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Population structure and genetic connectivity reveals distinctiveness of Irish harbour seals (*Phoca vitulina*) and implications for conservation management

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Title

Population structure and genetic connectivity reveals distinctiveness of Irish harbour seals (*Phoca vitulina*) and implications for conservation management

Authors, affiliations

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Abstract

1. The identification of discrete intraspecific units, such as genetically informed Management Units (MUs), is important to effectively develop and implement conservation strategies for protected species. Harbour seals (*Phoca vitulina*) occurring in Irish waters are currently viewed as a single nationwide panmictic population (and hence MU), though this assumption is not based on knowledge of population structure, due to a lack of available genetic data.
2. Thus, the present study used mitochondrial control region sequences and 9-11 microsatellite loci from harbour seals from Ireland and Northern Ireland (up to n = 123) and adjacent UK/European waters (up to n = 289) to provide insights into the genetic population structure and diversity of harbour seals in the studied areas.
3. Within the island of Ireland, genetic analyses revealed the presence of three genetically distinct local populations characterized by high genetic diversity, hereby defined as: Northwest and Northern Ireland (NWNI), Southwest Ireland (SWI) and East Ireland (EI).
4. Using previously published and newly generated data, a subsequent wider scale analysis revealed that the SWI and EI local populations were genetically distinct from neighbouring UK/European areas, whereas seals from the NWNI area could not be distinguished from a previously identified northern UK metapopulation. Migration rate estimates showed that NWNI receives migrants from Northwest Scotland, with NWNI acting as a genetic source for both SWI and EI.
5. The present study provides the most comprehensive genetic assessment of harbour seals in European waters to date, with findings indicating that conservation strategies for harbour seals in Irish waters should be amended to accommodate at least three genetically distinct local populations/MUs. The use of approaches considering both ecological and genetic parameters is recommended for future assessments and delineation of units of ecological relevance for conservation management purposes.

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Key words

Genetics, Management Unit, marine mammal, microsatellite loci, mtDNA, non-invasive sampling, *Phoca vitulina*, seals

1. Introduction

Appropriate and effective wildlife management and conservation policies rely on the delineation of discrete intraspecific units (ICES, 2009; Coates, Byrne & Moritz, 2018; Hohenlohe, Funk & Rajora, 2021). Such units must be well defined in space and time and are essential for effective assessment of conservation status (as required by national and international legislation), as well as for the development of regional and local management or conservation strategies (Waples & Gaggiotti, 2006; Palsbøll, Berube & Allendorf, 2007; Funk et al., 2012). Discrete units are generally proposed to conserve key elements of intraspecific diversity, but definitions and types of such units can vary depending on resources, geopolitical context and national/international legislative context (Funk et al., 2012; Coates, Byrne & Moritz, 2018).

Contrasting to Evolutionarily Significant Units (ESUs) which aim to conserve historical lineages (Ryder, 1986; Moritz, 1994; Crandall et al., 2000), Management Units (MUs) focus primarily on recent gene flow and connectivity rather than the level of historical gene flow (Palsbøll, Berube & Allendorf, 2007). Thus, it is possible for multiple MUs to exist within a single larger ESU (Funk et al., 2012). These smaller intraspecific units can be useful when devising monitoring programmes and developing ecological indicators, synonymous to 'stocks' used in fisheries assessments (Laikre, Palm & Ryman, 2005) and marine mammals in the United States or within the work of the International Whaling Commission (Hayes et al., 2021).

Within Europe, the EU Marine Strategy Framework Directive (MSFD) (2010/477/EU) and Oslo Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR convention) require the delineation of discrete units for seal biodiversity indicators; known as Assessment Units (AUs) under OSPAR (OSPAR Commission, 2019). Some EU Member States are employing the same spatial units for reporting under both OSPAR and the MSFD (Palialexis et al., 2020). While the term AU has not been defined in practice, it has been recommended that the spatial scale of AUs should be of ecological relevance for the species concerned (OSPAR Commission, 2019; Palialexis et al., 2020), and therefore the identification of discrete and countable units could theoretically encompass units below the MU level as defined above. Thus, in the context of delineating units of ecological relevance for conservation management purposes, the use of approaches considering both ecological and genetic parameters has been recommended for marine mammals (Evans & Teilmann, 2009; Giménez et al., 2018; Martien et al., 2019).

Although several studies have been carried out on the population genetic structure of harbour seals (*Phoca vitulina*) in western European waters, significant knowledge gaps still exist, particularly in Irish waters (Goodman, 1998; Olsen et al., 2017; Carroll et al., 2020; Blanchet et al., 2021). Initial work based on individuals sampled during the 1988 Phocine Distemper Virus (PDV) outbreak (7 microsatellite loci) suggested the existence of six populations, or ESUs: Iceland, Scotland/Northern Ireland, English East Coast, Wadden Sea, Western Scandinavia, and East Baltic (Goodman, 1998). Olsen et al. (2017) then identified two main genetic groups or metapopulations (using 12 loci), the southern UK/mainland Europe (here Southern North Sea/SNS) and northern UK (NUK) metapopulations, where four smaller genetic clusters were identified for the latter metapopulation. A follow-up study (using 14 loci), identified even finer-

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scale structure within the northern UK metapopulation, including genetic differentiation, but with ongoing gene flow, between harbour seals sampled in Northern Ireland and Northwest Scotland (Carroll et al., 2020).

While these nuclear markers have been the preferred method for assessments of European harbour seals, work undertaken in Japan recently showed that mitochondrial differentiation was higher than nuclear differentiation over the same geographic scale (Mizuno et al., 2020). However, to date, mitochondrial data have rarely been used to assess harbour seal population structuring in European waters (but see Stanley et al., 1996), which is surprising given the fact that this is one of the most used markers (usually in conjunction with nuclear microsatellite loci) adopted for population genetic studies of a wide range of animal taxa (Waples & Gaggiotti, 2006). Due to the maternal inheritance of mitochondrial DNA, such markers are particularly relevant for assessments of species that display female breeding site philopatry, including pinnipeds. Population structure identified through mitochondrial markers in such cases can vary substantially to the overall structure observed and should be taken into consideration for management actions for instance when introducing protective measures around breeding sites.

Fourteen seal management units (SMUs) have been proposed in the UK (SCOS, 2014), ten of which have populations of >50 individuals which can be considered relatively large for the area given the species in question (Thompson et al., 2019). The delineation of these SMUs was based on multiple factors including the spatial variation in monitoring schedules and country boundaries as well as the distribution of haul-out sites for the species. The SMUs largely align with findings of genetic analysis (Carroll et al., 2020) though some SMUs are at a finer scale than local populations identified using genetic markers, particularly in South England and Northwest Scotland (Olsen et al., 2017; Carroll et al., 2020). These units are employed both nationally and within OSPAR for seal biodiversity indicator assessments within UK waters.

In Ireland, harbour seals are protected under national law (1976 Wildlife Act and its Amendments, 2011 Birds and Natural Habitats Regulations) and international legislation, including EU Directives and associated conservation-oriented measures (Habitats Directive, MSFD, OSPAR). Harbour seal numbers in Ireland have been estimated at a minimum of 4,007 based on aerial counts in 2017/2018 (Morris & Duck, 2019) which constitutes about one tenth of the abundance of harbour seals in neighbouring UK waters (SCOS, 2020). Under the Habitats Directive, 13 Special Areas of Conservation (SAC) for the species have been designated within Ireland and reporting under Article 17 of the Habitats Directive revealed that while numbers of this species appeared stable in the short term (2007-2018), long term trends (1994-2018) could not be estimated with confidence (NPWS, 2019).

Irish harbour seals are currently viewed as a single nationwide unit for conservation purposes, a policy that is practical from a jurisdictional point of view but is hampered by a lack of genetic data rather than being evidence based. Such a nation-wide approach for harbour seal conservation would be appropriate if seals across the national territory were part of a single panmictic (randomly breeding) population. However, discrete haul-out sites (used for resting, pupping and moulting) have been identified within Ireland (NPWS, 2019), while individually tagged seals have been shown to exhibit high levels of haul-out site fidelity and largely cover short distances for foraging trips (Cronin, 2011). Based

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on studies reporting fine-scale population structure in contiguous waters (such as the UK), sub-structuring at a finer geographic scale may also exist in Ireland (ICES, 2014; NPWS, 2019).

The aim of the current study was to analyse the most comprehensive genetic dataset of harbour seals in western European waters, in order to elucidate genetic structure and diversity of harbour seals in Irish waters as well as their genetic distinctiveness. It was then aimed to identify genetic connectivity between observed local populations and to assess whether any of these local populations may be more vulnerable to pressures due to genetic bottlenecks which may possibly require further management actions. Taking into account findings from the present study, recommendations are brought forward to enable evidence-based delineation of discrete MUs and assist in the effective conservation management of harbour seals in western European waters.

2. Methods

2.1 Sampling and DNA extraction

Samples (scat, moulted hair, urine, regurgitated milk, blood/saliva, skin of dead seals encountered) were collected from wild harbour seals using non-invasive techniques (where individual seals were not captured during the process; Carroll et al., 2018) and stored at -20 ˚C until further processing. The samples were collected at key haul-out sites across Ireland (Supplementary Table S1) during the pre-breeding and moult seasons between 2017 and 2019 under NPWS Licences C33/2017, C83/2017, C86/2018 and C179/2018. Additional samples (blood, saliva, plucked hair, scat, skin) were collected by collaborating rehabilitation centres and research institutes in Ireland, Northern Ireland and Germany, sampled between 2016 and 2020 (Supplementary Table S1, see Supporting Information Table S2 for further detail). Genomic DNA was isolated from scats, moulted/plucked hair, urine, blood, saliva and skin as described in (Steinmetz et al., 2021). A maximum of 205 and 374 samples were included for analysis of mtDNA and microsatellites, respectively. Here, sample size of local populations ranged between 6 (Shetland, East Scotland) and 60 (Northwest and Northern Ireland) for mtDNA and between 14 (Shetland) and 90 (Northwest Scotland) for microsatellites (Supplementary Table S1).

2.2 Mitochondrial and microsatellite data acquisition

A portion of the mitochondrial control region (421-488 bp long) was amplified using primers PvH00034 (5'-TACCAAATGCATGACACCACAG-3') (Westlake & O'Corry-Crowe 2002) and L15926 (5' -ACACCAGTCTTGTAAACC-3') (Kocher et al., 1989). Each PCR was carried out in 10 µl reactions as detailed in Steinmetz et al. (2021). All amplifications included positive and negative controls and amplification products were subsequently checked via 1.5% agarose gel electrophoresis in 1x TAE buffer with SYBR Safe (Invitrogen). PCR products of scats were purified where possible using the QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) and all PCR products were subjected to commercial Sanger sequencing out of house (Eurofins GATC Biotech, Cologne, Germany). Raw sequence

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reads and polymorphic sites were inspected visually and electropherograms were checked and trimmed by eye to ensure retention of only high-quality data before carrying out alignments with the ClustalW algorithm in MEGA 7.0.26 (Kumar, Stecher & Tamura, 2016).

Microsatellite amplification and genotyping was undertaken for 14 loci previously developed for seals (Hg0, Hgdii, Hg6.1, Hg6.3, Hg8.9, Hg8.10, SGPV3, SGPV9, SGPV10, SGPV11, Pvc19, Pvc43, Pvc78, TBPV2) (Allen et al., 1995; Coltman, Bowen & Wright, 1996; Goodman, 1998). Each singleplex PCR was carried out in 10 µl reactions including approximately 5-60 ng/µl of DNA template, 1x GoTaq™ green MasterMix (Promega) and 1 µM of forward and reverse primer and each PCR included negative controls to rule out potential contamination (see Supplementary Table S3 for PCR profiles). Unsuccessful reactions were repeated once. Where still unsuccessful, reactions were repeated using identical cycling conditions and a more powerful polymerase (Platinum II Taq Hot-Start DNA polymerase and associated buffer, Invitrogen). All forward primers were fluorescently labelled at the 5' end using either IRD700 or IRD800. The resulting products were pooled into groups of a maximum of four loci (Supplementary Table S3) – whose fluorescent label and/or allele size did not overlap – and were co-loaded and separated on 6% polyacrylamide gels using a LI-COR 4300 DNA Analyzer (LI-COR Biotechnology, USA). Size of amplicons was resolved manually by visual inspection of gel images and comparison to size standards (LI-COR Biotechnology, USA) as well as home-made size ladders consisting of mixtures of amplicons from previous runs to ensure genotyping consistency among runs. In order to assess genotyping error rate, 25 out of 127 samples (19.5%) were re-run and assessed for each locus. All microsatellite loci were tested in Micro-Checker ver.2.2.3 (Van Oosterhout et al., 2004) for large allelic dropout, presence of null alleles, stutter bands, and genotyping inconsistencies.

Additionally, microsatellite data from 14 loci were obtained from 247 individuals previously analysed by Carroll et al. (2020). DNA isolates from 19 Scottish seals (from samples collected by the Sea Mammal Research Unit (SMRU) under Animal (Scientific Procedures) Act, 1986 Home Office Licences issued to SMRU (PIL nos. 60/3303, 60/4009 and 70/7806)) were used for cross-laboratory calibration of 10 loci to enable the inclusion of data from the aforementioned study. These 10 loci were employed by both the previous and current study, while a further four loci were only employed by SMRU and hence not considered here. Following cross-validation, one locus was omitted due to scoring inconsistencies resulting in a final nuclear dataset (including Irish and non-Irish) of 9 loci, while 11 loci were analysed for Irish only data.

2.3 Definition of local populations

Analyses requiring *a priori* groupings (based on geographic origin of samples) were initially carried out using six geographic sub-regions. The following sub-regions were used, based on the distribution of harbour seal haul-out sites in Ireland and Northern Ireland: East Donegal & Northern Ireland, Northwest Ireland, West Ireland, Southwest Ireland, Southeast Ireland, and East Ireland (Figure 1).

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Once the main barriers to gene flow and local populations within the island of Ireland were identified among the six geographic sub-regions, levels of genetic diversity and differentiation requiring *a priori* population definitions were based on those identified (i.e. genetically distinct) within the current study for the island of Ireland, as well as local populations previously identified by earlier studies (see further definition of Irish local populations in the Results section and Figure 1). For the Northern (UK) metapopulation, local populations were defined as per Carroll et al. (2020): Northwest Scotland (West Scotland + Western Isles), Moray Firth and North Coast & Orkney (Moray Firth + North Coast + Orkney Islands), Shetland, and East Scotland. Local populations within the Southern North Sea (SNS) metapopulation included Germany, Southeast England, France, and the Dutch Wadden Sea. Due to low sample sizes outside of German waters, all samples of the SNS metapopulation were pooled into one local population for analysis.

2.4 Mitochondrial population structure and diversity

Analysis was carried out for the European dataset that included all international samples. The number of polymorphic sites, number of haplotypes, nucleotide diversity (π) and haplotype diversity (h) for the mitochondrial control region (mtDNA) were assessed for each local population (as described in Supplementary Table S1, Figure 1) using ARLEQUIN ver 3.5 (Excoffier & Lischer, 2010). In order to assess partitioning of variation within and among local populations, an AMOVA was performed in ARLEQUIN and population differentiation was analysed via pairwise comparisons using the exact test within the same software. Further, analysis of population structure was carried out implementing a spatial analysis of molecular variance within the program SAMOVA 2.0 (Dupanloup, Schneider & Excoffier, 2002). SAMOVA enables the identification of partitions of sampling locations that are genetically homogenous, but maximally differentiated from each other, enabling the detection of sub-populations without *a priori* group assignments. These analyses were repeated three times to ensure consistency of the number of clusters identified based on 100 simulated annealing steps with the number of clusters K ranging from two to five. The most likely number of clusters was inferred from the largest associated F_{CT} value for a given K. In addition, a statistical parsimony network (TCS network) of haplotypes for the mtDNA dataset was created in tcs (Clement et al., 2002) and visualized in tcsBU (Múrias dos Santos et al., 2016).

2.5 Nuclear (microsatellite) population structure and diversity

An identity analysis was carried out in CERVUS 3.0.7 (Kalinowski, Taper & Marshall, 2007) to ascertain any potential replicate genotypes, assumed to represent the same individual, present within the dataset, as the majority of the samples from Ireland were obtained via non-invasive sampling in the field. Of the nine loci assessed, a minimum of five loci was set as a requirement for a match between genotypes as per recommendations for an identity analysis within CERVUS (Kalinowski, Taper & Marshall, 2007) and following initial tests including data known to originate from the same individual (data not shown).

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Observed and expected heterozygosity (H_O and H_E) were estimated for each locus and inferred local population using ARLEQUIN ver 3.5 (Excoffier & Lischer, 2010). The number of alleles, allelic richness (A_R) and the inbreeding coefficient (F_{IS}) were estimated using FSTAT ver. 2.9.4 (Goudet, 2003) for each local population. The software has the advantage that it calculates A_R by standardizing for sample size by default. Deviation from Hardy-Weinberg equilibrium (HWE) for each locus using a Markov chain approach (10,000 dememorization steps, 100 batches, 10,000 iterations per batch) between all pairs of loci were tested in GENEPOP (Raymond, 1995).

Genetic structure of harbour seals across Ireland and adjacent European waters was assessed using Bayesian clustering approaches implemented in STRUCTURE v. 2.3 (Pritchard, Stephens & Donnelly, 2000; Hubisz et al., 2009) and in TESS v. 2.3 (Chen et al., 2007) to identify the most appropriate number of genetic clusters (K). STRUCTURE was used employing the admixture model and with correlated allele frequencies between clusters and locations as priors. The model was run five times for K ranging from 1 to 10, each run set to 100,000 initial repetitions of burn-in followed by 1,000,000 Markov chain Monte Carlo (MCMC) repetitions. Output data were analysed using the ΔK method by Evanno, Regnaut & Goudet (2005) in the 'pophelper shiny' package within R (Francis, 2017) to identify and visualize the most likely number of K. The algorithm in TESS was also employed as it considers the spatial distribution of samples. TESS was run six times under the CAR model for K ranging from 2-10, using a burn-in of 20,000 sweeps, a total of 120,000 sweeps per run and a spatial interaction parameter of 0.6 (default value). To identify the most appropriate number of K present in the dataset, the deviance information criterion (DIC), a Bayesian method for model comparison, was plotted against the number of K. The number of K was considered most appropriate when the associated DIC first reached a plateau. In some cases, this method selects an optimum number of K higher than the number of K present within the dataset. Therefore, output bar plots were investigated to identify where no further actual clusters were detected for a higher number of K. Additionally, a multivariate statistical approach was investigated via DAPC analysis following a K means clustering approach implemented in the R package 'adegenet' (Jombart & Ahmed, 2011). The optimum number of K was assessed using the 'elbow' method.

Genetic differentiation within and between identified genetic clusters (i.e. local populations) was assessed using ARLEQUIN ver 3.5 (Excoffier & Lischer, 2010) for obtention of pairwise estimates of F_{ST}. Isolation by distance (IBD) between localities was investigated via regression of $F_{ST}/(1-F_{ST})$ and the log of geographic distances (Euclidian distance calculated within the 'adegenet' package in R (Jombart & Ahmed, 2011)) utilizing the ISOLDE approach within GENEPOP (Raymond, 1995).

2.6 Bottleneck and migration rate analysis

In order to assess vulnerability of local populations to threats, their demographic population status including any recent decrease in effective population size was assessed via the software BOTTLENECK 1.2 (Piry, Luikart & Cornuet, 1999) using the Wilcoxon and the sign tests. Analyses were carried out for local populations employing both the stepwise mutation model (SMM) and the two-phase mutation model (TPM); using 95% single-step mutations, 5% multi-step mutations and variance of 12% based on recommendations by Piry et al. (1999).

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Migration rates between local populations were assessed using BayesAss (Wilson & Rannala, 2003). The median across four runs was taken using 10,000,000 iterations per run and a burn-in of 1,000,000 - sampling every 1,000 iterations. Convergence was confirmed by visual inspection of results to ensure that independent runs converged on similar values. The software calculates migration rates for the last two generations via a gametic disequilibrium signal that is created by immigrating individuals (or descendants thereof). Migration rates can hence be utilized to assess connectivity over the past two generations. Generation time for harbour seals, i.e. the average interval between the birth of an individual and the birth of its offspring, has been reported between 8.75 and 15 years in western European waters (Nikolic et al., 2020; Silva et al., 2021). Given that samples from Ireland and Germany in the current study were collected between 2016 and 2019, and other international samples were collected between 1993 and 2007, information on migration rates using this approach gives relatively recent information in this regard, i.e. within the last few decades.

For all statistical analyses, sequential Bonferroni correction was used to adjust significance levels for multiple comparisons (Rice, 1989).

3. Results

3.1 Microsatellite genotyping success

Loci Hg0 and Pvc43 failed to amplify after optimization and were omitted from all analyses. As locus SGPV3 showed high genotyping inconsistencies during cross-laboratory validation it was also excluded from the analysis, and this resulted in a total of 11 loci for all biological samples processed by the current study (Hgdii, Hg6.1, Hg6.3, SGPV9, SGPV10, SGPV11, Pvc19, Pvc78, TBPV2, Hg8.9, Hg8.10) and nine loci for the European dataset (Hgdii, Hg6.1, Hg6.3, SGPV9, SGPV10, SGPV11, Pvc19, Pvc78, TBPV2) considered for analysis following cross-laboratory calibration as outlined above. Missing data were allowed for a maximum of three loci (≤7.48% missing data within a given dataset) and the genotyping error rate, judged from 19.5% of re-analysed samples, was <0.01% and hence considered negligible.

3.2 Definition of local populations (island of Ireland)

Samples collected within Ireland/Northern Ireland were first analysed based on pairwise comparisons between six geographic sub-regions (Figure 1) using both mtDNA (n=123, length=488bp) and microsatellite markers (n=83, 11 loci) in order to identify local populations that would be used for *a priori* definitions of the analysis on a European level. Mitochondrial data showed significant differentiation between Southeast Ireland and all other geographic areas apart from East Ireland, as well as significant genetic differentiation between East Ireland and the West and Southwest of Ireland (Supplementary Table S4). This differentiation was not observed on a nuclear level, where the

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only significant differentiation was found between the Southwest and the West of Ireland (Supplementary Table S4). Based on results of both marker types (Supplementary Table S4), the following three local populations were proposed and used in the subsequent analysis of the European dataset: Northwest and Northern Ireland (NWNI), Southwest Ireland (SWI) and East Ireland (EI) (Figure 1). The genetic population structure of the European dataset including these local populations (i.e. genetic MUs), and how they relate to the existing MUs of the harbour seal metapopulation in the UK and Europe, is discussed further below.

3.3 Genetic diversity

A total of 27 mitochondrial haplotypes were observed (n=205, 421bp) and for each local population, between 3-10 haplotypes and 5-11 polymorphic sites were identified. Haplotype diversity ranged from 0.600 for East Scotland (ESC) to 0.858 for the Southern North Sea (SNS) (Table 1). Within Ireland, haplotype diversity ranged from 0.622 for SWI to 0.758 for NWNI, with a maximum of 10 haplotypes and 11 polymorphic sites identified in NWNI. Overall, 13 previously unidentified haplotypes were observed within the dataset. Further, four observed haplotypes were previously identified by Stanley et al. (1996), three were reported by Andersen et al. (2011) and seven were previously identified in UK waters (SMRU, University of St Andrews, unpublished data). DNA haplotype sequences are presented in the Supporting Information along with accession codes to GenBank (Table S5).

Using microsatellite loci, there was no evidence of any individual being sampled more than once within the dataset (n=374). Evidence for the presence of null alleles was observed in all local populations, for 1-2 loci, apart from ESC and Shetland (SH). Individual locus tests for each local population identified deviations from HWE across several microsatellite loci within all local populations and using global tests, significant deviations from HWE caused by heterozygote deficiencies were observed for four local populations: Northwest & Northern Ireland (NWNI), Southwest Ireland (SWI), Moray Firth/North coast & Orkney (MFNCO) and the Southern North Sea (SNS). However, these results did not affect the analysis of population structure, tested by undertaking the analysis with and without the loci exhibiting departures from HWE (data not shown). Thus, all loci (n=9) were retained for subsequent analysis. The mean observed heterozygosity (H_o) for the local populations ranged from 0.278 to 0.446, with SNS and EI showing the lowest and highest levels of diversity, respectively, (Table 2) and the mean number of alleles ranged from 2.1 for MFNCO to 3.2 for NWNI.

3.4 Population structure

3.4.1 Mitochondrial data

Assessments of population structure based on mtDNA data showed evidence of genetic differentiation within the studied areas. Results from the AMOVA confirmed a moderate level of genetic differentiation across the whole sample set for *a priori* partitioning using local populations (F_{ST}=0.24, P<0.001), with 23.72% and 76.28% of variation

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explained between and within local populations, respectively. Pairwise comparisons between local populations showed evidence of statistically significant genetic differentiation between the island of Ireland and adjacent areas, as well as within the island of Ireland, with pairwise F_{ST} estimates ranging between 0.16 (NWNI-EI) to 0.44 (SWI-SNS). Within Ireland and Northern Ireland, differentiation was highly significant between EI and both the NWNI (F_{ST}=0.16, *P*<0.001) and SWI (F_{ST} = 0.39, *P*<0.001) local populations but not between NWNI and SWI (Table 3). The Southern North Sea (SNS) showed significant differentiation to all local populations of the Northern UK metapopulation (NUK) as well as to all Irish/Northern Irish local populations. Further, Eastern Ireland (EI) was significantly differentiated from North-west Scotland (NWS), the Moray Firth/North Coast & Orkney (MFNCO), and East Scotland (ESC). No significant differentiation was observed between northern UK local populations, previously identified by Carroll et al. (2020) using 14 microsatellite loci, or between those and NWNI and SWI (see Table 3).

SAMOVA reported a maximized F_{CT} value for K=2 after which F_{CT} estimates reached a plateau (Table 4) indicating that the optimum number of genetic clusters present in the dataset was two (F_{CT}=0.44, *P*=0.01, Table 4) – though this only separated one sample from Eastern Ireland (cluster 1) from all other samples (cluster 2). Upon investigation of higher cluster numbers (K>2), the software clustered German samples and partially separated the East and Southeast of Ireland from other areas in Ireland and Scotland, while clustering the Southwest, West and North of Ireland with Northern Ireland and Scotland.

The statistical parsimony network showed that 15 of the abovementioned unique haplotypes were observed in Irish and Northern Irish samples (Figure 2). Furthermore, data comprising individuals from Ireland and Northern Ireland included 10 haplotypes that were not present in samples from either Scotland (incl. Shetland) or Germany. The network showed three common haplotypes for Ireland and the northern UK, which were surrounded by multiple low frequency haplotypes in a star-like pattern. Two of the dominant haplotypes and surrounding low frequency variants occurred in Ireland, Northern Ireland and Scotland. The third, however, was exclusively observed in Ireland/ Northern Ireland, which is at the basis of the observed distinctiveness of Irish seals. These three dominant haplotypes were found in over half of the samples (131/205). No star-like pattern was evident for the Southern North Sea (samples from Germany), which showed several haplotypes at similar intermediate frequencies (blue pies in Figure 2). This local population was spatially separated from all other local populations including Ireland, though three of ten German haplotypes were also identified in samples from Ireland (particularly in the East) and one of those was also identified in Shetland.

3.4.2 Microsatellite data

For microsatellite data, AMOVA also reported highly significant structuring though levels of genetic differentiation were lower than for mitochondrial data (F_{ST}=0.10, *P*<0.001, $F_{ST(mtDNA)}$=0.24), with 10.11% of variation observed between local populations and 89.89 % of variation within local populations. Based on pairwise comparisons between local populations, genetic differentiation within Ireland/Northern Ireland was significant between NWNI and SWI (FST=0.108, *P*< 0.001), but not between EI and NWNI or SWI (Table 3). Internationally, statistically significant

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genetic structuring was observed between Ireland/Northern Ireland and adjacent areas in Scotland, with the highest level of significant differentiation found between SWI and ESC (F_{ST}=0.33, *P*<0.001, Table 3), as well as among most Scottish local populations (Table 3). Interestingly, Shetland was only significantly different to SWI (F_{ST}=0.23, *P*<0.001, Table 3). Within western European waters, significant genetic differentiation was found largely between SNS (encompassing Southeast England, France, the Dutch Wadden Sea and Germany) and local populations of the Northern (UK) metapopulation (apart from Shetland), as well as between SNS and local populations in Ireland/Northern Ireland.

When clustering microsatellite data, the two Bayesian approaches identified two and three distinct clusters using STRUCTURE (Figure 3A, no account of geographic origin) and TESS (Figure 3C, accounts for geographic origin), respectively, and the DAPC analysis based on a K means clustering approach showed three distinct clusters (Figure 4, no account of geographic origin). Specifically, STRUCTURE results confirmed the division into two main metapopulations (NUK, SNS) with strong support by ΔK values and some support from the negative log likelihood (Figure 3A). There were indications for further structuring via this approach though these were not as clear. Fine-scale STRUCTURE analysis of reduced datasets (i.e. Scottish/English data only, Irish/Northern Irish data only) found three genetic clusters within Scotland and two genetic clusters within Ireland/Northern Ireland (though with little support, data not shown). However, TESS identified an optimum number of three genetically distinct clusters (Figure 3C). Similarly, three clusters were identified by the DAPC analysis via K means clustering. All approaches found clear distinction between the previously identified NUK and SNS metapopulations. NWNI displayed the largest proportion of membership to the NUK metapopulation cluster in all analyses (Figures 2a, 2c, 3) and similarly to STRUCTURE/TESS it was observed that DAPC also clustered Scottish individuals and Irish/Northern Irish individuals separately (Figure 4). In contrast, SWI and EI local populations formed a distinct genetic cluster as shown with TESS (Figure 3C) supporting their genetic distinctiveness.

Several locus-population pairs showed evidence of null alleles and deviations from HWE. Global analysis revealed four local populations to display a heterozygote deficiency (NWNI, SWI, MFNCO, SNS). Isolation by distance (IBD) was observed using the ISOLDE approach in GENEPOP indicating a correlation between F_{ST} and geographic distances of haul-out sites (Figure 5), which was most pronounced when analysing Scottish samples on their own (Supporting Figure S2). On the other hand, no IBD was detected when only assessing samples from the SNS metapopulation (Supporting Figure S2), though samples from outside of German waters were limited. These findings were in line with the results of the discriminant analysis, which clearly separated the NUK metapopulation from the SNS metapopulation along linear discriminant 1; and showed evidence towards IBD within the NUK metapopulation and Ireland/Northern Ireland along linear discriminant 2 (Figure 6).

3.5 Genetic bottleneck and migration rate analysis

BOTTLENECK analysis showed no evidence for a recent genetic bottleneck within any of the local populations. Inspection of the traces for all four runs of BayesAss to estimate migration rates indicated that convergence was

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achieved, and the migration rate estimates were consistent across all runs. The Northwest and Northern Ireland local population was shown to be a source population for both Southwest Ireland and East Ireland (Table 5, Supporting Figure S1). The Northwest and Northern Ireland local population in turn was shown to receive migrants from Northwest Scotland. Within Scotland, NWS and MFNCO were identified as sources for NWNI/SH/MFNCO and ESC, respectively. The SNS metapopulation was not connected to other areas and showed very high internal recruitment over the last two generations, despite the sharing of haplotypes with East Ireland.

4. Discussion

4.1 Genetic diversity

Mitochondrial genetic diversity was similar for Irish, Northern Irish, Scottish and German samples. Reported genetic diversity values were similar or higher than those presented in previous studies for more northern populations of this species, including populations in Greenland, Iceland, Northern Norway, and Svalbard assessed for the same mitochondrial region (d-loop) (Andersen et al., 2011). Nuclear diversity based on microsatellites provided further confirmation, with Irish samples displaying comparable nuclear diversity to other European populations (Andersen et al., 2011; Olsen et al., 2014; Olsen et al., 2017). However, the expected heterozygosity for UK local populations of harbour seals was lower than previously reported by Olsen et al. (2017), which may indicate that certain nuclear loci employed by the former study had higher diversity, and potentially more discriminatory power.

Additionally, it is worth noting that a number of locus-population pairs departed from HWE and showed evidence of null alleles. Further, global analysis also identified that four local populations departed from HWE, displaying a heterozygote deficiency (NWNI, SWI, MFNCO, SNS). Any evidence for null alleles and/or deficit of heterozygotes can have several underlying causes and the presence of null alleles is known to inflate tests for Hardy-Weinberg proportions (Andersen et al., 2011). However, no locus/local population pair displayed both null alleles and heterozygote deficiency departing from the HWE suggesting that these may be indicative of genome-wide patterns within the local populations or a 'Wahlund effect' (i.e. where a population is not in HWE due to the existence of further sub-populations with differing allele frequencies) rather than any issues related to sample sizes or scoring errors. In practical terms, the heterozygote deficiency observed indicates the presence of further population structuring in areas showing significant deviations from HWE. Heterozygote deficiency was previously observed across European and North American harbour seal populations (Andersen et al., 2011; Huber et al., 2012; Olsen et al., 2014; Olsen et al., 2017; Carroll et al., 2020), though in contrast, a recent study on harbour seals sampled in Japan did not observe any such deviations (Mizuno et al., 2020). Heterozygote excess was not observed for any locus or local population within the present study, results akin to previous studies, where such an occurrence was rare (Andersen et al., 2011; Huber et al., 2012).

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4.2 Population structure

Within the island of Ireland, analyses of both mitochondrial and nuclear data revealed that barriers to gene flow exist among three main areas, Northwest and Northern Ireland (NWNI), Southwest Ireland (SWI), and East Ireland (EI), indicating significant reproductive isolation and the existence of three distinct local populations. Harbour seals from EI were genetically distinct from NWNI based on mtDNA (only), and harbour seals from SWI were significantly differentiated from NWNI based on microsatellite data (only). These inconsistencies between mitochondrial and nuclear markers may be attributed to differing sample sizes (Reiner, Lang & Willems, 2019) or can be indicative of sex-related differences in dispersal and migration (Lyrholm et al., 1999; Escorza-Trevino & Dizon, 2000; Herreman et al., 2009; Sonsthagen et al., 2012; Roycroft, Le Port & Lavery, 2019), considering that mitochondrial DNA is inherited maternally and thus reflects gene flow (or lack thereof) of females only. Here, geographic sampling differences between mitochondrial and nuclear data for NWNI exist with mitochondrial data primarily including samples from Northwest Ireland, whereas nuclear data include substantially more samples from Northern Ireland. This geographic inconsistency may contribute to observed inconsistencies between marker types. It may especially explain why differentiation was observed between SWI and NWNI from nuclear data but not from mitochondrial data. This is because geographic distance for the majority of samples was larger between the two local populations using microsatellite data and isolation by distance is known to occur in the species (see below). However, it could also be caused by female-mediated gene flow between these local populations.

Results from the current study reflect findings for harbour seals in other areas. For instance, a long-term freeze-branding study in Sweden demonstrated that female harbour seals exhibit stronger breeding site fidelity than males (Härkönen & Harding, 2001). While such demographic findings are not directly comparable to genetic results, these patterns would be reflective of stronger mitochondrial structuring provided that movement patterns are effective towards gene flow. Additionally, genetic studies of harbour seals in Alaska identified stronger differentiation between populations based on mitochondrial data, indicative of male-mediated gene flow (Burg, Trites & Smith, 1999; Herreman et al., 2009). Similarly, within the current study, the differentiation of East Ireland, as identified by mitochondrial data only, may indicate higher female philopatry to haul-out and/or breeding sites compared to males within the region. Support for this hypothesis is provided by the distribution of known breeding and haul-out sites, which are particularly sparse in the East of the country due to a general lack of suitable undisturbed habitat (NPWS, 2011; NPWS, 2013; Morris & Duck, 2019), thereby limiting available breeding sites. On the other hand, microsatellite findings indicate genetic differentiation between SWI and NWNI, which is supported by telemetry data that have shown a high degree of adult philopatry to haul-out sites in Ireland. Whereby harbour seals of both sexes tagged in Kenmare Bay (SWI) were shown to stay within 20km of their tagging sites (Cronin & McConnell, 2008) and more than 50% recorded foraging trips were <5km from tagging sites (Cronin, Kavanagh & Rogan, 2008; Cronin, Jessopp & Del Villar, 2011).

On a European scale, findings from the present study corroborated those of previous studies that identified two genetically distinct metapopulations based on microsatellite markers; a Northern UK (NUK) metapopulation

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comprising Scotland (incl. Shetland) and Northern Ireland, and a Southern North Sea (SNS) metapopulation including the southern English North Sea and the southern continental North Sea (incl. France, and the Dutch Wadden Sea) (Goodman, 1998; Olsen et al., 2017; Carroll et al., 2020). Through the incorporation of additional samples from the North Sea (Germany) and samples from Ireland/ Northern Ireland to the same nuclear dataset and employing mitochondrial analysis, the present study confirmed the existence of these two metapopulations.

Novel insights into Irish harbour seals in a transnational context were revealed, showing some level of connectivity (genetic membership) to both metapopulations (though less so for the SNS metapopulation) as well as displaying a unique (Irish/Northern Irish) genetic component. Specifically, the East Irish (EI) and Southwest Irish (SWI) local populations exhibited a uniquely Irish genetic signature. A lack of genetic differentiation and a high proportion of genetic group membership based on microsatellite data suggests, however, that harbour seals from NWNI are part of the contiguous NUK metapopulation. Mitochondrial population structure uncovered three common haplotypes for Ireland and the northern UK within the haplotype network, surrounded by multiple low frequency haplotypes in a star-like pattern reflecting recent rapid population expansion from a small number of founding individuals (Slatkin & Hudson, 1991; Corrales, Pavlovska & Höglund, 2014), as well as ongoing population expansion and migration into other areas (Doorenweerd et al., 2020). While an expansion was also supported by the bottleneck analysis, it needs to be kept in mind that the timing of this expansion is unknown. Since 1997, some UK SMUs have shown declining numbers of harbour seals (Thompson et al., 2019) with ongoing investigations into the drivers of the declines (Thompson et al., 2019; SCOS, 2020; Carroll et al., 2020). However, such recent demographic changes are unlikely to be picked up by the genetic analysis within the current study. Additionally, other events can also result in a star-shaped network such as events under the metapopulation model where subpopulations may individually and repeatedly be affected by bottleneck events (Mardulyn & Milinkovitch, 2005) or a so-called ‘sweep’ where an advantageous mutation leads to a rapid increase in frequency for a particular haplotype (Excoffier, 1990). However, the formation of such patterns due to bottleneck events is highly unlikely in this case because nuclear bottleneck analysis clearly indicated there was no evidence for bottlenecks within the three assessed sub-regions.

4.3 Connectivity and migration

The identification of source-sink relationships and connectivity between populations is crucial for the management of a species (Watson et al., 2011) and therefore the present study evaluated genetic migration rates between local populations, based on microsatellite data to investigate such relationships using new and previously published data (Olsen et al., 2017; Carroll et al., 2020). The timespan of the analysis covers the last two generations and therefore provides relatively recent information in this regard.

Within the island of Ireland, migration analysis showed that the NWNI local population is a source population for both Southwest Ireland (SWI) and East Ireland (EI). While NWNI and SWI showed genetic differentiation on a nuclear level, some migration was still shown to occur. NWNI and EI showed no significant differentiation on a nuclear level, indicating migration between the two, supported by results from telemetry studies, of adult harbour seals tagged in

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Strangford Lough (Northern Ireland), where some movements to the east of the Republic of Ireland were observed (Carter et al., 2020). In a transnational context, Carroll et al. (2020) identified Northwest Scotland as a source population for Northern Ireland. This was confirmed by results of the current study, which included a larger sample size from the respective local population (NWNI), reflecting data acquired through telemetry studies in the area (Cunningham et al., 2009; Carter et al., 2020; Carroll et al., 2020). The addition of German samples to the SNS metapopulation revealed high internal recruitment. Interestingly, no evidence for migration was observed between the SNS metapopulation and other local populations, despite the sharing of some haplotypes with other areas.

4.4 Methodological considerations

While the present study provided the first fine-scale insight into Irish harbour seal genetic structuring, a few considerations regarding methodological improvements can be made going forward. For instance, a lower number of loci were employed in the current study compared to previous work on the species, which led to reduced discriminatory power as evident from direct comparisons to the UK subset. Previous analysis identified five local (local) populations within the UK based on 14 loci (Carroll et al., 2020) whereas the reduced dataset of nine loci as employed within the present study only identified three local populations over the same geographic area (data not shown). It is further recommended to employ reduced representation or whole genome approaches to assess population structure using higher numbers of loci. Such approaches would also facilitate genetic population structure assessments in relation to selection (Funk et al., 2012) as well as additional assessments such as pathogen susceptibility (Olsen et al., 2017) or fitness and demography (Cammen et al., 2016).

In addition, haul-out sites differed in sample size with lowest numbers sampled within the EI local population and the West Ireland sub-region (forming part of SWI), and a lack of samples from some haul-out sites with known aggregations of harbour seals, such as Clew Bay in the West of Ireland. It is hence recommended to increase sample size for local populations in Ireland, particularly for the East and the West of Ireland in order to assess fine-scale genetic structure more appropriately and robustly (Fogelqvist et al., 2010; Hale, Burg & Steeves, 2012; Reiner, Lang & Willems, 2019). One additional step towards enhancing sample numbers in Ireland would be the establishment of a dead seal tissue sample bank as is the case for other countries including the UK.

4.5 Implications and recommendations for management

In the absence of information on population structure, harbour seals in Ireland are currently viewed as a single nationwide population (and hence MU). This study, however, has demonstrated the presence of three genetically distinct local populations of harbour seals within the island of Ireland, based on the best available genetic data and availability of samples. Thus, these results support the proposal of at least three genetically distinct MUs: Northwest & Northern Ireland (NWNI), Southwest Ireland (SWI) and East Ireland (EI).

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On a European scale, it is evident that confusion and inconsistency still exist around terminology and definitions of discrete intraspecific units for marine mammals. A recent review of Member State reports under Descriptor 1 of the MSFD highlighted the lack of harmonization on reporting under legislation (including the Habitats Directive and the Regional Seas Conventions), particularly regarding the spatial scale of employed assessment units/areas (Palialexis & Boschetti, 2021). While work is progressing towards better alignment of the Habitats Directive and MSFD (Palialexis et al., 2020), it is recommended that relevant stakeholders together with scientific experts agree on definitions and methodologies for delineation of discrete units; as was recently proposed for other descriptors of the MSFD (Konstantinos et al., 2021). Following such discussions, a guidance document should be created, as exists for instance for the delineation of discrete units of marine mammals within the US (Martien et al., 2019) and IWC (Waples et al., 2018), and/or such agreed terminology should be included within revised reporting guidance documents under the MSFD (European Commission, 2019) and OSPAR (OSPAR Commission, 2019).

It further remains to be considered that while traditional definitions rely solely on genetic approaches for the identification of discrete units (e.g. Moritz, 1994), genetic markers alone may not be able to appropriately delineate effective MUs at shorter time-scales (Taylor & Dizon, 1999). For example, genetic indices often used to infer population structure may not reflect current demographic connectivity (Palsbøll, Berube & Allendorf, 2007), which is important for conserving discrete intraspecies units that are of ecological relevance. This is evident for harbour seals within the UK where foraging ranges and breeding sites are known to overlap and individuals that have been assigned to different genetic MUs may be exposed to overlapping stressors. For instance, genetic differentiation has been observed between the Orkney Islands and the Moray Firth (Carroll et al., 2020) while movements are known to occur between the two areas (Russell, Jones & Morris, 2017; Carter et al., 2020). Hence, combining information from genetics (long-term time scale) and other complementary methods (short-term time scale) has been recommended for other marine mammal species for the delineation of Ecological Management Unit (EMUs) (Evans & Teilmann, 2009; Esteban et al., 2016; Giménez et al., 2018). Complementary methods include telemetry and ecological tracers such as stable isotopes, fatty acids, and/or pollutants, as well as assessing life-history parameters (Murphy, Pinn & Jepson, 2013; Giménez et al., 2018; Carroll et al., 2020). In a recent example, Carroll et al. (2020) took a multidisciplinary approach and combined population trajectories, genetic differentiation and telemetry data to assess the UK harbour seal metapopulation across timescales.

Going forward, an interdisciplinary approach is recommended for the ongoing refinement of discrete intraspecific units for harbour seals in Ireland, particularly for the identification of appropriate AUs of ecological relevance for indicator assessments under OSPAR. While such interdisciplinary data are not fully available for harbour seals in Ireland at the moment, the present study demonstrates that current management is not reflective of the species' population structuring and the following recommendations can be made: National assessments should be carried out at an assessment scale of the three identified genetic MUs rather than employing a single nationwide assessment scale. The three MUs should then also be utilised as ecologically relevant AUs until further evidence may become available to delineate more appropriate AUs. Hence any population assessments and reporting both under OSPAR

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as well as the MSFD should be carried out at the scale of these three proposed MUs. Annual monitoring by the National Parks and Wildlife Service during the species' moult should be maintained at the more local scale considering ecological relevance of specific sites and potential logistical constraints.

5. Conflicts of interest

The authors declare no conflicts of interest.

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Tables

Table 1. Genetic diversity based on mtDNA shown for each local population of harbour seals, including the number of haplotypes, haplotype (h) and nucleotide (π) diversity (standard deviation, SD) and the number of polymorphic sites. Acronyms for local populations are shown in Figure 1.

Local population	N	No. haplotypes	π (SD)	h (SD)	No. polymorphic sites
NWNI	60	10	0.005 (0.003)	0.758 (0.032)	11
SWI	34	8	0.004 (0.003)	0.622 (0.082)	9
EI	29	6	0.004 (0.003)	0.724 (0.051)	8
NWS	15	5	0.006 (0.004)	0.705 (0.088)	8
MFNCO	15	4	0.005 (0.003)	0.619 (0.120)	5
SH	6	4	0.007 (0.005)	0.867 (0.129)	5
ESC	6	3	0.006 (0.004)	0.600 (0.215)	5
SNS	40	10	0.005 (0.003)	0.858 (0.025)	8
Total	*205*	*27*	*0.005*	*0.841*	*21*

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Table 2. Genetic diversity of harbour seals based on 9 microsatellite loci showing the number of alleles (N_o), allelic richness (A_r), expected and observed heterozygosity (H_e, H_o) and the inbreeding coefficient (F_{IS}). Evidence for the presence of null alleles and deviations from HWE are indicated via asterisk and a cross, respectively. Acronyms for local populations are shown in Figure 1.

Locus	Parameter	NWNI (n = 46)	SWI (n = 20)	EI (n = 19)	NWS (n = 90)	MFNCO (n = 79)	SH (n = 14)	ESC (n = 28)	SNS (n = 78)	All (mean)
Sgpv9	N_a	4	3	3	2	1	2	1	3	2.375
	A_r	2.344	2.339	2.364	1.79	1	1.63	1	1.833	1.788
	H_e	0.345	0.396	0.437	0.181	NA	0.077	NA	0.109	0.193
	H_o	0.278	0.25	0.474	0.2	NA	0.077	NA	0.114	0.174
	F_{IS}	0.197	0.375	-0.087	-0.103	NA	0.0	NA	-0.046	0.042
Hgdii	N_a	3	3	2	3	3	1	1	6	2.75
	A_r	1.925	2.389	1.996	1.23	1.488	1.783	1	1.767	1.697
	H_e	0.188	0.522	0.387	0.033	0.075	NA	NA	0.115	0.22
	H_o	0.068	0.167	0.25	0.011	0.0	NA	NA	0.105	0.100
	F_{IS}	0.640	0.687	0.362	0.665	1	NA	NA	0.083	0.573
		*†	* †		*	* †				
Hg6.1	N_a	6	3	3	4	3	3	3	5	3.75
	A_r	2.718	2.951	2.758	2.855	2.946	2.498	2.983	2.963	2.834
	H_e	0.553	0.565	0.525	0.539	0.627	0.561	0.659	0.452	0.560
	H_o	0.564	0.444	0.632	0.494	0.595	0.429	0.720	0.368	0.531
	F_{IS}	-0.020	0.218	-0.21	0.083	0.052	0.243	-0.095	0.186	0.057
Hg6.3	N_a	5	6	5	7	5	3	4	7	5.25
	A_r	4.093	4.746	4.269	3.744	3.373	3.517	2.516	3.828	3.761
	H_e	0.716	0.766	0.758	0.658	0.594	0.558	0.476	0.598	0.641
	H_o	0.487	0.563	0.625	0.590	0.631	0.571	0.519	0.492	0.560
	F_{IS}	0.323	0.272	0.18	0.103	-0.063	-0.025	-0.091	0.178	0.110
		*	†						*	†
Sgpv10	N_a	4	4	3	2	2	2	1	3	2.625
	A_r	2.475	3.923	2.636	1.97	1.795	2.019	1	2.287	2.263
	H_e	0.438	0.712	0.541	0.667	0.185	0.409	NA	0.233	0.398
	H_o	0.317	0.357	0.545	0.379	0.179	0.385	NA	0.184	0.293
	F_{IS}	0.28	0.508	-0.008	-0.127	0.031	0.063	NA	0.211	0.120
		†	* †							†
Sgpv11	N_a	4	5	4	2	3	2	2	5	3.375
	A_r	2.299	3.566	2.733	1.968	1.924	2.987	1.971	2.437	2.486
	H_e	0.390	0.623	0.474	0.333	0.217	0.369	0.321	0.585	0.414
	H_o	0.386	0.5	0.263	0.326	0.179	0.308	0.321	0.487	0.346
	F_{IS}	0.009	0.203	0.451	0.022	0.173	0.172	0.0	0.168	0.150
				* †						†
Tbpv2	N_a	4	4	4	4	4	1	4	5	3.75
	A_r	2.746	3.235	3.348	2.073	1.748	2.513	3.319	3.181	2.770
	H_e	0.306	0.502	0.587	0.178	0.111	NA	0.558	0.289	0.316
	H_o	0.289	0.474	0.625	0.172	0.068	NA	0.412	0.241	0.285
	F_{IS}	0.056	0.058	-0.068	0.033	0.386	NA	0.268	0.168	0.113

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Pvc19	N_a	3	3	2	3	3	3	2	3	2.75
	A_r	2.301	2.467	1.835	2.473	2.605	2.095	1.952	2.426	2.270
	H_e	0.528	0.545	0.175	0.501	0.512	0.603	0.284	0.509	0.457
	H_o	0.512	0.467	0.188	0.469	0.415	0.429	0.167	0.351	0.375
	F_{IS}	0.03	0.148	-0.071	0.065	0.191	0.297	0.418	0.311	0.174
									*	
Pvc78	N_a	2	4	3	2	2	2	2	5	2.75
	A_r	2	3.318	2.383	1.999	1.97	2.058	1.259	2.196	2.148
	H_e	0.484	0.625	0.348	0.466	0.336	0.198	0.037	0.177	0.334
	H_o	0.465	0.611	0.412	0.483	0.397	0.214	0.037	0.162	0.348
	F_{IS}	0.039	0.023	-0.191	-0.036	-0.185	-0.083	0	0.086	-0.043
N_a (mean ±SD)		3.89 ± 1.17	3.889 ± 1.054	3.222 ± 0.972	3.222 ± 1.64	3.125 ± 0.991	2.429 ± 0.535	2.833 ± 0.983	4.667 ± 1.414	
A_r (mean)		2.544	3.215	2.702	2.234	2.094	2.344	1.889	2.546	
H_e (mean ± SD)		0.439 ± 0.155	0.584 ± 0.112	0.470 ± 0.164	0.359 ± 0.201	0.332 ± 0.219	0.397 ± 0.199	0.389 ± 0.223	0.341 ± 0.197	
H_o (mean ± SD)		0.374 ± 0.154	0.426 ± 0.144	0.446 ± 0.176	0.347 ± 0.187	0.308 ± 0.236	0.345 ± 0.162	0.363 ± 0.245	0.278 ± 0.151	
F_{IS} (mean)		0.173	0.277	0.039	0.078	0.176	0.074	0.083	0.149	

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*Table 3. Pairwise genetic differentiation between harbour seal local populations. Acronyms are shown in Figure 1. F_{ST} values for mtDNA shown below the diagonal and for microsatellites above the diagonal. Asterix indicates a significant p value after sequential Bonferroni correction (*p < 0.00178).*

	NWNI	SWI	EI	NWS	MFNCO	SH	ESC	SNS
NWNI		0.108*	0.042	0.005	0.063*	0.085	0.161*	0.119*
SWI	0.084		0.038	0.200*	0.279*	0.226*	0.330*	0.187*
EI	0.160*	0.392*		0.088*	0.097*	0.074	0.149*	0.070*
NWS	0.046	-0.003	0.284*		0.039*	0.065	0.129*	0.120*
MFNCO	0.047	-0.005	0.312*	-0.053		0.026	0.056*	0.120*
SH	0.048	0.132	0.168	-0.011	-0.024		-0.011	0.021
ESC	0.014	-0.061	0.317*	-0.105	-0.105	-0.034		0.070*
SNS	0.363*	0.437*	0.311*	0.334*	0.357*	0.236*	0.342*	

Table 4. Summary of SAMOVA output for harbour seal mitochondrial cluster analysis. The best number of K to represent the data is chosen based on a maximised F_{CT} value.

K	Run number	F_{CT}	P (F_{CT})
2	1	0.44236	0.01271+-0.00366
	2	0.44236	0.01075+-0.00300
	3	0.44236	0.01662+-0.00393
3	1	0.40175	0.00196+-0.00136
	2	0.37479	0.00000+-0.00000
	3	0.37479	0.00000+-0.00000
4	1	0.37646	0.00000+-0.00000
	2	0.37939	0.00000+-0.00000
	3	0.37258	0.00000+-0.00000
5	1	0.38169	0.00000+-0.00000
	2	0.38103	0.00000+-0.00000
	3	0.38818	0.00000+-0.00000

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Table 5. Median estimates (and 95% HPD intervals) of the proportion of individuals within population i that was identified as being migrants from population j (per generation) across four runs of analysis in BayessAss for all local populations. Acronyms for local populations are shown in Figure 1.

		i						
J	NWNI	SWI	EI	NWS	MFNCO	SH	ESC	SNS
NWNI	0.706 (0.662, 0.750)	0.212 (0.144, 0.280)	0.198 0.122, 0.273)	0.006 (<0.0001, 0.019)	0.009 (<0.0001, 0.024)	0.022 (<0.0001, 0.061)	0.010 (<0.0001, 0.028)	0.011 (<0.0001, 0.028)
SWI	0.006 (<0.0001, 0.018)	0.679 (0.655, 0.703)	0.013 (<0.0001, 0.036)	0.003 (<0.0001, 0.010)	0.004 (<0.0001, 0.012)	0.015 (<0.0001, 0.043)	0.009 (<0.0001, 0.027)	0.005 (<0.0001, 0.014)
EI	0.006 (<0.0001, 0.019)	0.013 (<0.0001, 0.038)	0.679 (0.655, 0.703)	0.003 (<0.0001, 0.010)	0.004 (<0.0001, 0.011)	0.015 (<0.0001, 0.043)	0.009 (<0.0001, 0.027)	0.004 (<0.0001, 0.013)
NWS	0.244 (0.191, 0.297)	0.027 (<0.0001, 0.067)	0.022 (<0.0001, 0.065)	0.959 (0.927, 0.992)	0.269 (0.220, 0.318)	0.186 (0.099, 0.272)	0.020 (<0.0001, 0.052)	0.009 (<0.0001, 0.025)
MFNCO	0.0092 (<0.0001, 0.026)	0.01225 (<0.0001, 0.035)	0.01435 (<0.0001, 0.041)	0.0147 (<0.0001, 0.040)	0.69605 (0.653, 0.739)	0.04975 (<0.0001, 0.118)	0.2567 (0.208, 0.306)	0.00775 (<0.0001, 0.022)
SH	0.00615 (<0.0001, 0.018)	0.01285 (<0.0001, 0.037)	0.0124 (<0.0001, 0.036)	0.0034 (<0.0001, 0.040)	0.00385 (0.0001, 0.011)	0.68175 (0.653, 0.710)	0.0092 (<0.0001, 0.027)	0.0052 (<0.0001, 0.015)
ESC	0.00625 (<0.0001, 0.018)	0.0129 (<0.0001, 0.037)	0.0123 (<0.0001, 0.036)	0.0034 (<0.0001, 0.040)	0.0039 (<0.0001, 0.011)	0.01505 (<0.0001, 0.043)	0.67585 (0.658, 0.693)	0.0055 (<0.0001, 0.015)
SNS	0.01585 (<0.0001, 0.040)	0.0301 (<0.0001, 0.072)	0.04925 (<0.0001, 0.107)	0.006 (<0.0001, 0.017)	0.0107 (<0.0001, 0.027)	0.0158 (<0.0001, 0.045)	0.0095 (<0.0001, 0.028)	0.95225 (0.923, 0.982)

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Figure legends

Figure 1. Harbour seal sample distribution and genetic Management Units (MUs, i.e. local populations). Colours represent the following MUs: SWI – Southwest Ireland, EI – East Ireland, NWNI – Northwest & Northern Ireland, NWS – Northwest Scotland, MFNCO – Moray Firth, North Coast & Orkney, ESC – East Scotland, SH – Shetland, SNS – Southern North Sea. Symbols represent the following genetically identified metapopulations: star – Northern UK (NUK) metapopulation, triangle – Southern North Sea (SNS) metapopulation (identical to the MU shown here), circle – distinct Irish component that could not be assigned to the previous metapopulations. Black lines around the island of Ireland represent geographic borders of the following sub-regions: NWI – Northwest Ireland, EDNI – East Donegal & Northern Ireland, EIR – East Ireland, SEIR – Southeast Ireland, SWIR – Southwest Ireland, and WIR – West Ireland. Black lines around the UK represent geographic boundaries of currently delineated Seal Management Units: SW – Southwest (Scotland), WS – West Scotland, WI – Western Isles, NCO – North Coast, SH – Shetland, MF – Moray Firth, ESC – East Scotland, SEE – Southeast England.

Figure 2. Parsimony network showing relationships between haplotypes of harbour seals in Irish and adjacent waters (n=205, length=421bp). Each circle depicts one haplotype, and its size shows the frequency of this haplotype (size chart given on the left). An open circle without colour filling shows haplotypes absent in the sample. Local populations are shown by colour gradient: purple gradient for Irish samples, green gradient for Scottish samples, and German samples (Southern metapopulation) shown in blue.

Figure 3. Genetic structure of harbour seals shown as graphical outputs from STRUCTURE (A, B) and TESS (C). Vertical bars represent individuals and colouration shows the proportion of membership to each of K clusters. (A-B) Plots for K=2-3 shown for STRUCTURE analysis, (C) Plot for K=3 shown for TESS analysis. Abbreviations for local populations are shown in Figure 1.

Figure 4. Genetic structure of harbour seals as identified by DAPC analysis. The top graph shows individuals coloured by cluster assignment (navy = genetic cluster 1, green = genetic cluster 2, blue = genetic cluster 3). The bottom graph shows the same clusters but coloured by origin of local population (brown = SNS, green gradient = NWNI/SWI/EI, blue = NWS, purple = MFNCO, orange = ESC, red = SH).

Figure 5. Logarithm of Euclidian distances between haul-out sites plotted against F_{ST} to visualise isolation by distance.

Figure 6. Individual genotypes plotted by LD from the DAPC analysis conducted with samples grouped by inferred local population.

Supporting Information figure legends

Figure S1: Logarithm of Euclidian distances between haul-out sites plotted against F_{ST} to visualise isolation by distance. (a) Subset of the Southern North Sea metapopulation (b) Subset of Irish/Northern Irish samples (c) Subset of Scottish samples only (d) Subset of Scottish samples and the Southern North Sea metapopulation.

Figure S2: Map showing the proportion of cluster memberships for each of three identified clusters by TESS.

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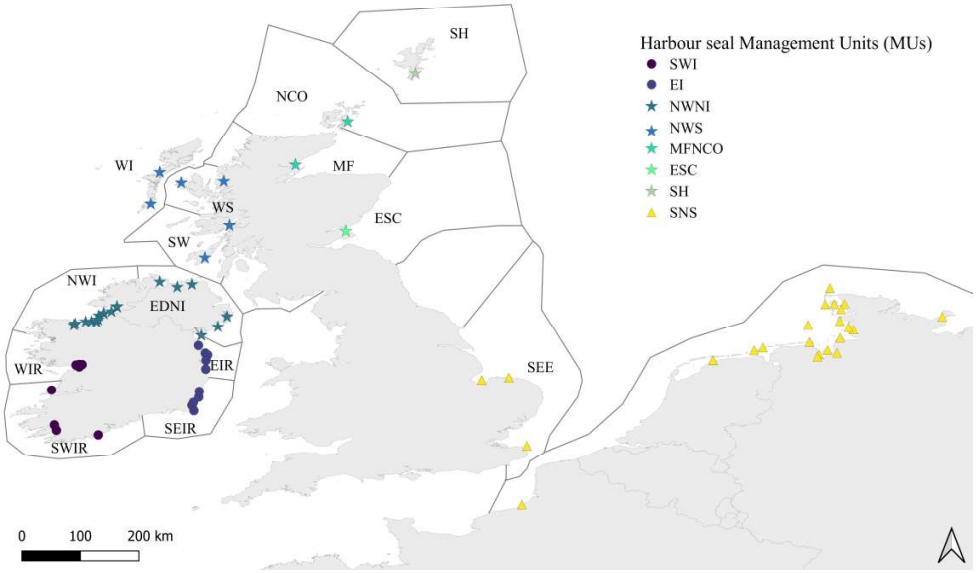


Figure 1. Harbour seal sample distribution and genetic Management Units (MUs, i.e. local populations). Colours represent the following MUs: SWI – Southwest Ireland, EI – East Ireland, NWNI – Northwest & Northern Ireland, NWS – Northwest Scotland, MFNCO – Moray Firth, North Coast & Orkney, ESC – East Scotland, SH – Shetland, SNS – Southern North Sea. Symbols represent the following genetically identified metapopulations: star – Northern UK (NUK) metapopulation, triangle – Southern North Sea (SNS) metapopulation (identical to the MU shown here), circle – distinct Irish component that could not be assigned to the previous metapopulations. Black lines around the island of Ireland represent geographic borders of the following sub-regions: NWI – Northwest Ireland, EDNI – East Donegal & Northern Ireland, EIR – East Ireland, SEIR – Southeast Ireland, SWIR – Southwest Ireland, and WIR – West Ireland. Black lines around the UK represent geographic boundaries of currently delineated Seal Management Units: SW – Southwest (Scotland), WS – West Scotland, WI – Western Isles, NCO – North Coast, SH – Shetland, MF – Moray Firth, ESC – East Scotland, SEE – Southeast England.

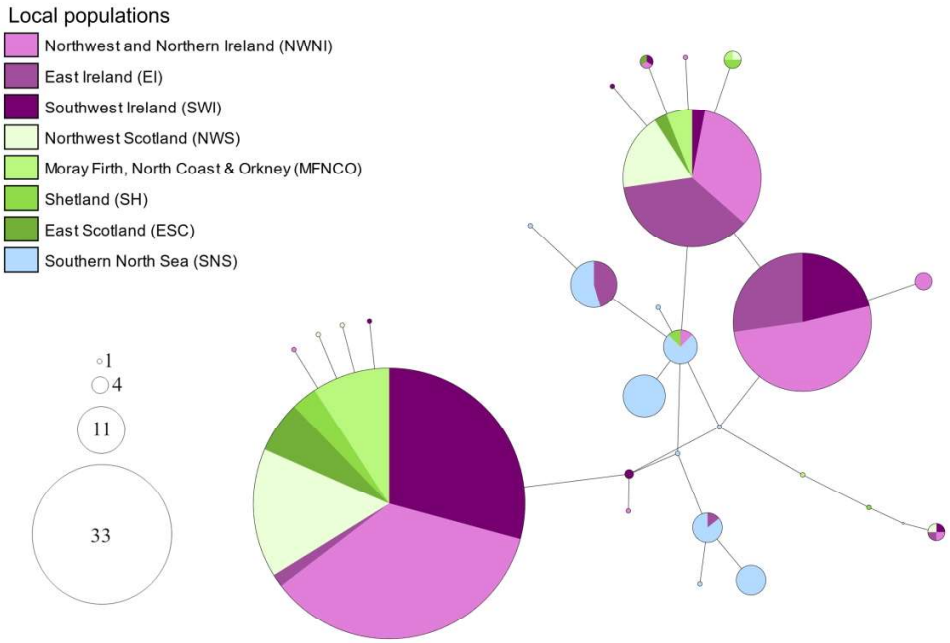


Figure 2. Parsimony network showing relationships between haplotypes of harbour seals in Irish and adjacent waters (n=205, length=421bp). Each circle depicts one haplotype, and its size shows the frequency of this haplotype (size chart given on the left). An open circle without colour filling shows haplotypes absent in the sample. Local populations are shown by colour gradient: purple gradient for Irish samples, green gradient for Scottish samples, and German samples (Southern metapopulation) shown in blue.

299x224mm (300 x 300 DPI)

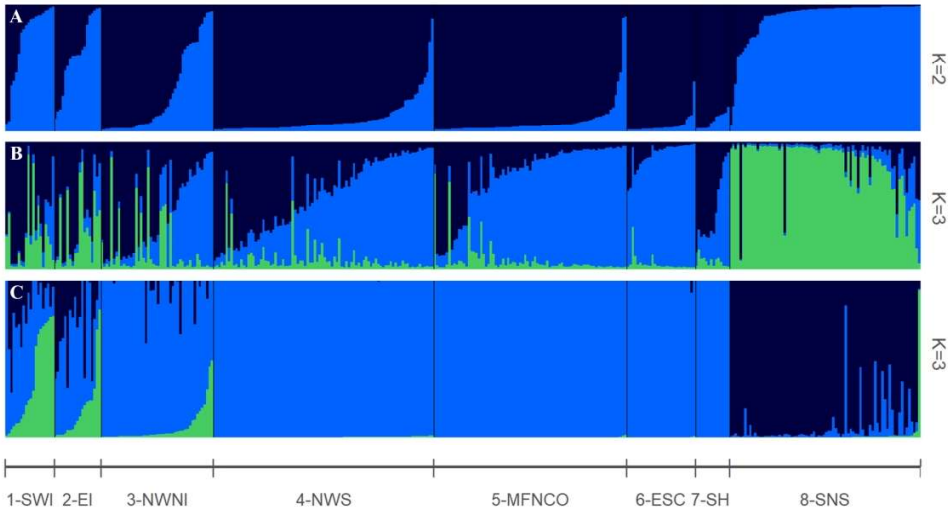


Figure 3. Genetic structure of harbour seals shown as graphical outputs from STRUCTURE (A, B) and TESS (C). Vertical bars represent individuals and colouration shows the proportion of membership to each of K clusters. (A-B) Plots for K=2-3 shown for STRUCTURE analysis, (C) Plot for K=3 shown for TESS analysis. Abbreviations for local populations are shown in Figure 1.

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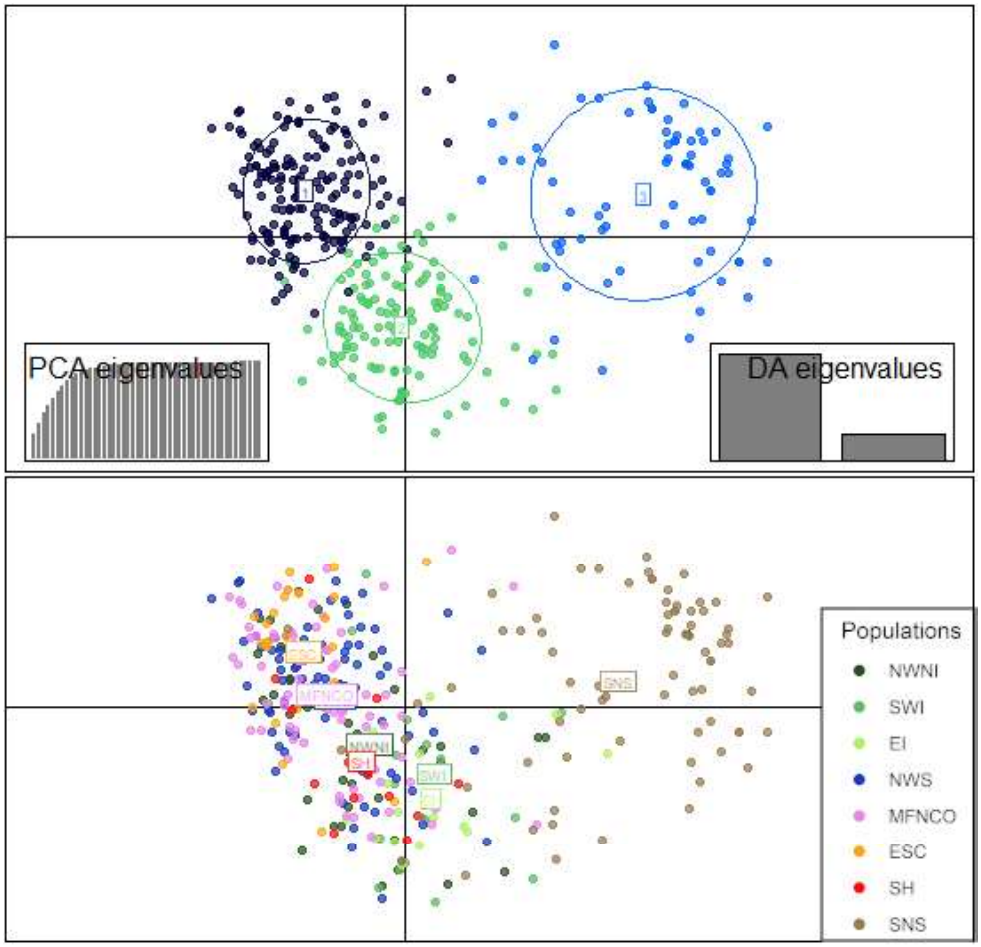


Figure 4. Genetic structure of harbour seals as identified by DAPC analysis. The top graph shows individuals coloured by cluster assignment (navy = genetic cluster 1, green = genetic cluster 2, blue = genetic cluster 3). The bottom graph shows the same clusters but coloured by origin of local population (brown = SNS, green gradient = NWNI/SWI/EI, blue = NWS, purple = MFNCO, orange = ESC, red = SH).

58x57mm (236 x 236 DPI)

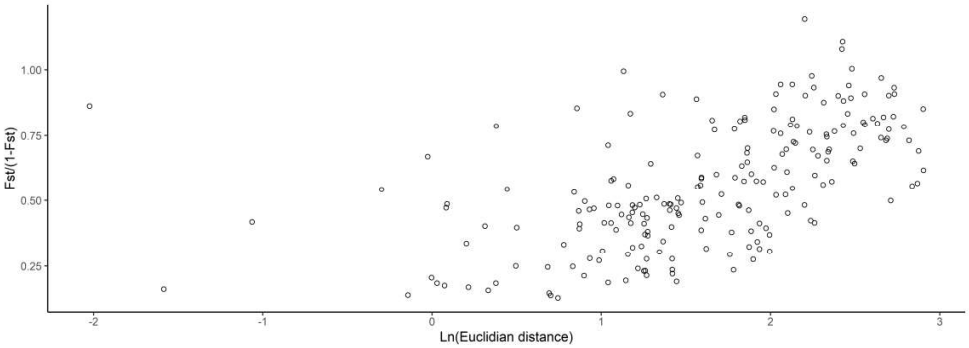


Figure 5. Logarithm of Euclidian distances between haul-out sites plotted against FST to visualise isolation by distance.

279x101mm (600 x 600 DPI)

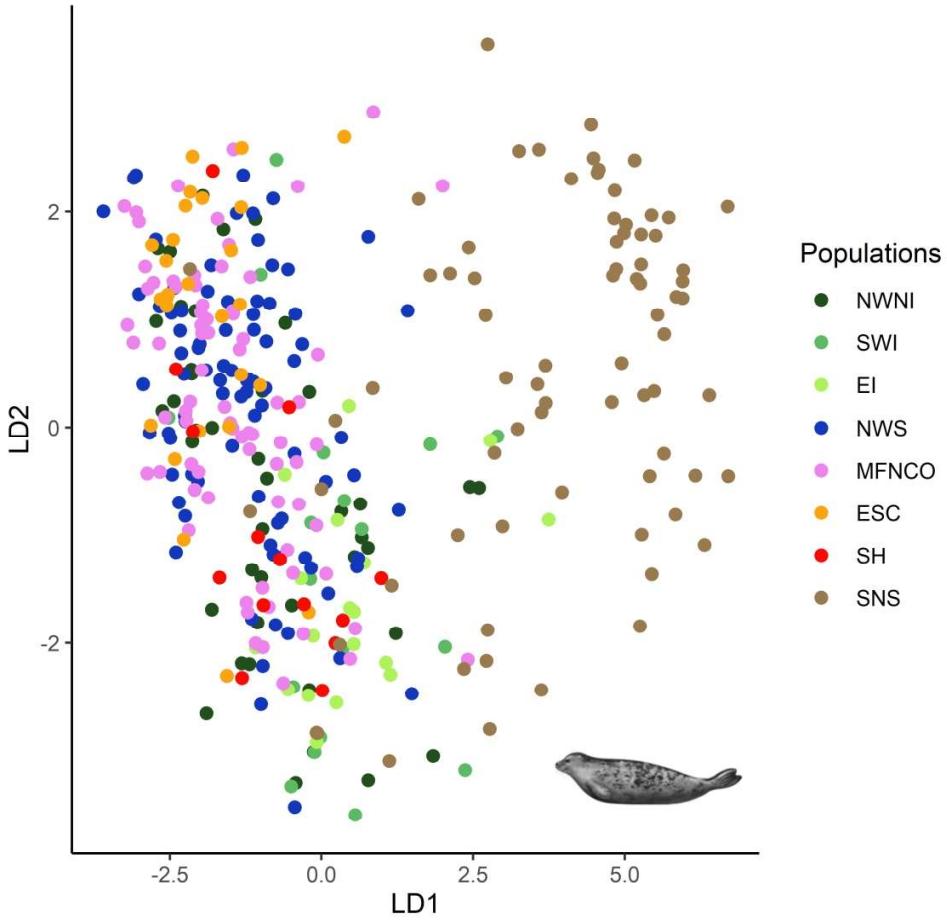


Figure 6. Individual genotypes plotted by LD from the DAPC analysis conducted with samples grouped by inferred local population.

139x134mm (600 x 600 DPI)

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Supplementary Table S1. Sampling parameters including localities sampled, sample sizes, s
(M, defined as either NUK (northern UK), I (Irish), SNS (southern North Sea incl. UK and Eu
Management Unit/Area shown by Carroll et al. 2020, geographic sub-regions based on haul-o
mitochondrial dataset (nmt) and numbers included in the final nuclear dataset (nnuc).

M	Local population	Sub-region	Locality	n_{mt}
NUK	Northwest & Northern Ireland (NWNI)	Northwest Ireland	Killala Bay	18
			Sligo Area	14
			Donegal	22
I	Southwestern (SWI)	West Ireland	Galway Bay	7
			Kilkee	1
I	Southwestern (SWI)	Southwest Ireland	Waterville	1
			Kenmare	4
			Bantry Bay	20
			Kinsale	1
I	Eastern (EI)	Southeast Ireland	Waterford	1
			Wexford	11
			Courtown to	3
I	Eastern (EI)	East Ireland	Dublin Area	8
			Dundalk	6
NUK	Northwest & Northern Ireland	East Donegal &	Northern	5
			Eastern	1
	Total (Ireland & Northern Ireland)			***123***
NUK	Northwest Scotland (NWS)	West Scotland	Lismore	5
			Loch	0
			Islay & Jura	5
			Skye	5
		Western Isles	Western	0
NUK	MFNCO	North Coast & Orkney (MFNCO)	Orkney	5
			Outer Hebrides	5
			Pentland Firth	0
			Moray Firth	5
NUK	SH	Shetland (SH)	Shetland	6
NUK	ESC	East Scotland (ESC)	Tay & Eden	6
			East Coast	0
SNS	-	English North Sea	Southeast England	0
SNS	-	European North Sea	Germany	40
			France	0

	Dutch Wadden Sea	0
Total (all localities)		***205***

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sampling years, tissue type and sample sources used in this study. Data a
urope)), local population inferred from Carroll et al. (2020) and this stuc
out sites chosen for Ireland) and locality. Sample sizes are shown for nu

n_{nuc}	Sampling years	Tissue type
6	2017-2019	Scat
10	2017, 2018	Saliva, scat, hair
5	2017, 2018	Scat
5	2017	Scat, skin, vomited milk, hair,
1		hair
0	2018	skin
5	2007	Skin
8	2017, 2018	Scat, moulted hair, urine
1	2018	Saliva
0	2016	Saliva
9	2017, 2018	Scat, saliva
2	2017	Scat, skin
7	2017, 2018, 2020	Scat, saliva
1	2017	Scat, skin
24	2018, 2019	Skin, saliva, hair
1	2018	Saliva
85		
22	2007	Skin
6	2011	Skin
30	2003, 2008, 2011,	Skin
17	2004, 2005, 2008,	Skin
15	2006, 2010	Skin
34	2003, 2008, 2009	Skin
0		Skin
13	2011	Skin
32	2003, 2008, 2009	Skin
14	2004, 2010	Skin
0		Skin
28	2003, 2008	Skin
24	2003, 2004, 2006, 2006, 2008	Skin
41	2017-2019	Blood, saliva
3	2006, 2007	Skin

10	2018	Blood

374

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are shown for metapopulation
dy, sub-region (equivalent to Seal
mbers included in the final

Source(s)
Fieldwork, Seal Rescue Ireland
Seal Rescue Ireland
Fieldwork, Seal Rescue Ireland
Fieldwork, Seal Rescue Ireland
Seal Rescue Ireland
Fieldwork
University College Cork
Fieldwork
Seal Rescue Ireland
Seal Rescue Ireland
Fieldwork, Seal Rescue Ireland
Seal Rescue Ireland
Fieldwork, Seal Rescue Ireland
-
DEARA, Seal Rescue Ireland, Seal Rescue Ireland
Carroll et al. (2020), SMRU
Carroll et al. (2020)
Carroll et al. (2020), SMRU
Carroll et al. (2020), SMRU
Carroll et al. (2020)
Carroll et al. (2020), SMRU (unpublished)
SMRU (unpublished)
Carroll et al. (2020)
Carroll et al. (2020), SMRU (unpublished)
Carroll et al. (2020), SMRU (unpublished)
SMRU (unpublished)
Carroll et al. (2020)
Carroll et al. (2020)
Seehundstation Friedrichskoog, Seehundstation Norden
Carroll et al. (2020)

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Carroll et al. (2020), Seehundstation Friedrichskoog

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Supplementary Table S2. Further sampling parameters for samples acquire sources. Data are shown for metapopulation (M, defined as either NUK (nor sources of samples where not collected during fieldwork.

M	Local population	Sub-region
N	Northwest Ireland & Northern Ireland (NWNI)	Northwest Ireland
I	Southwestern Ireland (SWI)	West Ireland
I	Southwestern Ireland (SWI)	Southwest Ireland
I	Eastern Ireland (EI)	Southeast
I	Eastern Ireland (EI)	East Ireland
NUK	Northwest Ireland & Northern Ireland (NWNI)	Northern Ireland & eastern Donegal
	Total (Ireland & Northern Ireland)	
SNS	Southern North Sea (SNS)	European North Sea
	Total (all localities)	

ed and proccessed as part of the present study, including latitude and longitude, mitochondrial (n_m
thern UK), I (Irish), SNS (southern North Sea incl. UK and continental Europe)), putative popula

Locality	Lat	Long	n_{mt}	n_{nuc}	Adult	Juv
Killala Bay	54.180851	-9.136391	18	6	0	1
Sligo Area	54.459285	-8.448949	14	10	0	0
Donegal Bay	54.612641	-8.139229	22	5	0	0
Galway Bay	53.214617	-9.012751	7	5	0	0
Kilkee	52.679117	-9.654556	1	1	0	0
Waterville	51.8288610	-10.1752350	1	0	0	0
Kenmare Bay	51.872498	-9.589686	4	5	5	0
Bantry Bay	51.749201	-9.542368	20	8	0	0
Kinsale	51.64211	-8.57095	1	1	0	0
Waterford	52.154376	-6.994405	1	0	0	0
Wexford	52.321082	-6.395612	11	9	0	0
Courtown to Wicklow	52.513881	-6.238473	3	2	0	0
Dublin Area	53.161491	-6.075348	8	7	0	0
Dundalk Area	53.997622	-6.319538	6	1	0	0
Northern Ireland	55.1710520	-6.7366160	5	24	0	0
Eastern Donegal	55.2923570	-7.1443610	1	1	0	0
			123	85	5	1
Germany	54.186657	7.9049	40	41	0	0
Dutch Wadden Sea	53.472342	6.736948	0	10	0	0
			163	136	5	1

nt) and nuclear (n_{nuc}) sample sizes, numbers of adults/juveniles/individuals with unknown age, fe
ation, geographic sub-regions based on haul-out sites chosen for Ireland, localities sampled, spec

Pup	Unknown age	F	M	Unknown sex	Sampling years
2	17	4	4	12	2017-2019
14	0	7	7	0	2017, 2018
2	20	7	2	13	2017, 2018
6	2	3	2	3	2017
1	0	0	1	0	
1	0	0	0	1	2018
0	0	0	0	5	2007
0	20	1	1	18	2017, 2018
1	0	1	0	0	2018
1	0	0	1	0	2016
1	13	0	2	12	2017, 2018
3	0	1	2	0	2017
6	4	4	1	5	2017, 2018, 2020
1	3	0	1	3	2017
3	1	2	1	1	2018, 2019
1	0	0	1	0	2018
43	80	30	26	73	
42	0	20	22	0	2017-2019
1	0	0	0	1	2018
86	80	50	48	74	

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emales/males/individuals of unknown sex, sampling years, tissue types and sample cific latidufe and longitude for each localitysizes and dates for the study as well as

Tissue type	Source(s)
Scat	Fieldwork, Seal Rescue Ireland
Saliva, scat, hair	Seal Rescue Ireland
Scat	Fieldwork, Seal Rescue Ireland
Scat, skin, regurgitated milk, hair, saliva	Fieldwork, Seal Rescue Ireland
hair	Seal Rescue Ireland
skin	Fieldwork
Skin	University College Cork
Scat, moulted hair, urine	Fieldwork
Saliva	Seal Rescue Ireland
Saliva	Seal Rescue Ireland
Scat, saliva	Fieldwork, Seal Rescue Ireland
Scat, skin	Seal Rescue Ireland
Scat, saliva	Fieldwork, Seal Rescue Ireland
Scat, skin	-
Skin, saliva, hair	DEARA, Seal Rescue Ireland, SMRU
Saliva	Seal Rescue Ireland
Blood, saliva	Seehundstation Friedrichskoog, Seehundstation Norden
Blood	Seehundstation Friedrichskoog

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Supplementary Table S3. PCR profiles for each microsatellite locus. Groups correspond to the pairing of loci for subsequent running on acrylamide gels.

Group	*Locus*	*Fluorescent label*	T_m	*Adapted from*	*PCR cycling conditions*
I I I	Pvc19 SGPV11 Hgdii	700 700 800	60	Olsen et al. (2017)	30 cycles: 95˚C 15min; 94˚C 30s; 60˚C 90s; 71˚C 45s; 72˚C 2min; 4˚C infinite
II II II	SGPV9 TBPV2 Hg8.9	700 700 800	58	Olsen et al. (2017)	30 cycles: 95˚C 15min; 94˚C 30s; 58˚C 90s; 71˚C 45s; 72˚C 2min; 4˚C infinite
II	SGPV10	800	54	Olsen et al. (2017)	30 cycles: 95˚C 15min; 94˚C 30s; 62˚C 90s; 71˚C 45s; 72˚C 2min; 4˚C infinite
I	Hg8.10	800	50 → 52	Allen et al. (1995)	7 cycles: 93˚C 2min; 94˚C 30s; 50˚C 90s; 71˚C 45s 25 cycles: 94˚C 30s; 52˚C 90s; 71˚C 45s 72˚C 2min; 4˚C infinite
III III	Hg6.3 Pvc78	700 700	58 → 60	Allen et al. (1995)	7 cycles: 93˚C 2min; 94˚C 30s; 58˚C 90s; 71˚C 45s 25 cycles: 94˚C 30s; 60˚C 90s; 71˚C 45s 72˚C 2min; 4˚C infinite
III	Hg6.1	800	60 → 62	Allen et al. (1995)	7 cycles: 93˚C 2min; 94˚C 30s; 60˚C 90s; 71˚C 45s 25 cycles: 94˚C 30s; 62˚C 90s; 71˚C 45s 72˚C 2min; 4˚C infinite
IV	SGPV10	800	51 → 53	Allen et al. (1995)	7 cycles: 93˚C 2min; 94˚C 30s; 51˚C 90s; 71˚C 45s 25 cycles: 94˚C 30s; 53˚C 90s; 71˚C 45s 72˚C 2min; 4˚C infinite

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Supplementary Table S4. Pairwise genetic differentiation between harbour seal geographic sub-regions. F_{ST} values for mtDNA (488bp) shown below the diagonal and for microsatellites (11 loci, minimum of eight scored) above the diagonal. Asterix indicates a significant p value after sequential Bonferroni correction (*p < 0.003).

	NWI	WIR	SWI	SEI	EI	NEI
NWI		0.017	0.098	0.074	0.059	-0.036
WIR	0.038		0.129*	0.116	0.132	-0.036
SWI	0.117	-0.020		0.131	0.089	0.015
SEI	0.132*	0.303*	0.402*		0.045	0.099
EI	0.178	0.295*	0.421*	0.119		0.096
NEI	0.035	-0.108	-0.082	0.359	0.350*	

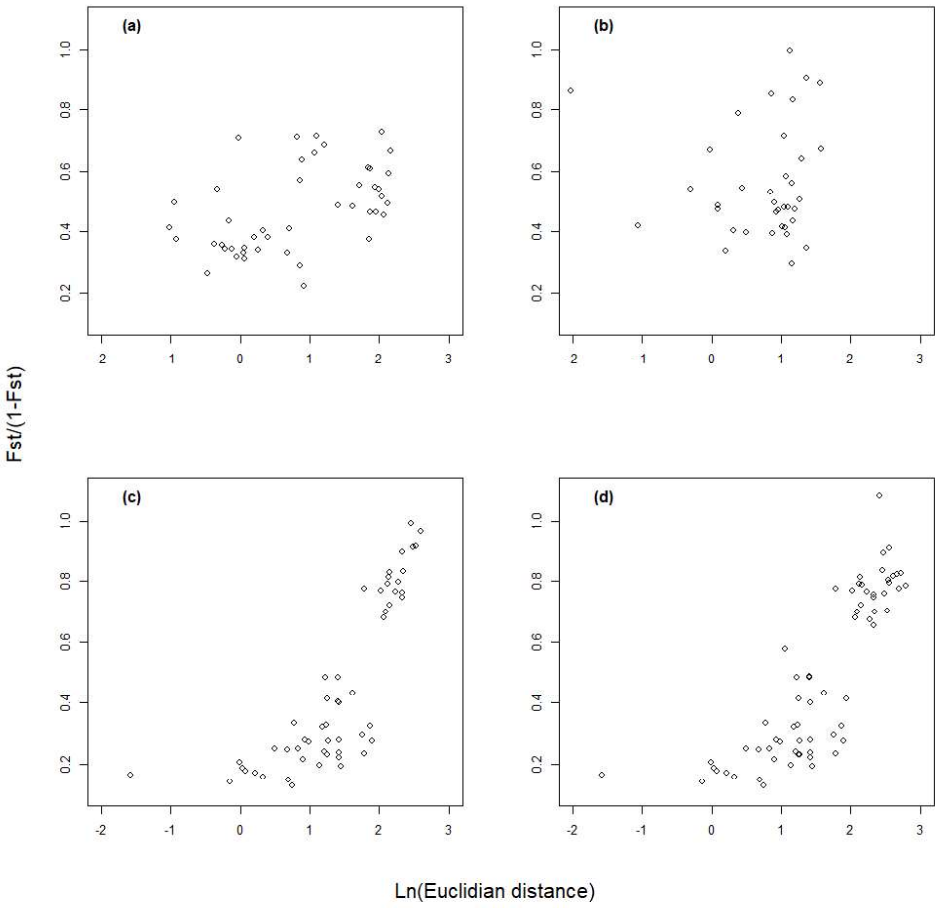
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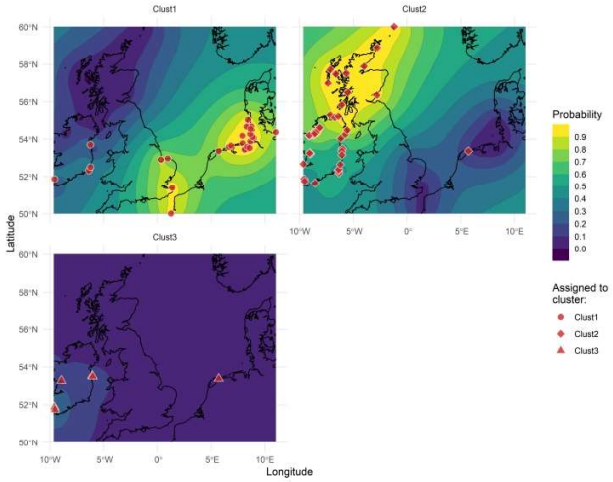
Supplementary Table S5. Haplotype information including previous matches on GenBank, accession number, study, whether a sequence was submitted to Genbank and haplotype frequencies per putative population.

	Match	Accession number	Study	Genbank submission	NWNI	SWI	EI	NWS	MFNCO	SH	ESC	SNS	Total
Hap_1	G3	U36365.1	Stanley et al 1996	no	11	1	12	5	3	0	1	0	33
Hap_2	none	OP807092	This study	yes	17	7	9	0	0	0	0	0	33
Hap_3	PV23	HQ153129.1	Andersen et al 2011	no	22	20	1	7	9	2	4	0	65
Hap_4	G24	U36359.1	Stanley et al 1996	no	1	1	0	0	0	0	1	0	3
Hap_5	none	OP807093	This study	yes	1	0	0	0	0	0	0	0	1
Hap_6	none	OP807094	This study	yes	4	0	0	0	0	0	0	0	4
Hap_7	none	OP807095	This study	yes	1	0	0	0	0	0	0	0	1
Hap_8	G24	U36354.1	Stanley et al 1996	no	0	0	1	0	0	0	0	6	7
Hap_9	G1	U36344.1	Stanley et al 1996	no	0	0	5	0	0	0	0	6	11
Hap_10	IJ3	OP807096	SMRU unpublished	yes	1	1	1	1	0	0	0	0	4
Hap_11	none	OP807097	This study	yes	0	1	0	0	0	0	0	0	1
Hap_12	none	OP807097	This study	yes	0	1	0	0	0	0	0	0	1
Hap_13	none	OP807099	This study	yes	0	0	0	0	0	0	0	1	1
Hap_14	none	OP807100	This study	yes	0	0	0	0	0	0	0	7	7
Hap_15	SH8	OP807101	SMRU unpublished	yes	1	0	0	0	0	1	0	6	8
Hap_16	none	OP807102	This study	yes	0	0	0	0	0	0	0	10	10
Hap_17	PV12	HQ153118.1	Andersen et al 2011	no	0	0	0	0	0	0	0	1	1
Hap_18	none	OP807103	This study	yes	0	0	0	0	0	0	0	1	1
Hap_19	none	OP807104	This study	yes	0	0	0	0	0	0	0	1	1
Hap_20	none	OP807105	This study	yes	0	0	0	0	0	0	0	1	1
Hap_21	PV19	HQ153125.1	Andersen et al 2011	no	0	2	0	0	0	0	0	0	2
Hap_22	none	OP807106	This study	yes	1	0	0	0	0	0	0	0	1
Hap_23	SK84	OP807107	SMRU unpublished	yes	0	0	0	1	0	0	0	0	1
Hap_24	OH56388	OP807108	SMRU unpublished	yes	0	0	0	0	2	2	0	0	4
Hap_25	OR59028	OP807109	SMRU unpublished	yes	0	0	0	0	1	0	0	0	1
Hap_26	LI76496	OP807110	SMRU unpublished	yes	0	0	0	1	0	0	0	0	1
Hap_27	SH11	OP807111	SMRU unpublished	yes	0	0	0	0	0	1	0	0	1

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42x42mm (600 x 600 DPI)



355x177mm (600 x 600 DPI)