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Genomics reveals the role of admixture in the evolution of structure among sperm whale populations within the Mediterranean Sea

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Abstract

In oceanic ecosystems, the nature of barriers to gene flow and the processes by which populations may become isolated are different from the terrestrial environment, and less well understood. In this study we investigate a highly mobile species (the sperm whale, *Physeter macrocephalus*) that is genetically differentiated between an open North Atlantic population and the populations in the Mediterranean Sea. We apply high-resolution single nucleotide polymorphism (SNP) analysis to study the nature of barriers to gene flow in this system, assessing the putative boundary into the Mediterranean (Strait of Gibraltar and Alboran Sea region), and including novel analyses on structuring among sperm whale populations within the Mediterranean basin. Our data support a recent founding of the Mediterranean population, around the time of the last glacial maximum, and show concerted historical demographic profiles in both the Atlantic and the Mediterranean. In each region there is evidence for a population decline around the time of the founder event. The largest decline was seen within the Mediterranean Sea where effective population size is substantially lower (especially in the eastern basin). While differentiation is strongest at the Atlantic/Mediterranean boundary, there is also weaker but significant differentiation between the eastern and western basins of the Mediterranean Sea. We propose, however, that the mechanisms are different. While post-founding gene flow was reduced between the Mediterranean and Atlantic populations, within the Mediterranean an important factor differentiating the basins is probably a greater degree of admixture between the western basin and the North Atlantic and some level of isolation between the western and eastern Mediterranean basins. Subdivision within the Mediterranean Sea exacerbates conservation concerns and will require consideration of what distinct impacts may affect populations in the two basins.

KEYWORDS
admixture, cetaceans, demography, evolution, genomics, population structure

1 | INTRODUCTION

In oceanic ecosystems, geographical barriers are often less relevant than oceanographic and other environmental factors in shaping the genetic structure of populations (e.g., Hoelzel, 1994; Palumbi, 2004; Van Cise et al., 2019; Westbury et al., 2022). Varying selection pressures, patterns of connectivity and genetic drift shape the diversity of species, potentially leading to greater geographical diversity in species with a wide latitudinal distribution range (Ralph & Coop, 2010). In general, cetaceans are highly mobile with high dispersal potential (see Mrusczok et al., 2021; Peres dos Santos et al., 2022; Stevick et al., 2011; Violi et al., 2021). Even so, they often show population genetic structure over relatively small geographical ranges (e.g., Hoelzel, 2009). In some cases, migratory whales from different stocks may mix on feeding grounds (Larsen et al., 1996; Palumbi & Baker, 1994). In conservation biology, the identification and protection of genetically distinct local populations, including those in mixed assemblages, is essential for conserving evolutionary potential and reducing extinction risks (e.g., Avise, 2009). Given the potential for population structure to be cryptic in the marine environment, both empirical data defining populations and an understanding of the evolutionary mechanisms generating structure are required to facilitate effective conservation and management (e.g., Hohenlohe et al., 2021; Holderegger et al., 2020; Luck et al., 2003).

In this study, we investigate fine-scale population structure in the sperm whale (*Physeter macrocephalus*), a species with a global range across ocean basins (see Whitehead, 2018), considered to play a fundamental role in the balance of oceanic ecosystems (Würtz & Simard, 2007). Worldwide and for up to three centuries, this species was the focus of two intensive hunting periods (Whitehead, 2002). From a pre-whaling abundance of over 1 million, today there are an estimated 844,761 individuals (Whitehead & Shin, 2022) and they are now globally classified as Vulnerable on the IUCN red list (Taylor et al., 2019).

Range-wide population genetic studies of sperm whales have identified several key features. Genetic variation is low especially at mitochondrial DNA (mtDNA) (Alexander et al., 2013, 2016; Morin et al., 2018), and low enough to suggest a historical population bottleneck (see Lyrholm & Gyllensten, 1998; Morin et al., 2018) or cultural hitchhiking (genetic variation cosegregating according to cultural structure; see Whitehead, 2017). In both the Atlantic and Indian Oceans, mtDNA structure was found despite the absence of clear geographical boundaries (Alexander et al., 2016; Engelhaupt et al., 2009). Within the Pacific Ocean, the lack of genetic structure at mtDNA was proposed to be consistent with the wider dispersal scale of females compared to Atlantic populations (Mesnick et al., 2011; Rendell et al., 2012; Whitehead et al., 2012). Discrepancy between mtDNA- and nuclear-DNA-based population structure was suggested to imply male-mediated gene flow (Engelhaupt et al., 2009; Mesnick et al., 2011).

Here, we focus on the populations of sperm whales within the Mediterranean Sea and in nearby Atlantic waters to investigate the mechanisms for maintaining fine-scale population structure in this region. Engelhaupt et al. (2009) reported differentiation between the Mediterranean Sea and North Atlantic at microsatellite DNA and mtDNA loci. Based on a mitochondrial genome phylogeny, Morin et al. (2018) found evidence for differentiation, but proposed that the Mediterranean lineage was founded from the Atlantic population only ~20,000 years ago, at the end of the last glacial maximum. Consistent with genetic differentiation, sperm whales show differential usage of vocal "codas" (see Frantzis & Alexiadou, 2008) within the Mediterranean compared to elsewhere (Drouot, Goold, et al., 2004; Pavan et al., 2000; Teloni, 2005).

Within the northeast Atlantic, sperm whales are regularly present (see Fais et al., 2016; Silva et al., 2014; Alves et al., 2018; Boys et al., 2019; Ferreira et al., 2022). Within the Mediterranean, sperm whales are widely distributed in both the western and eastern basins (see Aïssi et al., 2012, 2014; Ascheri & Fontanesi, 2023; Bellomo et al., 2019; Fiori et al., 2014; Frantzis et al., 2003, 2014; Mussi et al., 2014; Notarbartolo di Sciara et al., 2008; Pirotta et al., 2011; Pirotta, Brotons, et al., 2020; Pirotta, Vighi, et al., 2020; Rendell & Frantzis, 2016; Tepsich et al., 2014). In the Strait of Gibraltar, they are regularly seen both east and west of the Strait (de Stephanis et al., 2008), suggesting the potential for gene flow despite evidence for differentiation (e.g., Engelhaupt et al., 2009). Movement by males within the Mediterranean has been broadly documented (e.g., Alessi et al., 2014; Carpinelli et al., 2014; Mussi et al., 2014; Rendell et al., 2014) including movement from the western to the eastern basin (Frantzis et al., 2011), though such direct observations do not necessarily imply local mating success and effective gene flow.

To date, no studies have assessed sperm whale population genetics within the Mediterranean Sea, though this information is essential in support of effective conservation strategies (see Notarbartolo di Sciara, 2014). Conservation planning could furthermore benefit from information on current and past demographic trajectory. Ingestion of plastic debris, collisions with ships and entanglements put a strain on the Mediterranean population (Notarbartolo di Sciara, 2014), which numbers fewer than 2,500 mature individuals and is classified as Endangered in the IUCN Red List (Pirotta et al., 2021). Although this assessment is justified by the observed mortality levels at present, no robust data regarding population trends exist (Rendell & Frantzis, 2016).

Here we assess the pattern and mechanisms of demographic trends and population structure of a highly mobile species showing population structure within an oceanic basin. We use high-resolution nuclear genetic analyses (double digest restriction site-associated DNA sequencing: ddRADseq) to assess the pattern and level of gene flow within the Mediterranean Sea and between the Atlantic Ocean and the Mediterranean Sea. We also consider ongoing and historical population dynamics since the putative founding of the population, and implications for the effective conservation management of this endangered population. These data provide transferable inference supporting the conservation of other mobile marine species in similar, recently founded habitats.

2 | MATERIALS AND METHODS

Sperm whale tissue samples were obtained during various research projects between 1999 and 2018. Skin biopsy samples were collected in the Azores, Madeira, Strait of Gibraltar and the Ligurian–Provençal area, while skin and muscle samples were obtained from stranded individuals in the Canary Islands, Galicia, Tyrrenian Sea, Adriatic Sea and the Greek Seas. Altogether the Mediterranean samples were sourced from six different areas in four different countries (Greece, Italy, France and Spain) and the Atlantic samples were from four different areas (Galicia, Azores, Canary Islands and Madeira; Figure 1; Table S1).

2.1 | DNA extraction and library preparation

DNA was extracted both by using OMEGA BIOTEK and MN MACHEREY-NAGEL kits following the manufacturers' protocols, and by the phenol chloroform method (after Hoelzel, 1998). Genomic DNA concentration was quantified using the Qubit High Sensitivity kit (Thermo Fisher Scientific). We applied the ddRADseq methodology (Peterson et al., 2012) for sperm whales for the first time in this study. Two sequencing libraries, each of 80 samples, were constructed following the protocol of Peterson et al. (2012). DNA (500 ng) was digested using enzymes *Msp*I and *Hin*dIII (New England Biolabs). Adapters were ligated using T4 ligase in a reaction solution with 1× buffer, 400 units of T4 ligase and 1.5 μM of adapter that contained a unique in-line barcode for each individual. Uniquely barcoded samples were pooled, and cleaned using calibrated streptavidin-coated SpeedBeads (Sera-Mag). Fragments between 360 and 560 bp were size-selected using a Pippin Prep (Sage Science). Sixteen uniquely indexed libraries were prepared, each containing 10 individually barcoded samples, with randomized allocation of individuals across libraries. Fragment size distributions of resulting libraries were evaluated using a 2200 Tapestation (Agilent Technologies), and library DNA concentrations were estimated using qPCR (quantitative polymerase chain reaction). Two final libraries were then sequenced on an Illumina HiSeq 2500 using 125-bp paired-end sequencing at the DBS Genomics facility at Durham University (https://www.dur.ac.uk/biosciences/services/dna/dnasequencing/).

2.2 | SNP calling

Reads were trimmed to 110 bp, demultiplexed and filtered using process_radtags in STACKS version 2.2 (Rochette et al., 2019). Unpaired reads were discarded. Paired reads were aligned using the very-sensitive mode of BOWTIE version 2.2.5 (Reinert et al., 2015) against a sperm whale reference genome downloaded from the NCBI website (GCA_002837175.2). SAMTOOLS version 1.9 (Li et al., 2009) was used to filter out reads that aligned discordantly, as well as reads with a mapping quality below 20. Single nucleotide polymorphisms (SNPs) were called using the STACKS refmap pipeline with default settings. Sites were not filtered based on depth (an option which has purposely been removed from STACKS version 2.0 onwards), but instead based on a likelihood ratio test which weighs the evidence for heterozygous vs. homozygous calls (which depends partially on coverage), using the default *p*-value threshold of .05. The Populations command of STACKS was run with $r = .7$ and $p = 3$ (Rochette et al., 2019). We accepted multiple SNPs per read and thinned the data set downstream

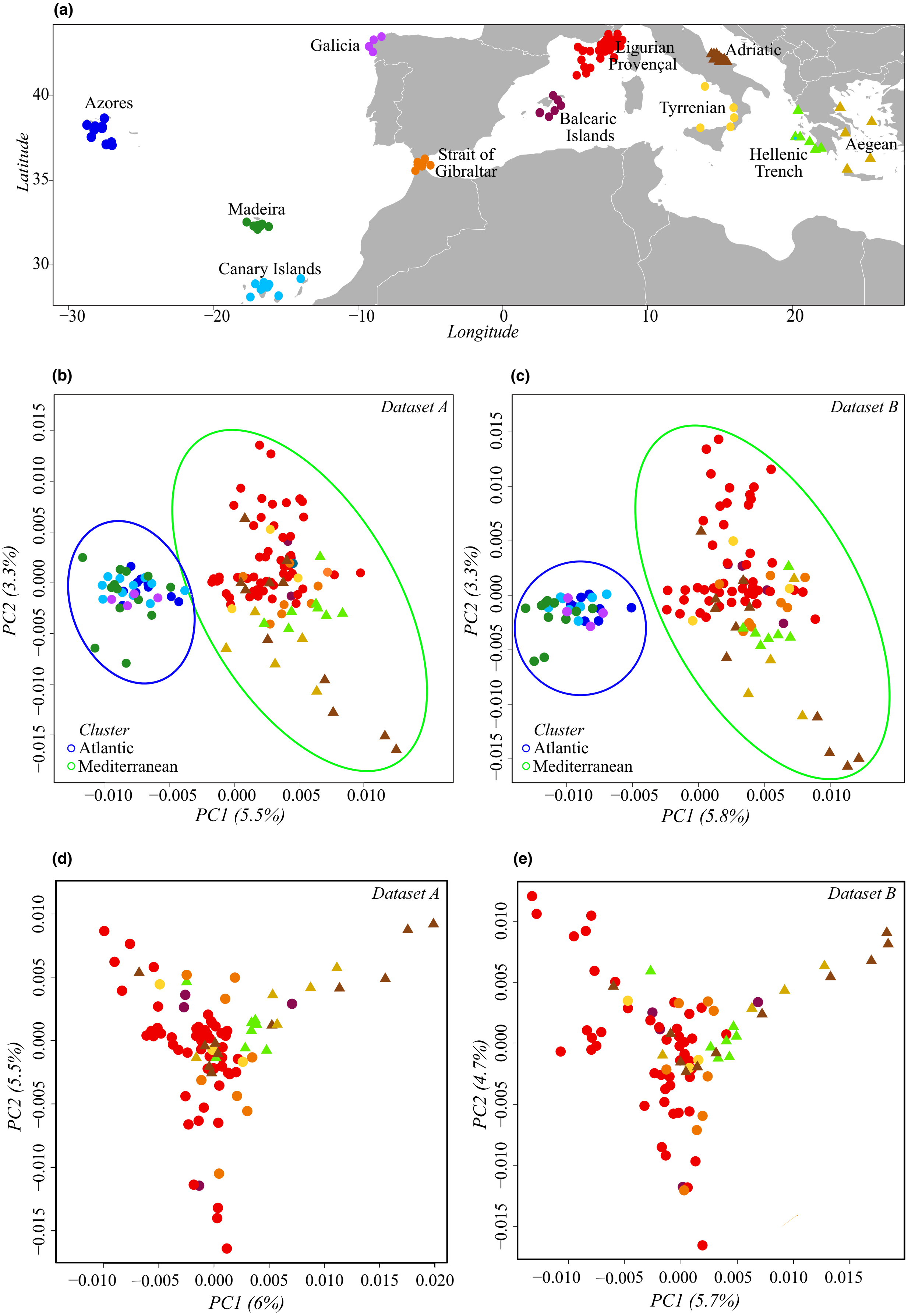

FIGURE 1 Population structure analyses. (a) Sampling locations, (b) principal coordinate analyses for data set A (4,422 SNPs) and (c) principal coordinate analyses for data set B (12,616 SNPs); (d) and (e) principal coordinate analyses on samples using data set A or B (respectively) restricted to the Mediterranean. Eastern basin samples are represented by triangles.

when appropriate. PGD-SPIDER (Lischer & Excoffier, 2012) or VCFTOOLS (Danecek et al., 2011) was used to convert the SNP data into PED and MAP format. Binary files (BED, RAW and BIM) were generated from PED and MAP files using PLINK (Purcell et al., 2007). The VCFTOOLS flags --depth and --site-depth were used to calculate read depth per individual and per SNP. SNP data management and analyses were performed in R version 3.6.2 (R Core Team, 2019) using wrapper functions of the R package SAMBAR (de Jong et al., 2021). The data were imported into R and stored in a genlight object using the function "read.PLINK" of the R package ADEGENET version 2.1.1 (Jombart & Ahmed, 2011).

2.3 | SNP and sample filtering

Two data sets were obtained using two different threshold filters. Data set A excluded samples with more than 50% missing data and all loci with greater than 5% missing data. Data set B excluded all samples with more than 25% missing data and all loci with greater than 5% missing data (see Table S2). Both data sets were thinned selecting one SNP per 500-bp region, in order to limit SNPs to one per paired-end read, and used for further structure and diversity analyses.

2.4 | Population structure

The two data sets were used in most of the analyses to test for divergence between Atlantic and Mediterranean populations and within the Mediterranean putative western and eastern populations. Replicates were detected using KING-robust score calculations (Manichaikul et al., 2010) and were excluded from the analyses. Kinship analyses were performed only on data set B due to the higher number of SNPs, using the pi_hat score of the software PLINK (Purcell et al., 2007). The background variation was based on the Atlantic and Mediterranean samples separately.

Population structure was assessed in R, using SAMBAR wrapper functions. Principal coordinate analysis (PCoA) was calculated using APE version 5.3 (Paradis & Schliep, 2019) for both data sets. PCoA is more suitable for the analyses of categorical data (like genotype data: 0, 1 and 2) than principal components analysis (PCA). Structure analyses were performed using two methods: the Landscape and Ecological Studies (lea 3.10.0) package (Frichot & François, 2015) setting the putative number of population (*K*) to 2–4, and ADMIXTURE 1.3 (Alexander et al., 2009), giving prior assignment to Atlantic and Mediterranean populations. We performed 20 repeat runs using PONG (https://github.com/ramachandran-lab/pong; see Behr et al., 2016), using a greedy approach to identify modes and default threshold (0.95) for determining modes. F-statistics were assessed in ARLEQUIN version 3.5 (Excoffier & Lischer, 2010) with 10,000 permutations and the level of missing data set to 0.05. F_{ST} was calculated for both data sets at three levels: (i) all sampled areas, (ii) Atlantic vs. Mediterranean populations and (iii) Atlantic vs. western vs. eastern putative populations.

2.5 | Migration rates and population modelling

Contemporary migration rates were calculated using BAYESASS3-SNPS (Mussmann et al., 2019) using both data sets comparing Atlantic vs. Mediterranean populations and Atlantic vs. western vs. eastern Mediterranean populations. BAYESASS3 uses allele frequency data and Markov chain Monte Carlo (MCMC) simulations to find individuals that are migrants or have migrant ancestry "over the last several generations." The analysis was run with 1,000,000 iterations and a burn-in of 100,000 iterations, seed of 10 and delta value of 0.1. Output matrices were converted into gene flow plots with the use of the R package CIRCLIZE version 0.4.6 (Gu et al., 2014) through SAMBAR.

We considered four scenarios in DIYABC (see Collin et al., 2021; https://github.com/diyabc) to test inference about one or two populations in the Mediterranean. These had the Atlantic as an ancestral population (at t2) and a split within the Mediterranean later at t1, the Western Mediterranean as ancestral, the Eastern Mediterranean as ancestral, and only two populations, Atlantic and Mediterranean. We excluded admixed individuals, and the number of simulations in the training set was to 120 while the number of trees was 500. The priors were default flat priors.

2.6 | Admixture and population dynamics

Patterns of admixture were interrogated using the Unix software TREEMIX version 1.13 (Pickrell & Pritchard, 2012) using the Atlantic population as an outgroup and testing for one migration event, while the parameter f3 was calculated using the R program ADMIXTOOLS version 2 using default settings (Patterson et al., 2012). Both analyses were performed on sample set B comparing the putative Atlantic, western Mediterranean and eastern Mediterranean populations. TREEMIX uses allele frequencies to construct a maximum-likelihood tree and builds a model which allows for both population splits and gene flow to infer the pattern of admixture among lineages. The analysis was run with bootstrap replicate over blocks of 500 contiguous SNPs (as suggested in the manual). The f3 statistic assesses the covariance of allele frequencies between a population pair with a shared outgroup. Measuring the shared drift between the population pairs in the context of drift that is specific to single populations allows inference about their relationship and the chance of admixture.

The historical demography of the Atlantic and the Mediterranean populations was inferred using Stairway plot analysis (Liu & Fu, 2020). Due to potential issues with admixture affecting demographic inference (e.g., Heller et al., 2013), we analysed pooled population samples that showed no evidence of differentiation. One sample from Galicia from the Atlantic sample was omitted due to missing data, and those individuals from Liguria with a clear signal for admixture with the Atlantic were also excluded. The folded site frequency spectrum (SFS) was calculated using the software package ANGSD version 0.938 (Korneliussen et al., 2014). The proportion of sites used for training was 0.67, and the number of input files created for estimations was

200. As in Morin et al. (2018), the mutation rate was set at 2.9 × 10^{-8} substitutions per nucleotide per generation based on Dornburg et al. (2012) and the generation time at 31.9 years based on Taylor et al. (2007). A comparative assessment that can estimate relatively recent N_e with greater accuracy was applied (using the same sample sets as for the stairway plots), drawing inference from linkage disequilibrium (LD). This was assessed using SNEP software version 1.11 (Barbato et al., 2015, after Corbin et al., 2012). N_e estimation was calculated with a minimum and maximum distance between SNPs of 50,000 and 4 Mb, respectively. Data were arranged in 30 bins of 50 kb distance each. The maximum number of SNPs per chromosome was set to 100,000. The formula used to estimate N_e from LD was as proposed by Sved and Feldman (1973), as recommended from a comparative analysis by García-Cortés et al. (2019).

3 | RESULTS

3.1 | Reads and SNPs

A total of 160 sperm whale individuals were sequenced. Both sequencing lanes combined produced 628.9 million reads. Up to 16.2 million reads had to be discarded due to either low quality, an ambiguous radtag or a missing read mate, resulting in an average of 1.9 million read pairs per sample (*SD*: 1.3 million, min: 0.003 million, max: 6.5 million). Individuals with less than 10%, 1%, 0.5% and 0.25% missing data had a minimum coverage of respectively 3, 8.6, 8.6 and 23.2 reads. The mean sequence depth per individual ranged from 3.2 to 184.4 reads per SNP, with a mean 12.4 reads. From a total of 46,717 SNPs, after filtering and thinning, 132 samples and 4,422 SNPs were retained for data set A and 117 samples, and 12,616 SNPs were retained for data set B. Ten samples found as replicates were excluded (Tables S1 and S2).

3.2 | Population structure and gene flow

Comparisons assessing genetic differentiation between the Atlantic and Mediterranean populations based on F_{ST} were significant for both data sets, as was the F_{ST} value comparing the western and eastern basins (Table 1). The F_{ST} value within the Mediterranean was lower than for comparisons with the Atlantic, but significantly greater than zero. A subdivision between the basins is sensible based on physical and oceanographic differences, and based on evidence of differentiation across this boundary for other highly mobile species (such as the bottlenose dolphin, *Tursiops truncatus*; see Natoli et al., 2005 and discussion therein). This established a pattern that we then further investigated using ordination (PCoA) and assignment (LEA and ADMIXTURE) methods.

PCoA revealed that Atlantic samples form a tight cluster (blue circle) separated from Mediterranean samples (green circle; both data sets are shown in Figure 1b,c). The Mediterranean samples are distributed over a broader range of Euclidian space in the plot, and there is only a weak pattern of differentiation among samples from the western and eastern basins (Figure S1). The pattern is somewhat clearer when only samples from within the Mediterranean are included, but there is still overlap (Figure 1d,e). Kinship estimations (pi-hat scores) are consistent with lower overall pairwise kinship among samples collected in the Atlantic than among samples collected in the Mediterranean Sea (Figure S2). While the values are consistently higher in the Mediterranean, they also extend over a broader range (Figure S2). Support values are low per axis, but this is typical when based on many loci.

LEA analyses with values of $K = 2$–4 revealed a clear differentiation between the Mediterranean and Atlantic samples and, for $K = 3$–4, inconsistent differentiation between the western and eastern Mediterranean basins (Figure 2). A cross-entropy analysis shows that both $K = 2$ and $K = 3$ are equally well supported (Figure S3). As expected, the pattern was somewhat clearer when only Mediterranean samples were included (Figure 2c). This is confirmed using PONG to repeat the ADMIXTURE Mediterranean-only analyses 20 times (Figure S4). For $K = 2$–4 for the Mediterranean-only samples (Figure S5) the lowest validation error is again $K = 2$ (*K*2 cv = 0.22375; *K*3 cv = 0.24256; *K*4 cv = 0.25900). For Bayesian modelling in DIYABC, three populations (Atlantic as ancestral followed by an east vs. west split within the Mediterranean) was as well supported as a model that considered only two populations (Atlantic and Mediterranean; Figure S6).

Mixed Atlantic/Mediterranean ancestry was found within Mediterranean areas consistently at all *K* values. The proportions of the admixture coefficient for $K = 2$ was estimated in ADMIXTURE, based on separate Atlantic and Mediterranean putative populations ($K = 2$), as $K = 2$ gave the lowest cross-validation error when all samples were included ($K = 2$: cv 0.2234; $K = 3$: cv 0.2318; $K = 4$: cv 0.2486). This analysis revealed a subset of Mediterranean samples with mixed Atlantic/Mediterranean ancestry, especially from the western basin

TABLE 1 Pairwise F_{ST} values calculated using ARLEQUIN version 3.5 and based on data set A (below the diagonal) and data set B (above the diagonal).

	Atlantic	West Mediterranean	East Mediterranean
Atlantic	–	.03009*	.04085*
West Mediterranean	.03099*	–	.01276*
East Mediterranean	.03583*	.01203*	–

*Values are significant at $p = .000$ (to the resolution provided by the program). Cell colour corresponds to comparisons between the Atlantic and Mediterranean (dark blue) or within the Mediterranean (light blue).

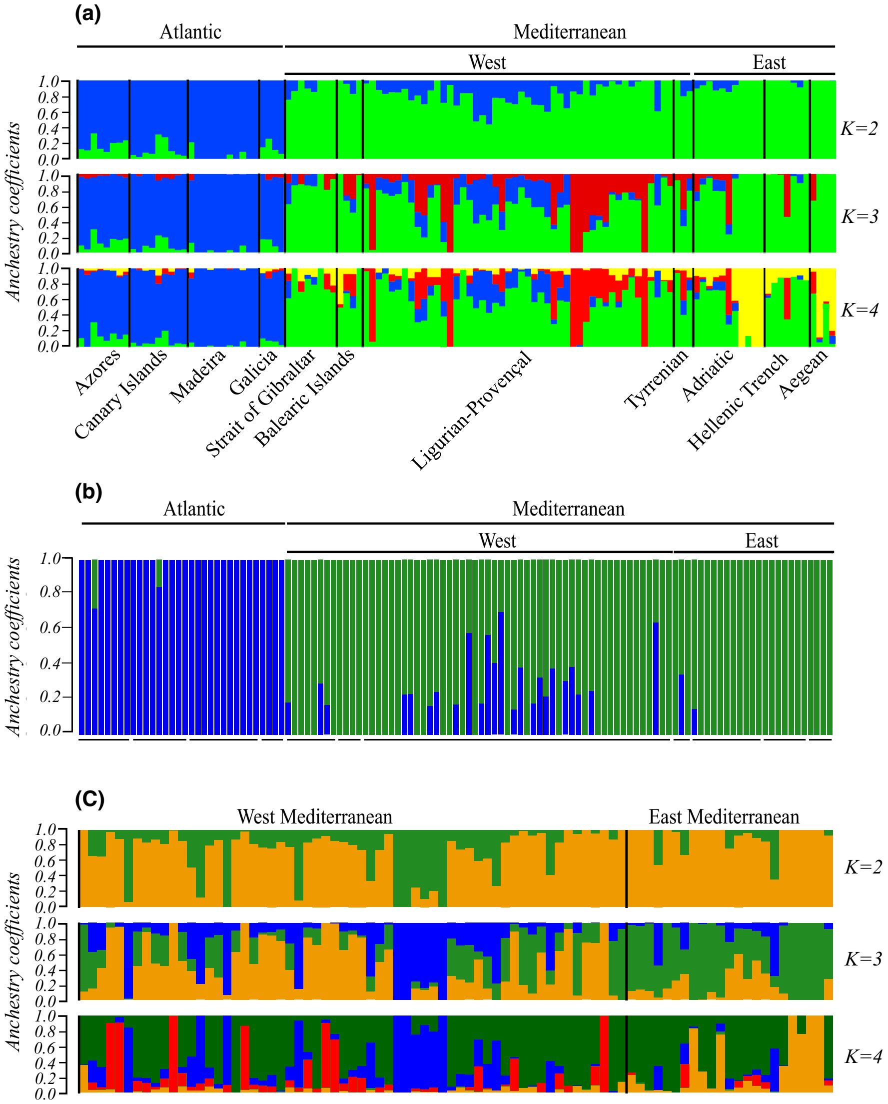

FIGURE 2 (a) LEA plots of population structure including all samples for $K = 2$–4. (b) Estimated proportions of admixture for $K = 2$. Atlantic assignment is in blue and Mediterranean in green. Estimates developed in ADMIXTURE and visualized in R. (c) LEA plots of population structure including only Mediterranean samples for $K = 2$–4.

(Figure 2b). That was the case for 29 samples (29/85 = 34%) overall, including three (3/85 = 3.5%) samples from the Strait of Gibraltar, 24 (24/85 = 28.2%) samples from the Ligurian–Provençal area, one (1/85 = 1.1%) sample from the Tyrrenian Sea and one (1/85 = 1.1%) sample from the Adriatic Sea. These samples showed assignment probabilities to the Atlantic population ranging from 14% to 74% (Table S3). Averaged over all samples within each basin, there was 12.6% admixture from the Atlantic in the western basin, and 0.7% in the eastern basin. Individuals showing some level of admixture in the ADMIXTURE analysis were marked as red dots in the PCoA shown in Figure S1b and all groups within the Mediterranean cluster. An f3 analysis showed marginal values, but a stronger indication of admixture for the western region with the Atlantic than for other possible combinations (Figure 3c; Table 4). The analysis in TREEMIX showed the same pattern, with admixture suggested from the Atlantic into the western region (Figure 3d), though the drift parameter is low for the tree overall. Neither of these methods specifically identifiesg only recent migrants (as in BAYESASS3). Also, while BAYESASS3 can consider bidirectional gene flow, in TREEMIX we could only test one migration edge due to the simplicity of the tree.

Investigations of contemporary migration rate were conducted with BAYESASS3-SNPs. A first analysis was run for both data sets between the Atlantic and Mediterranean populations and revealed an estimated proportion of migrants of ~1% through the Strait of Gibraltar in both directions (Figure 3a; Table 2), consistent with either low gene flow or the relatively recent cessation of gene flow. A second analysis, between the Atlantic, western and eastern Mediterranean putative populations, indicated recent or ongoing directional movement between the two basins from west to east (about 18%–26%), and low (~1%) gene flow between each basin and the Atlantic (Figure 3b; Table 3).

3.3 | Demography

All three stairway plot profiles (Figure 4) showed an apparent increase in population size during the early part of the Pleistocene, though recent time periods (within the last ~10,000 years) are less precise by this method and therefore should be interpreted with caution. All three regions showed a similar effective population

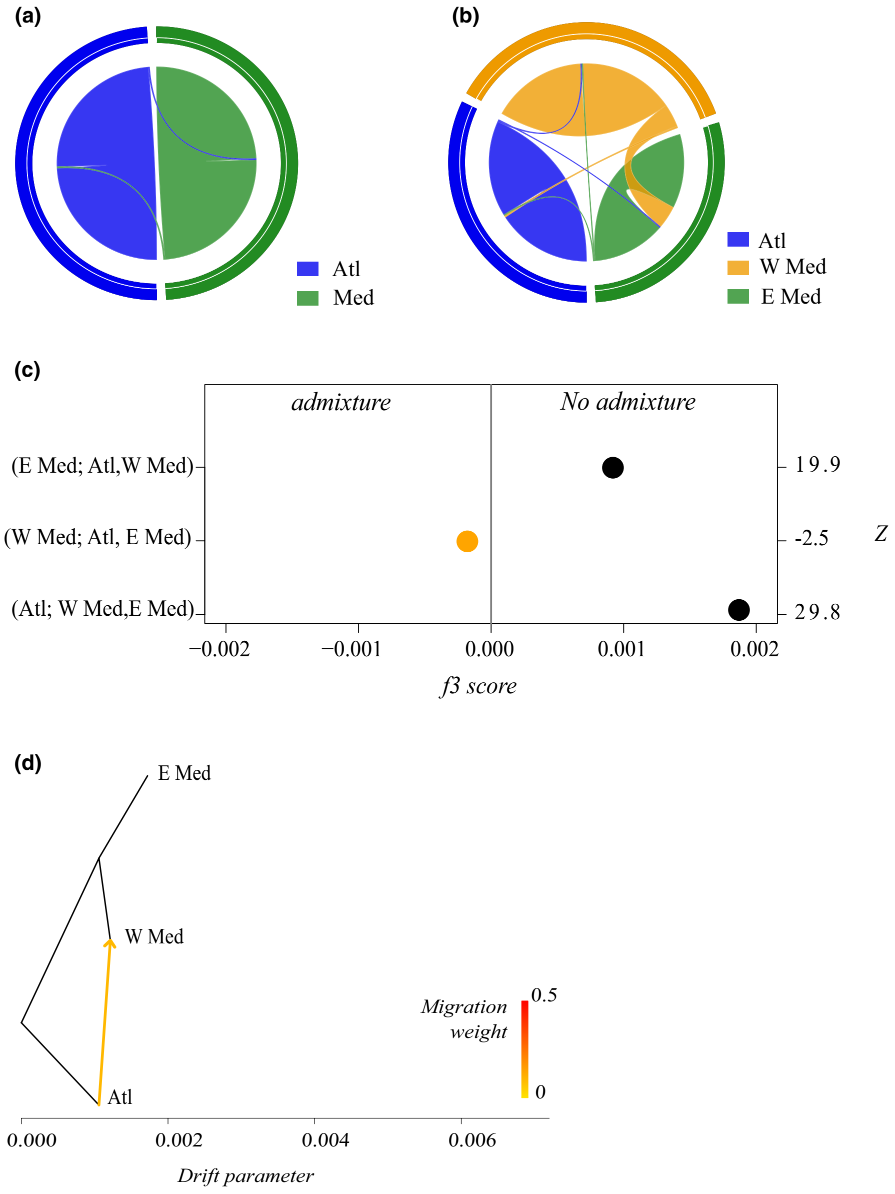

FIGURE 3 Gene flow analyses. Circos plots showing migration rates between populations as calculated by BAYESASS3-SNPS. (a) Atlantic (blue) and Mediterranean (green) putative populations. (b) Atlantic (blue) and Western (orange) and Eastern (green) Mediterranean putative populations. (c) f3 statistic values using Atlantic, west Mediterranean and east Mediterranean putative populations. All values are significant at $p < .05$ based on the Z-scores shown. (d) Maximum-likelihood tree generated with TREEMIX of Western and Eastern Mediterranean populations with inferred migration edges.

TABLE 2 Migration rates between Atlantic and Mediterranean populations using data set A (above) and data set B (below).

	Source	
Current	Atlantic	Mediterranean
Atlantic	0.9916 (0.0081) 0.9903 (0.0094)	0.0084 (0.0081) 0.0097 (0.0094)
Mediterranean	0.0063 (0.0044) 0.0088 (0.0074)	0.9937 (0.0044) 0.9912 (0.0074)

Note: Rates with standard deviations in parentheses defined as the proportion of individuals in a "current" population (in rows) that are migrants derived from a "source" population (in column), per generation. Values inferred in BAYESASS3-SNPS.

size (N_e) (in the range 10,000–20,000) and profile prior to the last glacial maximum (LGM ~20,000 years ago [ka]). After that, all populations showed a decline, but this was most severe within the Mediterranean (down to ~1,000 while in the Atlantic the decline was only to ~8,000). Our comparative assessment using inference from LD (in the program SNEP) showed a similar estimate for N_e at ~7 ka, but then a steeper decline to the present (Figure S7). The contemporary estimate of N_e was low by this method (~100; within the confidence limits of estimates from the stairway plots). N_e for the Atlantic was largest followed by the western and then eastern Mediterranean, but all had a similarly low level for the most recent estimates.

4 | DISCUSSION

4.1 | Atlantic vs. Mediterranean divergence

Our data support the differentiation between the North Atlantic and Mediterranean Sea described in previous genetic and acoustic studies (e.g., Drouot, Berube, et al., 2004; Engelhaupt et al., 2009;

TABLE 3 Migration rates between Atlantic West Mediterranean and East Mediterranean putative populations using data set A (values above) and data set B (values below).

	Source		
Current	Atlantic	West Mediterranean	East Mediterranean
Atlantic	0.9757 (0.0136) 0.9810 (0.0129)	0.0081 (0.0079) 0.0191 (0.0130)	0.0162 (0.0113) 0.0094 (0.0091)
West Mediterranean	0.0042 (0.0041) 0.0129 (0.0123)	0.9639 (0.0114) 0.9826 (0.0103)	0.0320 (0.0108) 0.0101 (0.0079)
East Mediterranean	0.0117 (0.0114) 0.0099 (0.0071)	0.2467 (0.0293) 0.2355 (0.0759)	0.7416 (0.0280) 0.7516 (0.0755)

TABLE 4 f3 statistic among Atlantic West Mediterranean and East Mediterranean putative populations.

pop1	pop2	pop3	est	*SE*	*z*	*p*
Atlantic	W Mediterranean	E Mediterranean	0.0018	6.25E-05	29.7818	<.00001
W Mediterranean	E Mediterranean	Atlantic	−7.9 E-05	3.16E-05	−2.4851	.00459
E Mediterranean	Atlantic	W Mediterranean	0.0009	4.58E-05	19.9024	<.00001

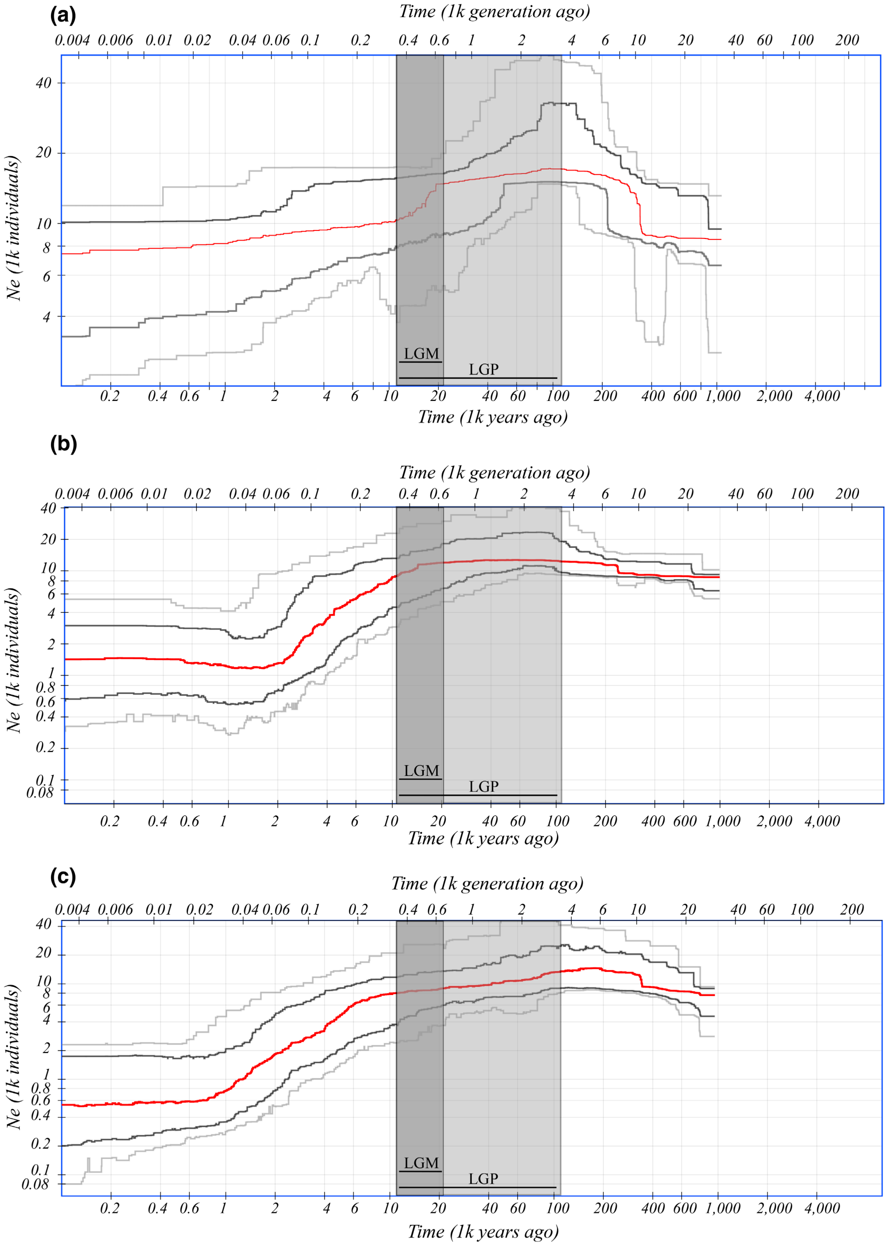

FIGURE 4 Demographic analyses using stairway plots. (a) Atlantic subset; (b) western Mediterranean subset; (c) eastern Mediterranean subset. Last glacial period (LGP) and last glacial maximum (LGM) are indicated by shading. Red line indicates the median values. Black lines indicate 12.5% and 87.5% percentile values, and grey margins indicate 2.5% and 97.5% percentile values.

Mesnick et al., 2011; Morin et al., 2018; Rendell & Frantzis, 2016). Pairwise comparison between these two areas in our study based on 12,616 SNP markers reveals a low but significant value ($F_{ST}=0.03454$, $p=.000$), consistent with that reported in Engelhaupt et al. (2009; where $F_{ST}=0.034$, $p=.022$ based on 16 microsatellite DNA markers). Significant genetic division between the Atlantic and Mediterranean areas has also been documented for other cetaceans such as fin whale (*Balaenoptera physalus*; Bérubé et al., 1998; Palsbøll et al., 2004), bottlenose dolphin (*T. truncatus*; Natoli et al., 2005) and Cuvier's beaked whale (*Ziphius cavirostris*; Onoufriou et al., 2022), and for other top predators such as swordfish (*Xiphias gladius*; Smith et al., 2015) and the blue fin tuna (*Thunnus thynnus*; Puncher et al., 2018). Our findings from LEA and ADMIXTURE were consistent with Atlantic ancestry of the Mediterranean populations. Estimates of contemporary migration patterns indicate low gene flow through the Strait of Gibraltar, ~1% migration per generation in both directions. Sperm whales have been routinely observed in the Strait of Gibraltar, both in summer (de Stephanis et al., 2008) and in winter (Gauffier et al., 2012, 2018). Some movements from the Strait of Gibraltar to the Balearic Islands and the Ligurian–Provençal area and vice versa have also been documented through photoidentification (Carpinelli et al., 2014). Historical reports from Bolognari (1949, 1950, 1951) suggested seasonal movements through the Strait of Gibraltar—entering in the winter and going out in spring. Movements from the Atlantic into the Mediterranean Sea have also been recorded for other cetacean species (e.g., fin whales: Castellote et al., 2010; Gauffier et al., 2018, 2020; Giménez et al., 2013; humpback whales *Megaptera novaeangliae*: Violi et al., 2021; killer whales *Orcinus orca*: Mrusczok et al., 2021).

A complex network of submarine canyons in the western Mediterranean act as corridors between continental shelf areas and deep-sea regions, enhancing oceanographic processes that enrich the deep-sea food web (De Leo et al., 2010) and create a favourable environment for the cephalopod species (e.g., Quetglas et al., 2000) that sperm whales prey on. Therefore, this region can support the energetic demands of sperm whales and other deep divers (Torreblanca et al., 2022). Distinct resource availability and resource specializations have been proposed as drivers of differentiation in various cetacean species (see Hoelzel, 2009; Louis et al., 2014). However, the umbrella squid *Histioteuthis bonnellii* is evidently the most common prey of sperm whales both in the Mediterranean Sea (Foskolos et al., 2020; Garibaldi & Podestà, 2014; Tonay et al., 2021) and in the Atlantic Ocean (Clarke et al., 1993), and so resource specialization may not be an important driver in this case. We note that in this study we have not tested for the various other environmental drivers that may be relevant, such as surface temperature and salinity.

4.2 | Structure within the Mediterranean

Within the Mediterranean, we report weak differentiation between the basins either side of Italy and the Strait of Sicily, as observed in other species (e.g., bottlenose dolphin in Gaspari et al., 2015; Natoli et al., 2005; striped dolphin in Gaspari et al., 2007; Cuvier's beaked whale in Onoufriou et al., 2022). The relatively shallow sill at the Strait of Messina and the Sicilian Channel, and the differential current patterns in the two basins probably reinforce this boundary. Our results from PCoA and LEA were largely consistent with F_{ST}, which showed significant differentiation between the western and eastern basins, though weaker than the differentiation between the North Atlantic and Mediterranean Sea. Assignment analysis (using LEA) restricted to the Mediterranean samples showed a weak pattern when $K=3$ or 4 (Figure 2), but this type of assignment method has low power to resolve a pattern when F_{ST} is lower than ~0.02 (Latch et al., 2006). Ordination (PCoA) showed some separation between western and eastern basin samples, but some overlap as well. We found evidence of admixture between the North Atlantic and Mediterranean Sea, based on analyses using the program ADMIXTURE (Figure 2), f3 statistics and TREEMIX (Figure 3). We propose that the signal for differentiation between the two basins is driven largely by the more frequent historical admixture between the Atlantic and the western basin than with the eastern basin. Signals for ongoing migration based on BAYESASS also show relatively low, bidirectional gene flow between the Atlantic and the Mediterranean, but relatively high, directional gene flow from the western into the eastern basin (see Figure 3). One possible interpretation would be that the signal for differential admixture from the Atlantic into the western basin pre-dates a more recent increase in gene flow from the western into the eastern basin (since BAYESASS should register only recent movement).

4.3 | Diversity and demography

Overall, the Mediterranean samples show higher pairwise kinship than the samples from the North Atlantic (Figure S2), reflecting reduced diversity overall (assuming random sampling). This is consistent with the results from demographic profiling, where the estimated contemporary N_e is approximately six times lower in the Mediterranean than in the Atlantic based on the stairway plots (Figure 4), though the difference was smaller based on SNEP (Figure S7). After a period of relative stasis through the latter part of the Pleistocene, all putative populations show a decline starting around the time of the LGM (Figure 4), and the overall profile is similar for all. However, the Mediterranean trajectories diverge from the Atlantic trajectory at that time. This would fit with a timeframe of within the last 20,000 years for the founding of the Mediterranean by sperm whales, as suggested by Morin et al. (2018). The decline in the Mediterranean might be explained by a relatively small founder population, a low rate of migration between the Atlantic and Mediterranean after founding, and a consequent reduction in N_e within the Mediterranean. At the same time, the parallel pattern of demography is interesting, suggesting that both populations were impacted by a similar process leading to population decline. Based on mtDNA mismatch distributions, Alexander et al. (2016) suggested a post-bottleneck expansion signal world-wide as recently as 10 ka. The stairway plots and SNEP gave similar N_e estimates between ~7 and 1 ka (especially within the Mediterranean), but the stairway plot estimates then level off, and are known to be less accurate for very recent timeframes (reflected in the confidence limit estimates; Figure 4). SNEP estimates continue to decline, suggesting low N_e in modern populations both in the Mediterranean and in the Atlantic.

Episodes of ocean cooling/warming are proposed to have influenced the distribution and abundance of various cetacean species and such events could have led to restricted home ranges (Gaskin, 1982). Morin et al. (2018) proposed this scenario for sperm whales, whose suitable habitat for females was reduced up to 50% since the LGM. Cold regions at high latitude were covered by ice, and sperm whale males, which usually feed in these regions, may have instead occupied the same home range as females and their social units, which normally do not range into cold waters (Best, 1979). This change of age/sex class distribution and overlap could have led to competition for resources and a reduction of food availability.

Some species known to be preyed on by sperm whales, such as the giant squid (*Architeuthis* spp.: Winkelmann et al., 2013), the Humboldt squid (*Dosidicus gigas*: Ibáñez et al., 2011) and the Patagonian longfin squid (*Doryteuthis gahi*: Ibáñez & Poulin, 2014) show low mitogenomic diversity and signatures of demographic/range expansions associated with the LGM. Some cetacean species such as long-finned pilot whale (*Globicephala melas*: Kraft et al., 2020), northern bottlenose whale (*Hyperoodon ampullatus*: Feyrer et al., 2019), white-sided dolphins (*Lagenorhynchus obliquidens*: Banguera-Hinestroza et al., 2010) and killer whales (Moura et al., 2014) also show a similar expansion pattern.

The Mediterranean Sea played a key role as a refuge for several marine species during the last glacial period (see Patarnello et al., 2007). During Pleistocene glaciations, sea level was up to 150 m lower (Lambeck & Purcell, 2005), changing oceanographic features that created isolated refugia and geographical barriers to gene flow, followed by post-glacial dispersal and expansion (Xue et al., 2014). Previous studies have suggested that Mediterranean biodiversity more generally is the result of endemism from glacial refugia (see Patarnello et al., 2007). During the LGM, changes in the pattern of deep-water availability were more pronounced in the eastern basin (Mikolajewicz, 2011; Thunell & Williams, 1989). In the western basin, sperm whales could have found suitable deep-water habitat to colonize where there was likely to be cephalopod prey.

4.4 | Conservation

Our data are consistent with previous studies showing significant genetic differentiation between the North Atlantic and Mediterranean sperm whale populations, and within the Mediterranean greater identity by descent and consequently lower N_e. Demographic profiles show a shared history until around the time of the LGM, after which the decline in N_e in the Mediterranean was probably greater than in the Atlantic. Our estimates of effective population size are low, consistent with the IUCN-Red list assessment, which classified the Mediterranean sperm whale population as Endangered (see Pirotta et al., 2021). Our data support the need to manage the Atlantic and Mediterranean populations separately. Estimates of gene flow suggest low contemporary levels between the Atlantic and the Mediterranean, but more historical admixture. Greater historical admixture in the west and some level of isolation between the basins appears to explain the pattern of differentiation between basins, though gene flow may have increased recently (from west to east based on the analysis in BAYESASS). Any persistent subdivision within the Mediterranean Sea would exacerbate conservation concerns and require consideration of what distinct impacts may affect populations in the two basins. Here we find a weak but significant subdivision between the eastern and western basins, probably driven by differential patterns of admixture with the Atlantic population (more in the west). We also find evidence for a lower N_e in the eastern basin (see Figure S7). We therefore would propose that the eastern and western populations be considered separate management units.

It has been proposed that sperm whales in the Mediterranean are not directly threatened by fisheries since their main prey is *H. bonnellii* (Foskolos et al., 2020; Garibaldi & Podestà, 2014), which is not a target of fishery activity. However, Mazzariol et al. (2011) found several hooks within stomach content analyses of stranded sperm whales, suggesting possible feeding activity along fishing lines as described in some oceanic regions (e.g., Jacobsen et al., 2010). At the same time, fishery activity can also impact sperm whales through entanglement in fishing nets (e.g., Blasi et al., 2021; Pace et al., 2008). Ghost nets, ship collision and the ingestion of plastic debris are the three main impacts that are seriously threatening these endangered sperm whales in the Mediterranean Sea (Alexiadou et al., 2019; de Stephanis et al., 2013; Frantzis et al., 2019).

5 | CONCLUSIONS

Marine systems can be a challenge for the identification of patterns of population structure and the units of conservation for effective management (e.g., Alves et al., 2019). Apart from obvious continental barriers, physical barriers are few, and even continental barriers can be overcome (by going around; see Hoelzel et al., 2021). However, oceanic structures, such as current systems (e.g., Knutsen et al., 2007) that move or concentrate larvae, prey or predators can determine distribution patterns that remain cryptic unless the relevant drivers are understood (see Goncalves da Silva et al., 2019). This reflects a need to combine genetics with ecological data to integrate evidence for both genetic and ecological management units (e.g., Esteban et al., 2016; Giménez et al., 2018). Historical changes in species distributions, such as those driven by the glacial cycles of the Pleistocene, can also shape modern-day population structure (e.g., Bracken et al., 2015). In our study, we find that a highly mobile species probably founded an isolated population around the end of the last glacial period, and that this founder population was further structured by some period of admixture between the source population and the region within the Mediterranean most proximate to the source. This may be a useful model for investigating other patterns of cryptic structure in marine systems.

AUTHOR CONTRIBUTIONS

B.V. and A.R.H. conceived the study and wrote the paper. B.V. undertook laboratory analyses. B.V. and M.d.J. performed bioinformatic analyses. A.F., P.A., C.T., D.O., R.d.S., J.G., G.L., M.A.S., C.O., F.A., A.D., M.T., A.F., M.A., M.A., A.L., and P.C. provided samples and useful feedback and advice. All authors read the manuscript and provided comments.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Sequences associated with ddRADseq analyses are deposited at GenBank under BioProject accession PRJNA936197. ddRADseq genotype files and relevant code are provided on Dryad at: https://doi.org/10.5061/dryad.dv41ns233. There are no restrictions on data availability.

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