecies, colonies and individuals ('include individual level' toggle turned on). Input files for ARLEQUIN (.arp) were generated using PGDSPIDER, and input files for AMOVA analyses were manually formatted to define hierarchical groupings. After ARLEQUIN applied internal filters, the AMOVA investigations retained > 3000 SNPs and all F_{ST} analyses retained > 300 SNPs. Colony-level F_{ST} values were adjusted for multiple testing in R v4.4.1 (R Core Team 2024) using the false discovery rate with the p.adjust() function.

Plotting per-locus F_{ST} across the genome provides a complementary perspective on subspecies differentiation. In theory, divergent selection acting in the presence of gene flow generates localised peaks of high differentiation, whereas neutral drift produces a more uniform, genome-wide pattern (Wolf and Ellegren 2017). Because this contrast can help distinguish potential selection-driven local divergence from drift-driven separation, per-locus F_{ST} values were estimated between subspecies using REALSFS (Korneliussen et al. 2014) following the methods in Note S3.

Population genomic structure was also investigated within and between subspecies using principal component analysis (PCA) in PCANGSD across the full, neutral and outlier BFS, CFS and AFS GL BEAGLE datasets. First, genetic covariance matrices were estimated from genotype likelihoods in PCANGSD. Then, following the methods of Fumagalli et al. (2021; GitHub repository), output files were formatted into standard binary covariance matrices (.npy) and loaded into R using the 'RcppCNPy' package (Eddelbuettel and Yu 2016). Eigen decomposition was computed with the eigen() function (Guennebaud and Jacob 2010) in R, and eigenvectors were used to generate principal components for visualising genetic variation. PCA was visualised using 'ggplot2' (Wickham 2016).

2.9 | Putative Adaptive Differentiation Between CFS and AFS

To investigate localised signals of putative adaptive variation between the CFS and AFS, high-F_{ST} SNPs were linked to likely orthologous functional genes in the *A. gazella* reference genome (arcGaz4_h1, Hench et al. 2024). Previously annotated BUSCO genes, which represent conserved single-copy genes, and their

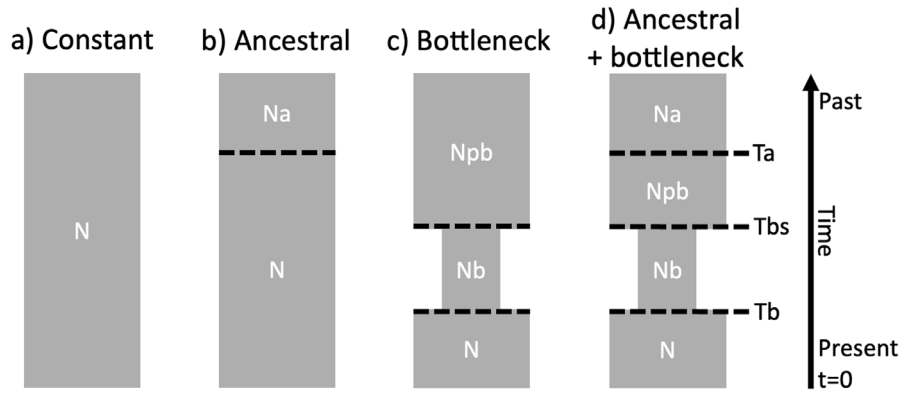

FIGURE 2 | A schematic representation of the four demographic scenarios used to compare support for a recent (< 500 years ago) population bottleneck in the Cape fur seal (*Arctocephalus pusillus pusillus*) and in the Australian fur seal (*A. P. doriferus*) in DIYABC-RF (Collin et al. 2021). These scenarios represent (a) constant population size over time ('constant'), (b) glacial or post-glacial ('ancestral') population size change, (c) a recent population size bottleneck ('bottleneck') and (d) glacial or post-glacial population size change followed by a recent population size bottleneck ('ancestral+bottleneck'). The scenarios were investigated for the CFS and AFS separately. Changes in population size are represented by dashed lines, and parameter labels correspond to parameter types and distributions in Tables S1 and S2.

associated Gene Ontology (GO) terms were obtained from the published genome (Dryad repository, doi: https://doi.org/10.5061/dryad.g1jwstqzn). SNPs were mapped to BUSCO genes by overlapping their genomic positions with annotated gene regions. The methods for this process and for downstream inference are outlined in Note S4.

2.10 | Gene Flow

Individual ancestry was inferred in NGSADMIX (Skotte et al. 2013) from the full BFS GL BEAGLE dataset, with the optimal number of clusters (K) evaluated across $K = 1$–8. However, because assessing support for $K = 1$ is difficult in NGSADMIX since model choice relies on evaluating the relative improvement of likelihood scores across clusters, optimal K was also evaluated within subspecies across $K = 1$–5 in the CFS and $K = 1$–3 in the AFS using the 'LEA' (Frichot and François 2015) R package, which permits inference of $K = 1$ (see Note S5 for methods in NGSADMIX and 'LEA').

Recent gene flow was estimated in BAYESASS v3.0.4 (Wilson and Rannala 2003; Mussmann et al. 2019) from the full BFS HG dataset. Mossel Bay and Sinclair's Island were again excluded due to small sample sizes (see Meirmans 2014; Wilson and Rannala 2003). To reduce noise in the dataset, only SNPs with no missing data were randomly selected in BCFTOOLS and filtered for MAF ≥ 0.05. A subset of 3000 SNPs was used for analysis to ensure computational tractability within time constraints (see Mussmann et al. 2019). Two analyses were performed in BAYESASS: recent gene exchange was investigated at global and local scales by grouping samples according to subspecies and colony, respectively. Details for BAYESASS methods are outlined in Note S6.

2.11 | Demographic History

The timing and magnitude of historic effective population size (N_e) changes were estimated for the CFS and AFS separately using Stairway Plot v2.1.1 (Liu and Fu 2015, 2020). A species-specific SNP-based mutation rate of 1.10×10^{-8} per site per generation (Peart et al. 2020) and generation time of 9.1 years (Hofmeyr 2015) were applied to these analyses. In order to estimate medians and 95% pseudo-confidence intervals, the default number of 200 bootstrap replicates were generated. The neutral SFS values used as input were generated according to the methods in Note S7.

The timing and magnitude of divergence and population size change events in the history of *A. pusillus* were also investigated using approximate Bayesian computation (ABC; Beaumont et al. 2002) with a machine learning random forest approach in DIYABC-RF v1.2.1 (Breiman 2001; Collin et al. 2021). The neutral SNP dataset used for ABC analyses was generated according to methods in Note S8.1; Figure S1. Two ABC analyses were implemented. First, support for an anthropogenically-induced population bottleneck was investigated separately in the CFS and the AFS across four simple demographic scenarios (Figure 2). These scenarios represented (a) a constant population size over time ('constant'), (b) a glacial or post-glacial ('ancestral') population size change, (c) a recent (≤ 500 years ago) population size bottleneck ('bottleneck') and (d) a glacial or post-glacial population size change followed by a recent population size bottleneck ('ancestral+bottleneck'). Bottleneck and non-bottleneck scenarios were tested with and without ancestral population size changes to account for potential confounding effects of older demographic fluctuations on signals of a recent population bottleneck (Malan et al. 2022; Stoffel et al. 2018). For initial investigations, individuals were 'sampled' from a single population representing the metapopulation. However, in order to allow shallow population structure among colonies to be incorporated within scenarios and to increase the number of summary statistics available to inform model performance, two demographic scenarios that visually displayed the best model fit to the observed data were further explored by 'sampling' individuals from their respective colonies. The second ABC analysis investigated the nature of divergence between the CFS and AFS. Two scenarios were compared: (i) a founder event, in which the AFS lineage was established by ≤ 999 individuals following a small

TABLE 1 | Pairwise fixation indices (F_{ST}; below diagonal) and associated p-values (above diagonal) calculated in Arlequin (Excoffier and Lischer 2010) for six brown fur seal (*Arctocephalus pusillus*) colonies based on the full SNP dataset.

	n	Kleinzee	Pelican point	Cape cross	Lady Julia Percy	The skerries	Seal rocks
Kleinzee	15	—	0.2693	0.5013	0.0000	0.0000	0.0000
Pelican point	17	0.001	—	0.0868	0.0000	0.0000	0.0000
Cape cross	16	−0.001	0.004	—	0.0000	0.0000	0.0000
Lady Julia Percy	18	0.112*	0.087*	0.108*	—	0.0252	0.2259
The skerries	10	0.108*	0.079*	0.104*	0.005*	—	0.2259
Seal rocks	12	0.106*	0.076*	0.099*	0.000	0.000	—

Note: Statistically significant pairwise F_{ST} values (p-values < 0.05) are marked by an asterisk (*). p-values are corrected for multiple comparisons using the False Discovery Rate (FDR) method. Estimates from Mossel Bay and Sinclair Island colonies are excluded due to small sample sizes ($n = 2$).

dispersal event, and (ii) a vicariance event, in which ≥1000 individuals became isolated from the ancestral BFS breeding population to form the AFS lineage. Methods for ABC analyses are detailed in Note S8.2–S8.5; Figures S2 and S3; Tables S1–S3.

3 | Results

3.1 | Data Cleaning and Putative Outlier Detection

A total of 80.0% of 861,040,308 original reads were retained after cleaning raw reads with the PROCESS_RADTAGS program in STACKS, with no noteworthy differences in data retention visible across colonies (Figure S4). Two individuals with < 1 million reads (TS2 from The Skerries, SR39 from Seal Rocks) were removed from the data such that 92 individuals remained for downstream analyses. After alignment to the reference genome, mean read depth across covered sites was 5.87×. No individuals were identified as duplicate samples (in accordance with the fact that only pups were sampled over the sampling period) and none were missing more than 40% of data (minimum = 7.6%, maximum = 22.4%); all these individuals were included subsequently where appropriate. Outlier detection identified 364,548 candidate outlier SNPs. After filtering datasets for SNPs present in laboratory blanks, LD and MAF, and removing or isolating outliers as appropriate, the resulting full, neutral and outlier GL and HG BFS, CFS and AFS datasets ranged in size from 101,635 to 1,152,666 SNPs (Table S4). Across the GL dataset, the median per-individual per-site read depth was 12× (interquartile range: 4–22×), and across the HG dataset the median per-site read depth was 15.9× (interquartile range: 10.8–23.8×).

3.2 | Genomic Diversity and Population Structure

Across *A. pusillus* colonies, estimates of mean H_O ranged from 0.170 (±0.190) to 0.229 (±0.206) and mean H_E ranged from 0.148 (±0.135) to 0.200 (±0.137). Estimates of mean F_{IS} ranged from −0.149 to −0.118, and estimates of π ranged from 0.078 (±0.038) to 0.098 (±0.048; Table S5).

Per-colony pairwise F_{ST} values from ARLEQUIN indicated significant differentiation between all colony pairs between subspecies across both full (0.076–0.112, $p < 0.001$) and outlier (0.157–0.188, $p < 0.001$) datasets. Within subspecies, one AFS colony pair (Lady Julia Percy and The Skerries) showed low but significant differentiation based on F_{ST} values across full (0.005, $p < 0.05$) and outlier (0.003, $p < 0.05$) datasets (Table 1; Table S6b). Across the neutral dataset, pairwise F_{ST} values showed low but significant differentiation between one AFS colony pair (Lady Julia Percy and Seal Rocks; 0.003, $p < 0.05$) and one intersubspecies colony pair (Lady Julia Percy and Cape Cross; 0.002, $p < 0.001$; Table S6a). Visual inspection of per-locus F_{ST} values between subspecies across scaffolds showed a generally uniform pattern, with no distinct islands of elevated differentiation (Figure S5).

AMOVA revealed significant genomic variation between subspecies across full ($\Phi_{CT} = 8.95\%$, $p < 0.001$) and outlier ($\Phi_{CT} = 17.47\%$, $p < 0.001$) datasets, whereas the neutral dataset showed virtually no variation contained between subspecies ($\Phi_{CT} < 0\%$, $p > 0.95$). Among colonies within subspecies, low but significant variation was detected across full ($\Phi_{SC} = 0.10\%$, $p < 0.05$) and neutral ($\Phi_{SC} = 0.15\%$, $p < 0.005$) datasets. Across all datasets, most genomic variation was identified within individuals ($\Phi_{IT} \geq 90.47\%$; Table S7a).

PCA showed distinct genomic clusters for the CFS and AFS across full and outlier datasets (Figure 1b,h) but not the neutral dataset (Figure 1e). Within the AFS, the Lady Julia Percy colony formed a distinct genomic cluster across full and outlier datasets (Figure 1d,j). Two non-geographically structured clusters were apparent from PCA in both subspecies across all datasets (Figure 1b–j). A visual inspection of the PCA (Figure 1f,g,i,j) and AMOVAs performed separately on each subspecies (Table S7b,c) suggested among-colony variation was relatively higher in the neutral dataset for CFS and the outlier dataset for AFS, although AMOVA proportions from subspecies-specific analyses were not statistically significant.

3.3 | Putative Adaptive Differentiation Between CFS and AFS

Across the outlier dataset, 27,909 variants private to CFS, 11,271 variants private to AFS and 5083 SNPs with high pairwise F_{ST} between subspecies were retained as candidate loci likely to represent putative genomic variation between subspecies. Of these, 352 SNPs overlapped with 241 BUSCO genes linked to 19 GO terms in the closely related *A. gazella*. These GO terms have

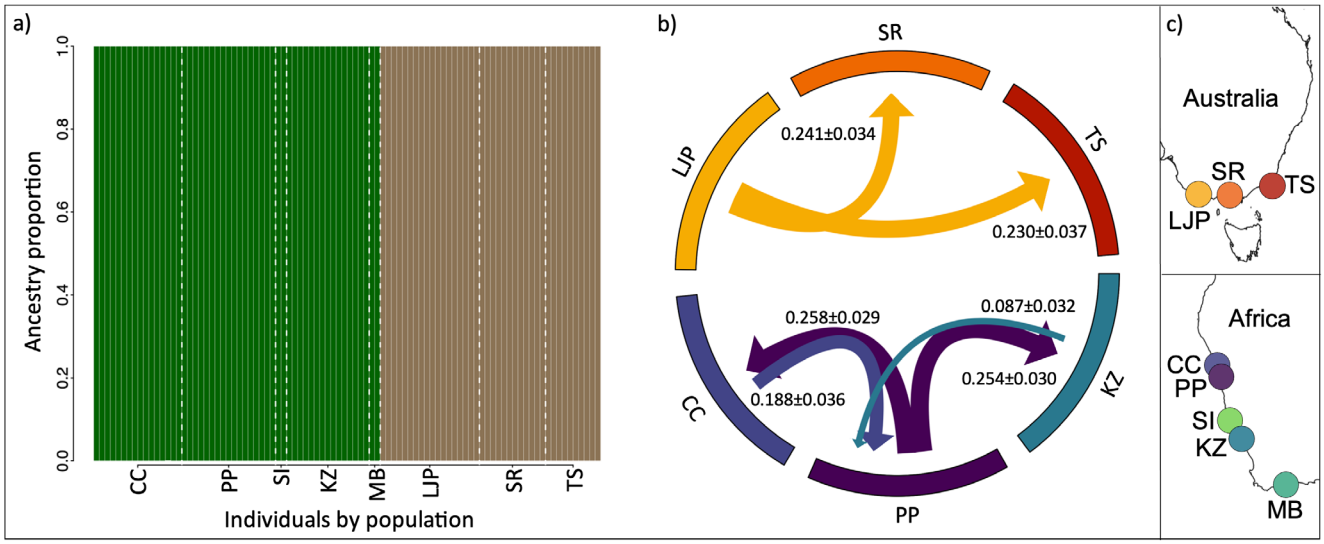

FIGURE 3 | (a) Individual ancestry proportions of 92 brown fur seal (BFS, *Arctocephalus pusillus*) individuals from admixture analysis in NGSADMIX (Skotte et al. 2013) across five Cape fur seal (CFS) colonies (KZ, Kleinzee; PP, Pelican Point; CC, Cape Cross; MB, Mossel Bay; SI, Sinclair's Island) and three Australian fur seal (AFS) colonies (LJP, Lady Julia Percy; SR, Seal Rocks; TS, The Skerries). Colonies are separated by white dashed lines. (b) Circleplot of recent migration rates (*m*) calculated in BAYESASS (Wilson and Rannala 2003; Mussmann et al. 2019) across six BFS colonies (Table S9); arrow direction and thickness represent the direction and magnitude of gene flow. Maps of the (c) AFS sampling localities in Australia and (d) CFS sampling localities in southern Africa are provided for reference.

been related to biological functions in *A. gazella* including cerebellum development (66 SNPs across 48 genes), cellular response to salt (36 SNPs across 26 genes), fatty acid metabolism (17 SNPs across 16 genes), respiratory gaseous exchange (25 SNPs across 16 genes) and blood pressure regulation (17 SNPs across 9 genes and 2 GO terms; Table S8).

3.4 | Gene Flow

Admixture analysis in NGSADMIX suggested two genetic clusters (K) across BFS samples (Figure S6), with the ancestries of individuals sampled in Africa and Australia assigned to non-overlapping lineages (Figure 3a). In agreement with this, admixture analyses in 'LEA' supported one genetic cluster (K) within each subspecies (Figures S7 and S8). In BAYESASS, recent gene flow events were detected among colonies within subspecies but not between subspecies. Of the eight colony-level chains, six converged reliably to high-probability solutions (log probability = −146,152 to −146,284). Summaries were based on the three highest-probability runs; these indicated that the non-zero proportion of first-generation 'migrants' (*m*) among colonies within subspecies averaged 0.087 (±0.032) to 0.258 (±0.029) over one to two generations preceding sampling (Figure 3b; Table S9).

3.5 | Demographic History

Estimates of CFS and AFS effective population size across time, generated with the stairway plot approach, showed a gradual population decline in the CFS between 2 and 15 kya and a sharp population decline in the AFS between 17 and 21 kya (Figure 4).

From demographic inference with ABC, the 'ancestral' and 'ancestral+bottleneck' scenarios provided clear visual overlap between the simulated and observed data among the AFS analyses 'sampled' from one population (Note S9.1; Figure S9f,k,l). Model choice between these scenarios did not provide distinguishing support for either scenario (Posterior Probability, PP = 0.63, in favour of the 'ancestral' scenario). For CFS analyses 'sampled' from one population, none of the scenarios provided visual overlap between the simulated and observed data (Note S9.1; Figure S9). In both the AFS and CFS analyses 'sampled' from three populations, the 'ancestral+bottleneck' scenario provided the only visual overlap between the simulated and observed data (Note S9.2; Figure S10). However, model choice between the 'ancestral+bottleneck' scenario and the next most-well-fitting scenario ('ancestral' scenario) did not show distinguishing support for either scenario for the CFS analysis (PP = 0.47, in favour of the 'ancestral+bottleneck' scenario) or the AFS analysis (PP = 0.60, in favour of the 'ancestral' scenario), possibly because the observed data overlapped only with the 'tail' of the simulated data (Figure S10). The posterior estimates for the three-population analyses suggested a divergence time ~23,000 kya and post-glacial population size declines in the CFS (12 kya) and AFS (11 kya; Table S10).

Model selection between divergence scenarios showed moderately strong support for a vicariance scenario compared to a founder event (PP = 0.77; Note S9.3; Figures S11 and S12). Among parameters estimated under the vicariance scenario, N1pb (pre-bottleneck population size for CFS), N2pb (pre-bottleneck population size for AFS) and Td (time of divergence) were characterised by relatively low Local Normalised Mean Absolute Error (NMAE ≤ 0.70) and narrow credible intervals (Relative 90% CI ≤ 1.10; Table S11). This suggests that these posteriors were relatively well-informed by the data, yielding the most robust median estimates.

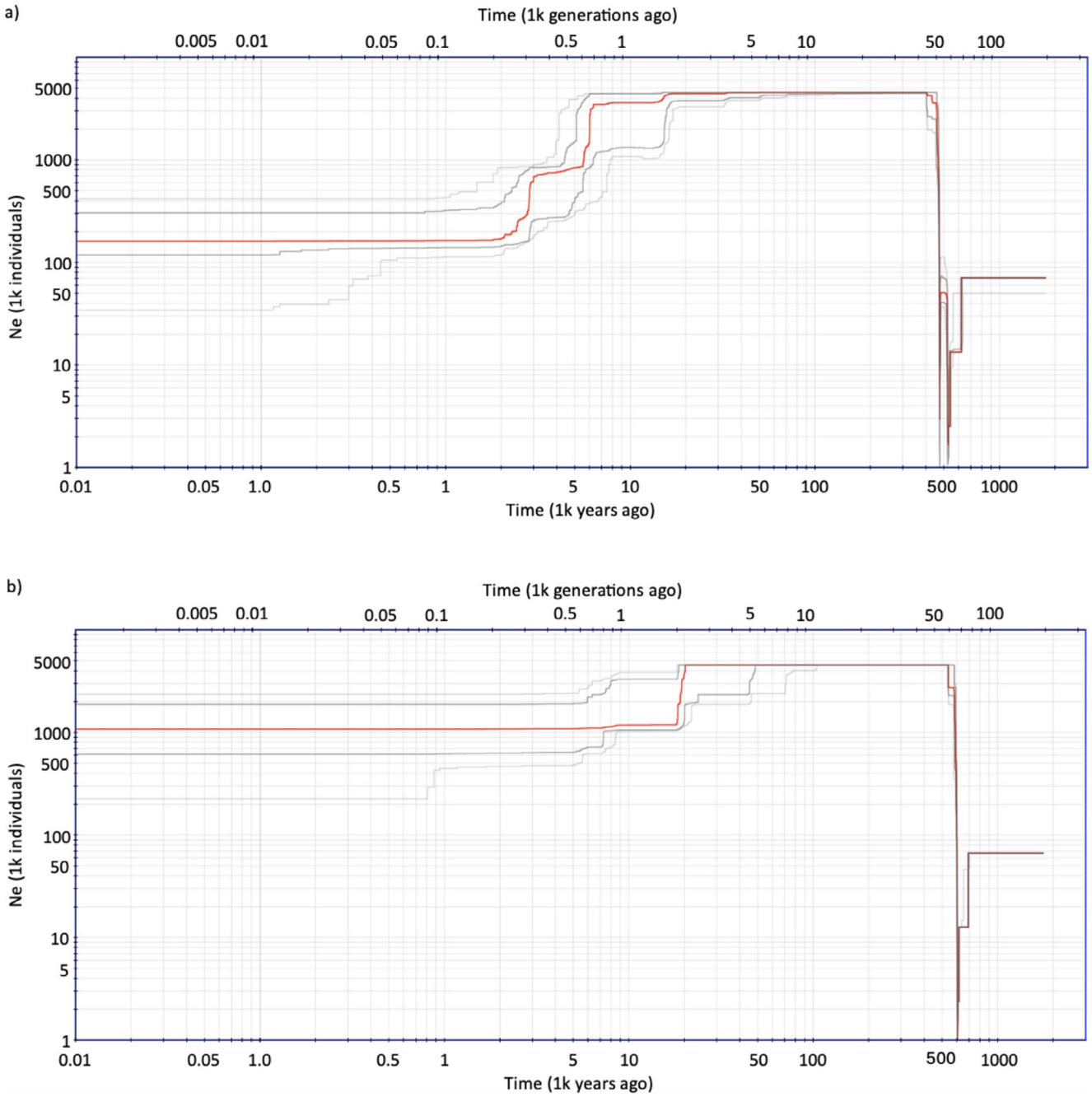

FIGURE 4 | Estimated effective population sizes (N_e) across time in thousands (k) of years for (a) the Cape fur seal (CFS, *Arctocephalus pusillus pusillus*) and (b) the Australian fur seal (AFS, *Arctocephalus pusillus doriferus*) lineages using Stairway Plot v2.2 (Liu and Fu 2015, 2020).

4 | Discussion

This genome-wide study reinforces evidence for clear lineage divergence between the CFS (*A. p. pusillus*) and AFS (*A. p. doriferus*; Malan et al. 2022), with no extant gene flow detected between the two subspecies. It also offers more detailed insights into the demographic histories of the two BFS (*A. pusillus*) subspecies. The subspecies show post-Last Glacial Maximum (LGM) population declines and shallow population genomic substructure within the AFS.

The genomic data revealed limited interpopulational divergence among colonies. In fact, the Lady Julia Percy (LJP) colony is the only colony that forms a distinct genomic cluster in the AFS across full and outlier PCAs and shows differentiation from The Skerries (TS) and Seal Rocks (SR) based on full, neutral and/or outlier F_{ST} values. This distinct genomic signal for LJP suggests that it may contribute unique genomic variation within the subspecies. Differentiation, whether adaptive or neutral, could persist between AFS colonies if gene flow between them is sufficiently limited by mechanisms such as site fidelity, which is presumed for the species, or geographic distance (Shaughnessy and Warneke 1987; Lancaster et al. 2010; Allendorf et al. 2010). In the AFS, inferred gene flow from BayesAss was primarily unidirectional from LJP to TS and SR, suggesting that incoming gene flow to LJP may be limited,

which could account for LJP's differentiation from the other colonies. Lancaster et al. (2010) found some evidence of isolation by distance across 12 AFS colonies, suggesting that geographic distance may partly restrict gene flow between AFS colonies. Moreover, tagging data for Australian fur seals from Kanowna Island and Seal Rocks suggest that AFS females spend > 90% of their time foraging < 100 km of their home colony (Arnould and Hindell 2001), while AFS males spend 64%–98% of their time-at-sea foraging at distances < 200 km from their home colony (Kirkwood et al. 2006). Given that the LJP colony is located > 200 km from the SR colony and > 600 km from TS colony, movement and gene exchange may be limited between LJP and the other two colonies. The phylogeographic pattern we detected for the Lady Julia Percy (LJP) colony is most likely attributed to female philopatry and harem-based breeding; in other pinnipeds, relatively fine-scale genomic structure is often attributed to these drivers, alongside environmental barriers (Sutherland et al. 2024; Ahonen et al. 2016; Chaves et al. 2022; Valtonen et al. 2014; de Oliveira et al. 2017; McCarthy, Cammen, et al. 2025; McCarthy, Refoyo Martínez, et al. 2025). Resolving the extent and drivers of substructure in the AFS may benefit from examining genomic connectivity and structure across a broader geographic range, including recently colonised sites that have expanded the AFS breeding range over the past two decades (e.g., North Casuarina, South Australia; Montague Island, New South Wales; Illes de Phoques and Maatsuyker Island, southern Tasmania; Shaughnessy et al. 2010; McIntosh et al. 2018; McIntosh et al. 2022), alongside environmental data.

In sharp contrast to the comparisons within subspecies, clear genomic differentiation between the CFS and AFS was apparent in both full and outlier datasets across PCA, F_{ST}, AMOVA and individual ancestry proportions. The neutral dataset, however, showed weaker genomic differentiation between subspecies; this can possibly be ascribed to the large effective population sizes in recent divergent lineages (Cutter and Payseur 2013; Corbett-Detig et al. 2015; Allendorf et al. 2010). The Cape fur seal shows overlapping levels of genomic differentiation ($F_{ST} = 0.08–0.11$) to that observed between subspecies of the bearded seal ($F_{ST} = 0.12–0.20$, McCarthy, Refoyo Martínez, et al. 2025) and ringed seal ($F_{ST} = 0.04–0.08$, Löytynoja et al. 2023), supporting their taxonomic distinctness at the subspecies level. The generally uniform, genome-wide differentiation observed across per-locus F_{ST} values between subspecies plotted across scaffolds is consistent with allopatric separation, followed by drift acting in the absence of sufficient gene flow to homogenise lineages (Wolf and Ellegren 2017; Vijay et al. 2016; Jasper and Yeaman 2024). Small peaks or individual loci with elevated F_{ST} across the plot may reflect local adaptation (Vijay et al. 2016; Jasper and Yeaman 2024).

Although the proportion of true adaptive variation in the outlier dataset is not always robust (Excoffier et al. 2009; Lotterhos and Whitlock 2014; Bierne et al. 2013; Hoban et al. 2016), mapping outlier loci to functional genes in the *A. gazella* reference genome highlights potential adaptive differentiation between the CFS and AFS putatively related to lipid metabolism, respiratory exchange, blood pressure regulation and brain development, among other processes. Some of these functions correspond with known ecological and morphological differences between the CFS and AFS, including average dive depth, skull shape, habitat productivity and diet (Kirkman et al. 2019; Repenning et al. 1971 in Hofmeyr 2015; Lutjeharms 2006; Ridgway and Dunn 2003; Pearce and Phillips 1988; Shannon and Nelson 1996; Knox et al. 2017; Kirkwood and McIntosh 2021). In other pinnipeds, cranial variation is associated with dietary differences, suggesting the important role of feeding ecology in pinniped evolution (Kienle et al. 2022). If these functional gene associations hold, it seems reasonable to suggest that the two fur seal subspecies have recently diverged through allopatric separation and subsequently experienced distinct selective pressures which are further contributing to their divergence. Important to note, however, while some outlier loci (identified here based on associations with population structure) likely reflect genuine adaptive signals, others may be false positives arising from demographic history or drift (Meisner and Albrechtsen 2018; Beaumont and Balding 2004; Allendorf et al. 2010; Nielsen et al. 2005). Our use of a conservative neutral dataset allows robust inference of demographic patterns, but some truly neutral loci may be included in the outlier dataset, potentially producing slightly conservative estimates of differentiation across both datasets. Additional validation through genotype-environment association studies, for example, is needed to confirm adaptive divergence.

The separation of the two fur seal subspecies is further corroborated by the absence of recent gene flow detected between subspecies in BayesAss (i.e., within two generations preceding sample collection in 1998–2019; Faubet et al. 2007); suggesting that contemporary exchange between subspecies is likely rare or absent (also see Malan et al. 2022). Given that the CFS and AFS are separated by > 8000 km of ocean and appear to function as independent evolutionary units, and their current subspecies classification may merit re-evaluation (Kollár et al. 2021; Hey and Pinho 2012). Within subspecies, there are clear signals of gene flow among colonies. Among the three CFS colonies investigated here, gene exchange occurs more strongly in a southern direction. Our findings as a whole suggest that gene exchange among the southern African sampling sites ($m = 0.087–0.258$) may be sufficient to prevent significant genetic differentiation among them (Matthee et al. 2006; Malan et al. 2022). It has been suggested that populations become demographically interdependent when migration rates (m) fall around 10% (Hastings 1993 in Waples and Gaggiotti 2006), and several of our estimates of m are considerably larger than this threshold. Our estimates of m are comparable with estimates made for other pinnipeds using BayesAss, where $m \leq 0.26$ (Nikolic et al. 2020; Ahonen et al. 2016; Klimova et al. 2014). It is worth noting that BayesAss shows reduced accuracy in assigning individuals to correct source populations when populations are very weakly differentiated ($F_{ST} < 0.05$) and when $m > 0.01$ (Faubet et al. 2007). Because these conditions apply among colonies within each subspecies, estimates of recent gene flow within subspecies should be interpreted cautiously, particularly with respect to absolute values. In a study by Mardulyn et al. (2008), BayesAss was also shown to overestimate migration (m), although true values were within reported confidence intervals (Mardulyn et al. 2008 in Meirmans 2014).

Demographic inference based on Stairway Plot2 and ABC analyses supports a vicariant divergence between the CFS and AFS lineages between 12 and 23 kya, along with post-glacial

population size declines in both subspecies. ABC analyses estimate this divergence at approximately 23 kya. In stairway plots, we attribute the steep population decline observed in the AFS stairway plot from ~4.5 to 1 million individuals between 17 and 21 kya to separation of the AFS population from the BFS lineage. This signal is mirrored in the CFS stairway plot by a decline from ~4.5 to 3.5 million individuals between 12 and 15 kya. Together, the population sizes of the resulting lineages approximate the ancestral population size (~4.5 million individuals). Collectively, these results support a vicariant separation of an ancestral *A. pusillus* lineage into geographically isolated populations in Australia and Africa, rather than the establishment of the AFS lineage through a small founding event, as previously inferred from genetic diversity estimates (Malan et al. 2022). Low sea surface levels during the LGM 19–25 kya may have facilitated the dispersal of *A. pusillus* to Australia as Antarctic and sub-Antarctic islands gained supplementary habitats and submerged seamounts transformed into shallow feeding grounds (Clark et al. 2009; Camoin et al. 2004; Ray and Adams 2001; Fraser et al. 2012; Malan et al. 2022). Subsequent post-glacial sea level rise likely reversed these changes (Camoin et al. 2004).

ABC analyses suggest post-glacial population size declines in both the CFS and AFS, and stairway plots support this pattern for the CFS lineage, which shows a population decline between ~2 and 15 kya. Although speculative, it is interesting to note that simulations suggested that the population size of the African penguin (*Spheniscus demersus*) may have declined precipitously since the LGM (between ~15 and 7 kya) due to sea level rises and the subsequent loss of almost 90% of island habitats around the southern African coastline (Beckett et al. 2023). Because it is thought that CFS breeding colonies were restricted to coastal islands in the pre-harvesting era (Rand 1972 in Kirkman et al. 2013), such environmental change could have similarly affected breeding habitat available to the CFS with possible consequences for abundance.

The demographic history of *A. pusillus* that emerges across our findings differs from previous studies primarily in revealing post-glacial population declines in the CFS and AFS, rather than the population expansion inferred from microsatellites (~18 kya, Malan et al. 2022) or mtDNA (~37–18 kya, Matthee et al. 2006). More broadly, evidence for a decline in *A. pusillus* abundance during this period contrasts with the post-glacial population size expansions reported for many pinniped species (Liu et al. 2022; Ruiz-Puerta et al. 2023; Klimova et al. 2014; Dickerson et al. 2010; Stoffel et al. 2018; Bender et al. 2023; Berg et al. 2025; Weinberger et al. 2021; de Oliveira et al. 2017). However, many of these taxa occupy higher-latitude ranges that would have experienced more extensive and later glacial retreat, potentially facilitating greater and later post-glacial range expansion than in *A. pusillus* (Cleary et al. 2021). Although our results broadly align with Malan et al. (2022) in supporting recent lineage divergence between the CFS and AFS (~12–23 kya here; ~13 kya in Malan et al. 2022), the ancestral population size inferred for the AFS differs between studies, with ~5500 individuals estimated by Malan et al. (2022) and 2.2 million individuals estimated here (Table S11). Despite this discrepancy, our results are more consistent with a vicariance scenario involving allopatric separation than with the founder scenario inferred by Malan et al. (2022), since even the estimate from Malan et al. (2022) is too large to reflect a typical founder event involving a small colonising group.

A visual assessment of ABC model fit for CFS and AFS analyses suggested that simulations under a glacial or post-glacial population size change combined with a recent bottleneck ('ancestral+bottleneck' scenario) provided the best model fit across analyses 'sampled' from three populations. However, model choice between scenarios did not provide decisive support for anthropogenically-induced population declines. This result is consistent with previous microsatellite-based studies (Malan et al. 2022; Stoffel et al. 2018). In the CFS, our visual assessment of model fit suggests that the simulated priors or demographic scenarios do not adequately capture the true demographic history of the subspecies. In the AFS, the lack of quantitative support for bottleneck scenarios may reflect either methodological limitations or a genuinely weak genomic signal. The work of Cabrera and Palsbøll (2017) and Cammen et al. (2018) suggests that DIYABC approaches have high accuracy in identifying bottleneck scenarios when simulated population reductions are two orders of magnitude in size but have limited power to detect weaker bottlenecks. In our AFS simulations, bottlenecks corresponded to reductions in effective population size of one order of magnitude. Additionally, it is also possible that harvested populations remained large enough, and bottlenecks brief enough, to allow considerable genomic diversity to be replenished in the subspecies (Allendorf and Luikart 2005; Matthee et al. 2006). A comparative analysis of 30 pinniped lineages observes such resilience in the heavily harvested South American sea lions and northern fur seals, suggesting that short-term bottlenecks do not necessarily substantially erode genetic diversity (Stoffel et al. 2018). However, ongoing anthropogenic pressures, including pollution, climate change and prey depletion, pose cumulative, ongoing risks that extend beyond historic losses in genomic diversity (Kirkwood and McIntosh 2021; Hofmeyr 2015).

This study advances our understanding of the genomic landscape and demographic history of *A. pusillus* across distinct southern African and Australian subspecies. Our findings indicate clear, genome-wide differentiation between the CFS and AFS, which may reflect recent allopatric separation, and provide support for recognising these subspecies as separate Evolutionary Significant Units. Divergence is more pronounced at a subset of outlier loci, some of which may reflect localised putative adaptive variation between subspecies. The divergence may have accumulated in response to distinct ecological drivers in the absence of sufficient gene flow to homogenise populations. At a regional scale, our results show evidence of regular, sometimes unidirectional gene flow between colonies within each subspecies, along with some genomic substructure among AFS colonies. Our demographic analyses fail to detect quantifiable signals of anthropogenically-induced population bottlenecks in the CFS or AFS, perhaps suggesting genomic resilience to these losses or poor compatibility of the observed data with scenario specifications. Finally, our findings support a post-glacial population size contraction in *A. pusillus*, advancing our understanding of pinniped demographic responses to deglaciation in the Southern Hemisphere. Collectively, our findings broaden our understanding of evolutionary processes that have shaped genomic diversity in *A. pusillus*, underscoring the

utility of genome-wide data in resolving these patterns and drivers across fine and broad evolutionary scales.

Author Contributions

Charlotte A. Spanjaard: investigation – data analysis, writing – original draft, writing – review and editing. Amoré Malan: sample acquisition, conceptualisation, investigation – laboratory analysis, writing – review and editing. Tess Gridley: sample acquisition, writing – review and editing. John P.Y. Arnould: sample acquisition, writing – review and editing. Roger Kirkwood: sample acquisition, writing – review and editing. Conrad A. Matthee: conceptualisation, supervision, funding acquisition, sample acquisition, project administration, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The raw sequence data generated in this study are available from the NCBI Sequence Read Archive (BioProject ID: PRJNA1354663). Analysis scripts are available at https://github.com/Charlotte7654/a-pusillus-evolutionary-genomics.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The frequency of false discovery rate-corrected Q-value scores across sites. For demographic inference in DIYABC-RF v1.2.1 (Breiman 2001; Collin et al. 2021), candidate outlier sites were identified from PCANGSD (Meisner and Albrechtsen 2018; Rosemeis 2025) as those with a Q-value < 0.01 (represented to the left of the red line). **Figure S2:** A schematic representation of the four demographic scenarios used to compare support for a recent (< 500 years ago) population bottleneck in the Cape fur seal (*Arctocephalus pusillus pusillus*) and the Australian fur seal (*A. p. doriferus*) in DIYABC-RF (Collin et al. 2021). These scenarios represent (a) constant population size over time ('constant'), (b) glacial or post-glacial ('ancestral') population size change, (c) a recent population size bottleneck ('bottleneck') and (d) glacial or post-glacial population size change followed by a recent population size bottleneck ('ancestral+bottleneck'). Individuals from each subspecies are 'sampled' from their respective colonies. Changes in population size are represented by dashed lines, and parameter labels correspond to parameter types and distributions in Tables S1 and S2. **Figure S3:** The two demographic scenarios used to compare support for (a) a 'founder' and (b) a 'vicariance' divergence event in the brown fur seal (BFS, *Arctocephalus pusillus*) in DIYABC-RF (Collin et al. 2021). The models differ based on the population size prior ranges of N2f (2–999 individuals) in the 'founder' scenario and N2v (1000–500,000 individuals) in the 'vicariance' scenario. The Cape (CFS) and Australian (AFS) fur seal lineages are depicted diverging at time 'Td'. Changes in population size are represented by dashed lines, and parameter labels correspond to parameter types and distributions in Table S3. **Figure S4:** The (a) proportion and (b) number, in millions (M), of Genotyping-by-Sequencing (GBS) reads retained in sequence library after cleaning and filtering in process_radtags (Rochette et al. 2019) for 94 brown fur seal (*Arctocephalus pusillus*) individuals and two laboratory blanks (Blank1, Blank2). **Figure S5:** Per-locus pairwise fixation index (F_{ST}, light grey) between South African and Australian samples calculated in RealSFS (Korneliussen et al. 2014) and plotted across the genome for the full dataset. Coloured points show sliding-window mean F_{ST} (window size = 50 SNPs, step size = 10 SNPs). Scaffolds are distinguished by colour. **Figure S6:** The difference between the best log likelihood scores for genetic clusters (K) 1–8 for 92 brown fur seal (*Arctocephalus pusillus*) individuals in NGSADMIX (Skotte et al. 2013). **Figure S7:** Cross-entropy scores for genetic clusters (K) 1–5 for 92 Cape fur seal (*Arctocephalus pusillus pusillus*) individuals in LEA (Frichot and François 2015). **Figure S8:** Cross-entropy scores for genetic clusters (K) 1–3 for 92 Australian fur seal (*Arctocephalus pusillus doriferus*) individuals in LEA (Frichot and François 2015). **Figure S9:** Principal Component Analysis (PCA) of summary statistics from simulated datasets across four demographic scenarios ('constant', 'ancestral', 'bottleneck' and 'ancestral+bottleneck'; Figure 1) used to explore support for bottleneck scenarios, with individuals 'sampled' from one population. Neutral SNP data for the Cape fur seal (*Arctocephalus pusillus pusillus*) and Australian fur seal (*Arctocephalus pusillus doriferus*) were explored in DIYABC-RF (Collin et al. 2021). The observed dataset is depicted by the red star. **Figure S10:** Principal Component Analysis (PCA) of summary statistics from simulated datasets across the two scenarios ('bottleneck' and 'ancestral+bottleneck'; Figure 1) used to explore support for bottleneck scenarios with individuals 'sampled' from three populations. Neutral SNP data for the Cape fur seal (*Arctocephalus pusillus pusillus*) and Australian fur seal (*Arctocephalus pusillus doriferus*) were explored in DIYABC-RF (Collin et al. 2021). The observed dataset is depicted by the red star. **Figure S11:** Principal Component Analysis (PCA) of summary statistics from simulated datasets across the pilot runs of divergence scenarios (Figure S3) with (a) log-uniform sampling and (b) uniform sampling. Neutral SNP data for the Brown fur seal (*Arctocephalus pusillus*) were explored in DIYABC-RF (Collin et al. 2021). The observed dataset is depicted by the red star. **Figure S12:** Principal Component Analysis (PCA) of summary statistics from simulated datasets across the divergence scenarios (Figure S3) for parameter estimation. Neutral SNP data for the Brown fur seal (*Arctocephalus pusillus*) were explored in DIYABC-RF (Collin et al. 2021). The observed dataset is depicted by the red star. **Table S1:** The parameters, parameter types (N = population size, T = time in generations) and scenario types ('Pops': one population, three populations, both) used with the scenarios in Figure 2 and Figure S2 to compare support for recent bottleneck scenarios with the Australian fur seal (AFS, *Arctocephalus pusillus doriferus*) data in DIYABC-RF (Collin et al. 2021). Notes about parameter estimates and sources, working estimates, adult estimates (derived using a species-specific pup-to-population-size conversion factor of 4.5×; Gibbens and Arnould 2009), lower and upper prior conversion factors and lower and upper prior distributions are provided. **Table S2:** The parameters, parameter types (N = population size, T = time in generations) and scenario types ('Pops': one population, three populations, both) used with the scenarios in Figure 2 and Figure S2 to compare support for recent bottleneck scenarios with the Cape fur seal (CFS, *Arctocephalus pusillus pusillus*) data in DIYABC-RF (Collin et al. 2021). Notes about parameter estimates and sources, working estimates, adult estimates (derived using a species-specific pup-to-population-size conversion factor of 4.5×; Gibbens and Arnould 2009), lower and upper prior conversion factors and lower and upper prior distributions are provided. **Table S3:** The parameters, parameter types (N = population size, A = rate of admixture, T = time in generations), prior distributions (lower and upper), posterior parameter estimates (median, 5th percentile, 95th percentile), Relative 90% Credible Interval (CI) and Local Normalised Mean Absolute Error (NMAE) generated in DIYABC-RF v1.2.1 (Collin et al. 2021) for the (a) Australian fur seal (*Arctocephalus pusillus doriferus*) and (b) the Cape fur seal (*Arctocephalus pusillus pusillus*) for

divergence investigations. **Table S4:** Summary of genotype-by-sequencing (GBS) dataset sizes for brown fur seal (*Arctocephalus pusillus*) analyses. The number of markers comprising each dataset is shown for full, neutral and putative outlier datasets across Cape (CFS, *Arctocephalus pusillus pusillus*), Australian (AFS, *Arctocephalus pusillus doriferus*) and brown (BFS) fur seals for genotype likelihood (GL) and hard genotype (HG) datasets. **Table S5:** Mean estimates of observed heterozygosity (H_O), expected heterozygosity (H_E), the inbreeding coefficient (F_{IS}) and nucleotide diversity (π) with standard error (±SE) calculated in Arlequin (Excoffier and Lischer 2010) for six brown fur seal (*Arctocephalus pusillus*) colonies across the Cape (CFS) and Australian (AFS) fur seal subspecies. The number of individuals (n) and number of loci (loci) used for each colony's estimates are included in the table. Estimates from Mossel Bay and Sinclair Island colonies are excluded due to small sample sizes ($n = 2$). **Table S6:** Pairwise fixation indices (F_{ST}; below diagonal) and associated p-values (above diagonal) calculated in Arlequin (Excoffier and Lischer 2010) for six brown fur seal (*Arctocephalus pusillus*) colonies based on (a) neutral and (b) outlier datasets. Statistically significant pairwise F_{ST} values (p-values < 0.05) are marked by an asterisk (*). p-values are corrected for multiple comparisons using the False Discovery Rate (FDR) method. Estimates from Mossel Bay and Sinclair Island colonies are excluded due to small sample sizes ($n = 2$). **Table S7:** Analysis of molecular variance (AMOVA) in Arlequin (Excoffier and Lischer 2010) across full, neutral and outlier datasets for (a) 92 brown fur seal (BFS, *Arctocephalus pusillus*) individuals from six colonies across both subspecies, (b) 48 Cape fur seal (CFS, *A. p. pusillus*) individuals from three colonies and (c) 40 Australian fur seal (AFS, *A. p. doriferus*) individuals from three colonies. Variance components may occasionally fall outside the 0%–100% range due to estimation artefacts: negative values are interpreted as no variance explained at that level, while values >100% indicate that nearly all variance is explained at that level, with negligible variance partitioned elsewhere. Statistically significant variation ($p < 0.05$) is indicated with an asterisk (*). **Table S8:** Number (n) of SNPs and associated genes in the filtered brown fur seal (*Arctocephalus pusillus*) outlier dataset that mapped to BUSCO genes in the *A. gazella* reference genome (Hench et al. 2024) with assigned Gene Ontology (GO) terms. To provide a relative weighting of putative adaptive responses per GO term, the mean and sum of allele frequency (AF) gaps are included for each GO term, along with GO term functions as reported in Hench et al. (2024). **Table S9:** Mean migration rates (m) with standard deviation (±SD) between brown fur seal (*Arctocephalus pusillus*) colonies from the Cape (CFS) and Australian (AFS) fur seal subspecies based a colony-level analysis of gene flow in BayesAss (Wilson and Rannala 2003; Mussmann et al. 2019). Migration rates (m) represent the proportion of individuals in the receiving colony that are first-generation 'migrants' from the donor colony, averaged over one to two generations preceding sample collection in 1998–2019 (Faubet et al. 2007). **Table S10:** The parameters, parameter types (N = population size, A = rate of admixture, T = time in generations), prior distributions (lower and upper), posterior parameter estimates (median, 5th percentile, 95th percentile), Relative 90% Credible Interval (CI) and Local Normalised Mean Absolute Error (NMAE) generated in DIYABC-RF v1.2.1 (Collin et al. 2021) for the (a) Cape fur seal (*Arctocephalus pusillus pusillus*) and (b) the Australian fur seal (*Arctocephalus pusillus doriferus*) from the 'ancestral+bottleneck with uniform sampling' scenarios for each subspecies, where individuals were 'sampled' from three colonies. **Table S11:** The parameters, parameter types (N = population size, A = rate of admixture, T = time in generations), prior distributions (lower and upper), posterior parameter estimates (median, 5th percentile, 95th percentile), Relative 90% Credible Interval (CI) and Local Normalised Mean Absolute Error (NMAE) generated in DIYABC-RF v1.2.1 (Collin et al. 2021) for the Cape fur seal (*Arctocephalus pusillus pusillus*) and Australian fur seal (*Arctocephalus pusillus doriferus*) from vicariance divergence scenarios (Figure S3).