Research in Association with New Seal Licensing System

Research on the population structure of harbour seals

Final Report

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Report to Marine Scotland Science

January, 2012

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1 Summary

The population structure of harbour seals (*Phoca vitulina*) around Scotland was investigated using different genetic markers and approaches. This allowed discrete population units or metapopulations to be identified. The population genetic structure is compared to the recently defined harbour seal management regions (SCOS, 2011), ensuring Scottish Government's regional management procedures and plans for harbour seals are based on genetic data as well as the currently employed ecological haulout and pupping site data. Analysis of DNA samples from a total of 453 individuals around Scotland including samples from comparative regions in the UK and Europe (including an out-group of Pacific harbour seals) was carried out. Following some initial trials the most appropriate population differentiation analysis comprised 10 putative populations across all the samples analysed. Focusing on Scotland, Bayesian clustering analysis clearly separated Scotland from England, France and the Dutch Wadden Sea. In this scenario 3 clusters were generally identified: a) Norway, b)West Coast of Scotland/Northern Ireland and c) Pentland Firth / Orkney / Shetland / Moray Firth / Tay and Eden with some degree of shared individuals between them.

Examining the Scottish populations alone indicated there might be some additional separation between the Tay and Eden compared to the other north and east coast groups.

Within the Scottish populations a number of harbour seal Management Areas have been assigned based on haul outs and breeding sites (SCOS, 2011). The result of the genetic analyses reported here clearly supports the designation and definition of these Areas.

Allelic diversity and heterozygosity are standard measures that assess the level of inbreeding which populations display as a reflection of their 'genetic health'. The populations with relatively good sample sizes and low levels of genetic diversity were Shetland (n=2.545, H_O=*0.363*) and the Outer Hebrides (2.467, H_O= *0.331*). It has been widely shown that inbreeding, translated as very low levels of genetic diversity in wild populations is correlated with disease such as cancer (Acevedo-Whitehouse et al. 2003) and with susceptibility to pathogens such as parasites (Rijks et al. 2008) among others.

2 Introduction

It is well recognised that information on the genetic population structure and levels of genetic variation within and between populations of a species are critical to its successful conservation and management. In particular, it enables the identification of discrete units within populations that may be of evolutionary importance and that may require different management strategies (Bruford and Wayne 1993; Waples et al. 2008). The distribution of the harbour seal throughout its range has most likely been influenced by glaciation (King 1983) and more recently, by human impact on habitat and population size. These processes may be reflected in the patterns of genetic differentiation and variation among harbour seal populations (Kappe et al. 1997). The extent of genetic sub-structuring in this species will depend on the levels of gene flow, through migration, between populations. Thus the identification of genetically distinct populations is vitally important for the identification of management units and the appropriate calculation of PBR (potential biological removal).

Microsatellites consist of short runs of nucleotide repeats (nucleotides are the individual molecules, adenine, thiamine, guanine and cytosine (A,T,G and C) that make up DNA, that are scattered throughout most of the genome (Goldstein and Pollock 1997). This means sequences of A, T, G and C are repeated one after another. For example, one might be a stretch of di-nucleotides, AC, another might be one of tri-nucleotides, CCA. The majority of microsatellites are found within non-coding regions of the genome, i.e. regions that do not encode for proteins, allowing for high mutation rates in these regions. These regions are highly polymorphic in size because the number of times the nucleotide sequence is repeated varies between individuals, within populations and/or between species. Thus, one population may have 13 AC's repeated in a stretch whilst another might have 18 repeated, at the same location within the genome. The sizes of the particular stretches (called alleles) can be measured using gel electrophoresis or more commonly now using capillary electrophoresis. Therefore a locus (i.e. a particular region within the genome) with 13 repeats is one allele and within another individual the same locus that contains 14 repeats is another allele.

Microsatellites have revealed substantial levels of polymorphism (differences among populations, groups or individuals) where other markers that were used historically did not and this has greatly increased their popularity and utility in molecular ecology (Hughes and Queller 1993) and population management.

Mitochondrial genetics on the other hand investigates variation in the DNA contained in the mitochondria. This is inherited from the mother and can be used to trace lineages over longer timescales. A variable control region of the mitochondrial DNA, usually ~500 base pairs long, is sequenced (i.e. the order of the nucleotide bases is determined) and the sequences compared among individuals. This results in the identification of different haplotypes or unique sequences within the population that can be used to determine the genetic divergence between individuals from different groups or putative subpopulations.

The phylogeographic study of UK harbour seals (i.e. the geographical distribution of genetic variation and genealogical lineages within the species) was first carried out in the late 1980's (Goodman 1998), using tissue samples collected from animals that died during the 1988 phocine distemper virus epidemic. The population structure of seals from the UK and Europe including Iceland and the Baltic was studied using microsatellite markers (Bruford and Wayne 1993). Six population units were identified from the 12 areas studied: Iceland, Scotland-Ireland, English east coast, Wadden Sea, Western Scandinavia and East Baltic. However, only two populations from Scotland and one from England were included in this study and the number of microsatellite markers used was very small (n=7) compared to the number potentially available today (Osborne et al. 2011).

2.1 Aim and Objectives

The aim of this study was to determine the population structure of harbour seals in Scotland on a finer spatial scale than has been previously published. By increasing the sample size, expanding the geographical scope and increasing the number of microsatellite loci examined, a finer scale and resolution of population structure could be established. Over 200 seal microsatellite sequences are now available (Osborne et al. 2011). We have chosen 28 of the most highly polymorphic markers. In addition mitochondrial DNA (mtDNA) studies will also be included to determine the phylogeographic relations between UK populations and its neighbours.

2.2 Approach and Output

The focus of the research is the Scottish populations and all the samples available in the SMRU archive (n=254, Fig. 1) were analysed. Additional effort to collect samples from regions not present in the archive was required. A capture trip to Shetland was carried out in August 2010 when 19 animals were captured and sampled.

In addition, samples from dead seals retrieved by the Scottish Marine Mammal Strandings Scheme throughout Scotland were included, although the true origin of these seals may not

be certain. The output from this study is an analysis of the population structure of harbour seals around Scotland using both microsatellite markers and mtDNA haplotypes and its relationship to the management regions currently identified.

Figure 1. Sample locations for harbour seals in the UK. Comparison groups from the Wash, Thames, Chichester, Wadden sea, France and Norway are also included together with Pacific harbour seals as an outgroup.

3 Methods

3.1 DNA Extraction

A total of 453 skin samples from harbour seals were extracted with a salt saturated DNA extraction technique (Sunnucks and Hales 1996) with some modifications. The extracted samples were quantified in a nanodrop ND-1000 spectrophotometer and diluted to a working concentration of 10ng/µl. Forty-one samples from Norway, donated by Anne Kirstine Frie from the Marine Mammal Group at the Institute of Marine Research in Tromsø, were also included in this study.

3.2 Microsatellite genotyping

A total of 28 polymorphic microsatellites from different species were selected from 7 different publications on pinnipeds population genetics for PCR amplification and tested in this study (Table 1).

Table 1. Twenty-eight pairs of primers tested in this study. Each pair shows the sequence for both (forward) and r (reverse) primers, the reported sizes in each original study and the original source.

Locus	Primer sequence 5' 3'	Reported Size	Author
Hg6.1f	TGCACCAGAGCCTAAGCAGACTG		
Hg6.1r	CCACCAGCCAGTTCACCCAG	141-166	(Allen et al. 1995)
Hgdiif	ACCTGCCATAGTGCTCATC		
Hgdiir	GAGCCAACTAAGACAAGCC	111-141	(Allen et al, 1995)
Hg6.3f	CAGGGCACCTGAGTGCITATG		
Hg6.3r	GACCCAGCATCAGAACTCAAG	230-242	(Allen et al, 1995)
Hg8.10f	AATTCTGAAGCAGCCCAAG		
Hg8.10r	GAATTCTTTTCTAGCATAGGTTG	175-189	(Allen et al, 1995)
OrrFCB2f	CCATTTCATCCGATGGAAGGAG	109-115	
OrrFCB2r	CAAGGACAAGATAGTGACCTAGAC		(Buchananet al. 1998)
OrrFCB7f	GAACCAGGGAGGAAGACAGAGTG	197-223	
OrrFCB7r	CAGACTGTATCAGGAGGCTTTGG		(Buchanane *et a.*, 1998)
OrrFCB8f	ATTTCTTCTACCTTACCCAGCCAG	175-177	
OrrFCB8r	CTGGGCTTGTTTGTGGGCATAG		(Buchanane *et al*, 1998)
OrrFCB16f	ATCACCTCAATGAGAGTTTCATAATC	197-215	
OrrFCB16r	CTCCAACGTAAGTCTACATCTGTA		(Buchanan *et al.* 1998)
Pvc30f	GCATGTGATCTTACAGCAAT	166-168	
Pvc30r	CATGGGTTCTCAATAGAAGA		(Coltman et al. 1996)
Pvc78f	GAGTATACCTCCATACTACAC	146-150	
Pvc78r	AGTTGTTCTCCTGACCCAAG		(Coltman et al. 1996)
Lw-7f	TGGGCTTTCTACAGTTC	159-180	
Lw-7r	ACATAACTCAAGGGACAA		(Davis et al. 2002)
Lw-20f	GACTCTTGCCCCCTTCAG	122-146	
Lw-20r	GTTTCACAGACCTGCCTCTAGTG		(Davis et al. 2002)
Hl-20f	CTCAACACAGGCGTAATATTG	93-125	
Hl-20r	GATCTTTGACAAGGAGAGTATGTT		(Davis et al. 2002)
Hl-15f	TCATCTTGTAGTGCCAAAAAC	119-139	
Hl-15r	ATCTTTCAGTTGACCCTTCT		(Davis et al. 2002)
Lc-18f	TATTCTCCTCTCACCCCTG	275-302	
Lc-18r	AATCGGCTGCTGGTAAAT		(Davis et al. 2002)
Lc-26f	CTCAAGGGACTGAGCCACTCA	285-319	
Lc-26r	ACGGCAGGATTCTGAAACACT		(Davis et al. 2002)
Lc-28f	TTCATATAATACCCACCTCTGTAAG	128-136	
Lc-28r	TGCCTCGTGATGAAAAACT		(Davis et al. 2002)
Lw11f	CTCTCCCTCTCACCTTCC	169-177	
Lw11fr	GGCAAATGAGGTGATGTC		(Davis et al. 2002)
Sgpv2f	TTGTATCAGTCACTAGCCTGGC	161-167	
Sgpv2r	CAAATCGAGATAACATTGCCC		(Goodman 1997)
Sgpv10f	TTCACTTAGCATAATTCCCTC	132-138	
Sgpv10r	TCATGAATTGGTATTAGACAAAG		(Goodman 1997a)
Sgpv11f	CAGAGTAAGCACCCAAGGAGCAG	155-167	
Sgpv11r	GTGCTGGTGAATTAGCCCATTATAAG		(Goodman 1997a)
ZcwF07f	TATTCCTAGAGGGGCAAGTCAAG	(148–156)	
ZcwF07r	CATTGACTCTCTGAAATGGTGTC		(Hoffman et al. 2006)
ZcwF09f	TGTTTATACATGTGGTATGCACCTA	(124–130)	
ZcwF09r	TCTGTATAACCCAGAGAGGTCCAAT		(Hoffman et al. 2006)
ZcwA12f	CCATTCCCCAGGTACATACTTCAG	(196–218)	
ZcwA12r	AATACAGTTGGGGAGGGTAGGAG		(Hoffman et al. 2006)
Agaz-1f	ACTCATGCCCTGCTTGAAAT	238–260	
Agaz-1r	CAGGAGACTTAGGCCAGCAC		(Hoffman 2009)
Agaz-2f	CCCAAGTTTGACCCTCGATA	230–244	
Agaz-2r	GGAAGGTGGGCCTTAGGTAT		(Hoffman 2009)
Agaz-8f	GGGGAGCCCTGATAGAAATC	136–164	
Agaz-8r	AGATTGATGGCCTGGGAAC		(Hoffman 2009)
Agaz-9f	TTCATGAGTTGCTCTCCTTCTTC	198–210	
Agaz-9r	CATGCCTTGTTTGCAGGTTA		(Hoffman 2009)

From the 28 pairs selected only 26 were successfully amplified in our samples. Those remaining 26 were amplified with a fluorescent marker (D2, D3 or D4) for further genotyping in the Beckman Coulterer sequencer. The 26 pairs were organized in 4 groups according to its size and colour to minimize the number of groups for Multiplex PCR amplification as seen in Table 2.

Table 2. Loci groups (LG). Twenty-six microsatellites were arranged in 4 loci groups according to their size (in brackets) combining them in three dyes (D2, D3 and D4).

	Blue (D4)	Green (D3)	Black (D2)
LG1	OrrFCB2 (109-115) Zcwf07 (148-156) Lc18 (275-302)	 Hl20 (93-125) OrrFCB8 (175-177)	 Pvc78 (146-150) Hg8.10 (183-201)
LG2	Lc28 (128-136) OrrFCB7 (197-223)	Zcwf09 (94-98) Sgpv11 (152-166)	Hl15 (119-139) Lw11 (169-177)
LG3	Pvc30 (166-168) Lc26 (285-303)	Lw7 (159-173) Sgpv10 (134-136)	Lw20 (122-146) Hg6.3 (219-229)
LG4	 Hg6.1 (141-166) ZcwA12 (196-218)	 Agaz8 (136-164) Agaz9 (198-210)	Hgdii (111-141) Sgpv2 (163-167) Agaz1 (238-260)

Initial PCR conditions were the same for the four loci groups (LG) and consisted of 20 ng of genomic DNA, 5 µl of Multiplex mix and 3µl of primer mix in a 10µl reaction. The PCR profile was as follows: 95°C for 15 min followed by 30 cycles of 94°C for 30 s, 60°C for 90 s and 71°C for 45s, with a final extension of 72°C for 2 min. The multiplex PCR kit recommends a minimum annealing temperature (Ta) of 60 °C for markers with Ta between 50 and 60 °C.

Results from each LG are shown in Figs 2 to 5. Although in the original tests all the microsatellites amplified in each group, once 96 well-plates were used to simultaneously amplify 96 samples (i.e. multiplexed), some microsatellites stopped showing in each run.

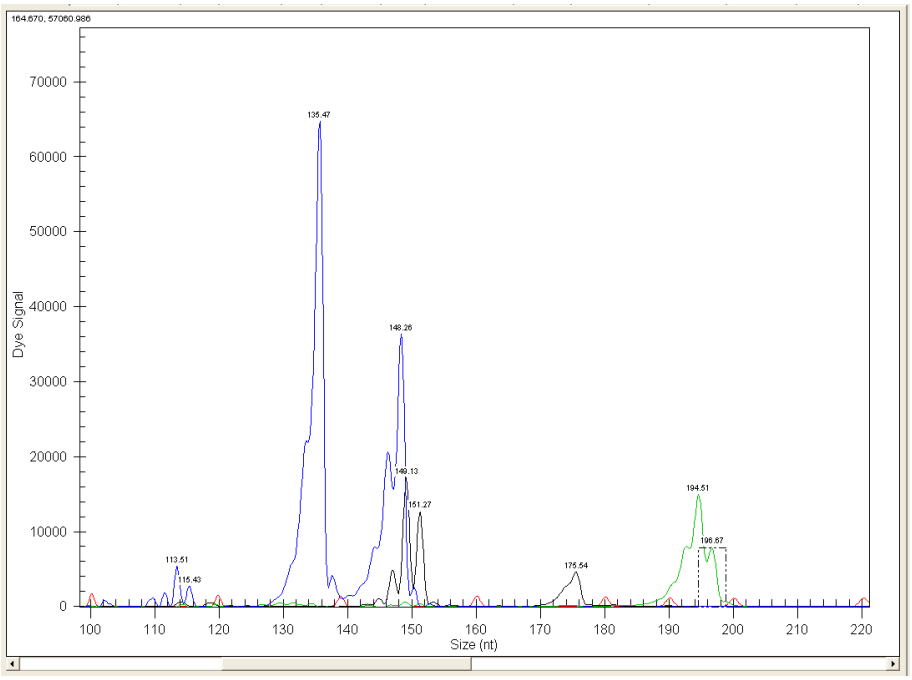


Figure 2. Locus Group 1 (LG1). Seven expected microsatellites and only five showed. Failed to amplify: Lc18 and HI20.

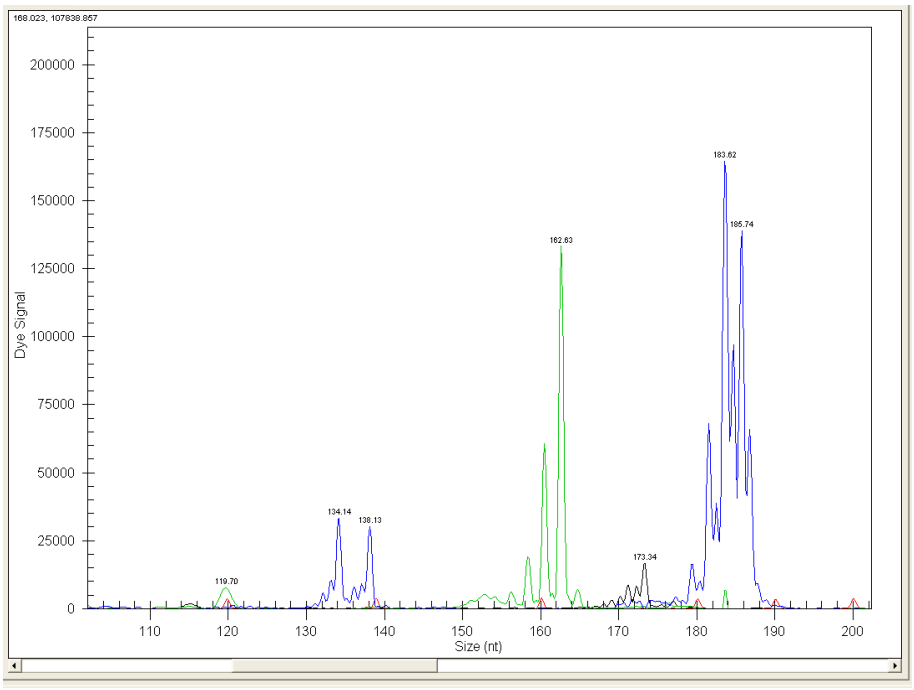


Figure 3. Locus Group 2 (LG2). Six expected microsatellites and only five obtained. Failed to amplify: HI15.

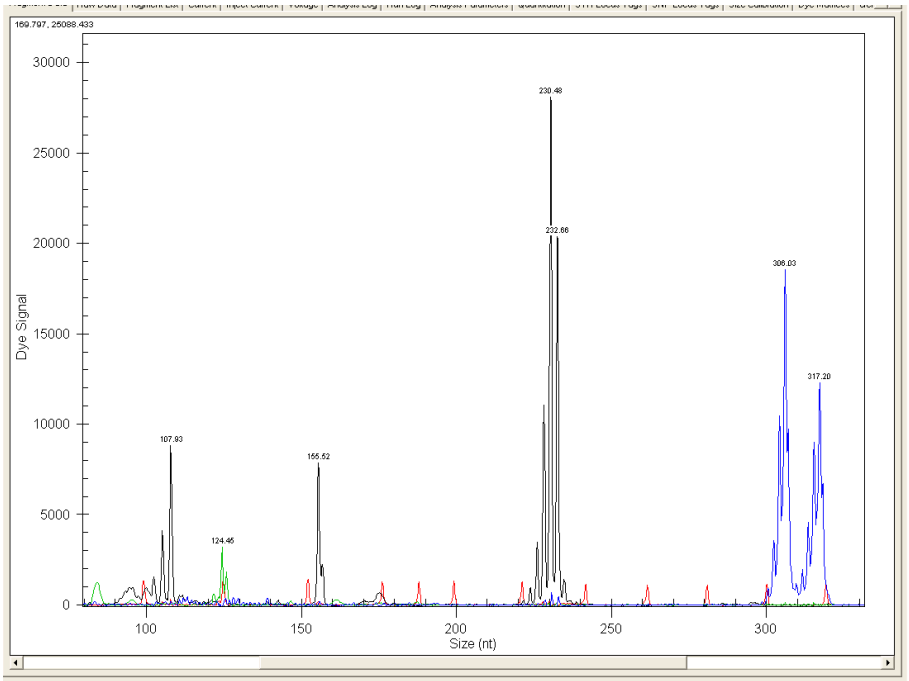


Figure 4. Locus Group 3 (LG3). Six microsatellites expected and four obtained. Failed to amplify: Lw7 and Pvc30.

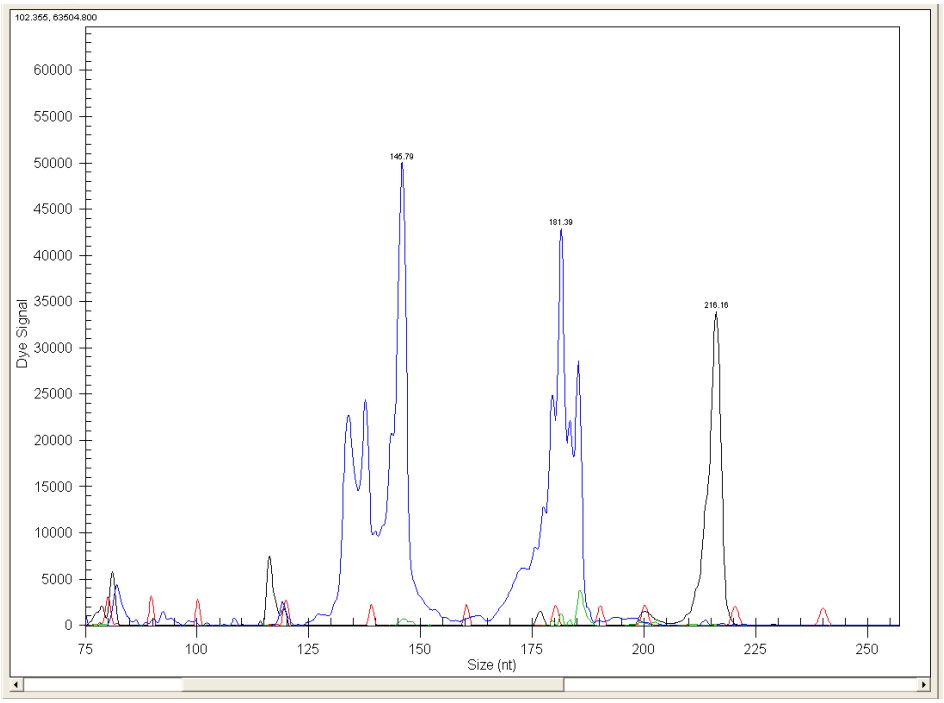


Figure 5. Locus Group 4 (LG4). Seven microsatellites expected and only three obtained. Failed to amplify: Agaz-8, Agaz-9, Hgdii and Sgpv2.

The nine remaining microsatellites have been re-grouped several times in different combinations and different temperatures. Finally seven of them amplified with a Ta of 50°C and the other two (Agaz-8 and Agaz-9) failed to amplify consistently in all the individuals analyzed and therefore eliminated from this analysis. The raw data obtained for each of the successfully amplified microsatellites, is plotted to determine the minimum and maximum boundaries for each allele, as shown in Fig. 6. Once the boundaries were determined for each allele of each locus, all the data was transformed to be converted into multiple input files for the following population genetic analyses.

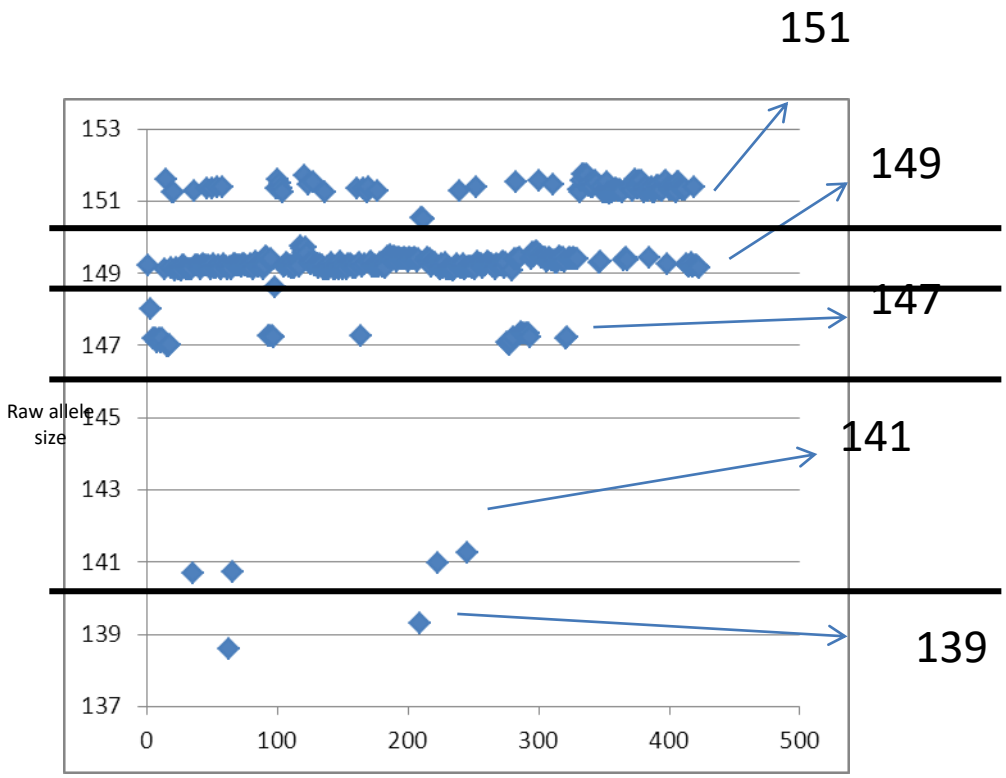


Figure 6. OrrFCB2 raw data. The boundaries between five alleles obtained for OrrFCB2 and the corresponding character state.

3.3 Microsatellite Analyses

All loci were run in Micro-checker (Van Oosterhout, Hutchinson et al. 2004) to check them for null alleles, missed genotyping and stutter bands. A random sample of 10% of the individuals was repeated to calculate the error rates of each locus. If the error was higher than 10% the loci were eliminated from the analysis. The remaining loci were used to assess the patterns of genetic structure and genetic diversity.

Genetic diversity was calculated as average number of alleles and expected and observed heterozygosity (H_E and H_O). Deviation from H-W equilibrium was calculated as the differences between H_E and H_O with the program ARLEQUIN 2.0 (Schneider et al. 2000). Hardy-Weinberg equilibrium states that, if there are no evolutionary forces, such as mutation, migration, natural or sexual selection acting on the populations, the allele frequencies from one generation to the other should not change (Hartl and Clark 1997[1]). Pairwise comparisons of genetic differentiation (F_{ST}) were conducted with the program GENEPOP (Raymond and Rousset, 1995) and FSTAT 2.9.3.2 (Goudet, 1995) was used to test the significance of the resulting estimates. As F_{ST} has proven to be restricted to show high levels of differentiation when loci show high values of heterozygosity, the index (D_{EST}) (Jost 2008), was also calculated. D_{EST} was calculated with the program SMOGD (Crawford 2009) and compared with F_{ST}. The linkage disequilibrium for each locus was also calculated with GENEPOP. A sequential Bonferroni correction (Rice 1989) was applied later to assess significance values.

Population structure was analyzed with a Bayesian clustering method in the program Structure 2.3.1 (Pritchard et al. 2000). The settings for this analysis were the following; burn in period was set to 150 000 iterations and the probability estimates determined using 5 000 000 Markov chain Monte Carlo (MCMC) iterations. Runs were conducted with K set from 1 to 10 with 5 runs for each value of K with both the admixture and no admixture models and correlated frequencies. The selected value of K represents the minimum number of clusters or populations, represented in our dataset. To obtain the true value of K from the log probability of the data LnP(D) Evanno et al. (2005) developed an ad hoc statistic called ΔK that calculates the second order rate of change of Ln P(D) between the values of K.

3.4 Mitochondrial DNA

To have a representative sample of the harbour seal management areas, a total of five individuals were sequenced from each of the following locations: Shetland, Orkney, Moray Firth, Tay and Eden Estuary, Outer Hebrides, Skye and IslayJura.

PCR conditions for amplification of Mitochondrial DNA were obtained from (Andersen et al. 2011). Primers L15926 (5'-ACACCAGTCTTGTAAACC-3') and PvH00034 (5'-TACCAAATGCATGACACCACAG-3') were amplified with the following conditions: 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl 2, 0.8 mmol/L (dNTPs), 1.5 units TaqDNA polymerase, 0.3 mmol/L of each primer, and 70 ng DNA template. The PCR profile consisted of initial denaturation of 90°C for 2.5 min, 37 cycles at 94 °C for 30 s, 48 °C for 60 s, and 72 °C for 60 s. A final cycle included 5 min at 72 °C, then cooling to 4 °C. The PCR products were purified with a QIAGEN-QIAquick gel extraction kit and quantified for further automated sequencing. Chosen individuals were sequenced in both directions (*forward and reverse)* to verify the identity of each. Sequences were edited, checked and aligned by eye with BIOEDIT 7.0.5.3**.**

Nucleotide (π) and haplotypic (h) diversities (Nei 1987) were calculated for each sample with the program ARLEQUIN 2.0 (Schneider et al. 2000.

MtDNA sequences of other populations of harbor seals were obtained from the GenBank. Duplicate haplotypes across the sample were obtained with the program COLLAPSE 1.2 (Posada © 1998-2006)). Individual haplotypes were analyzed to obtain a substitution model for the amplified region with the program MODELTEST 3.05 (Posada and Crandall 1998).

A Bayesian consensus tree was constructed with MrBayes 3 (Huelsenbeck and Ronquist 2001) using the substitution rates obtained from Modeltest. An autocorrelation test of the Ln function of the parameters obtained by MrBayes was used to determine the sampling frequency of each tree.

4 Results

4.1 Microsatellite markers

A total of 414 individuals from all the 20 populations were successfully amplified for 23 microsatellites: OrrFCB2, OrrFCB7, OrrFCB8, Pvc30, Pvc78, Sgpv2, Sgpv10, Sgpv11, Lw7, Lw11, Lw20, Lc18, Lc26, Lc28, HI-15, Hg6.1, Hg6.3, Hg8.10, Hgdii, Zcwf07, Zcwf09, ZcwA12, and Agaz-1. Approximately 10% of the individuals were replicated to estimate the error rate for each locus. Microsatellite loci with an error rate higher than 10% were eliminated from the analysis (OrrFCB2, OrrFCB7, Zcwf07, Zcwf09, Hg6.1, Agaz1, and Pvc30). The remaining 16 loci were analysed with Micro-checker (Van Oosterhout, Hutchinson et al. 2004) to check for null alleles, mis-scoring and stutter bands; only Hgdiii presented null alleles and was dropped out of the analysis. Fifteen microsatellite loci were analysed for total number of alleles (n), Observed and Expected Heterozygocity (H_O, H_E) and Hardy-Weinberg equilibrium in 20 putative populations (Table 3). These included the comparison populations and outgroup of Pacific Harbour seals.

After Bonferroni correction which set the significance level at $p = 0.00016$ only two loci (Lc18 and Sgpv2) were out of equilibrium the former in the Tay and Eden population and the latter in Norway and California. This indicates that overall all populations seemed to be in equilibrium. The average number of alleles (n) was highest in California (n=5.33) and Dutch Wadden Sea (n=3.667) and lowest in Thames and Chichester (n=2.273) followed by the Scottish populations of Loch Sheildag (n= 2.375) and Shetland (n=2.545). It has to be noticed that Loch Sheildag only has 4 individuals so it was expected to show low levels of genetic diversity. The rest of the populations were in between these values with higher values for the East Coast of Scotland, Islay/Jura and Norway, followed by the West Coast of Scotland and Northern Ireland and lastly the English and French populations (Table 3).

The genetic structure of these putative populations was investigated with Structure 2.3.1. Unfortunately the large amount of data made it impossible to run all the populations with all the loci, so we sub-divided the data set. The first run compared the East Coast of Scotland with the English, European and Pacific populations: Moray Firth, Tay and Eden, the Wash, Blakeney, Thames, Chichester, Normandy Dutch Wadden Sea and California (Fig. 6)

	OrrFCB8	Pvc78	Lc28	Sgpv11	Lw11	Sgpv10	Lw20	Hg6.3	Lc26	Sgpv2	ZcwA12	Lw7	Lc18	HI15	Hg8.10	
Strangford Lough, Northern Ireland N=23	*n=4* H_O=0.523 H_E=0.520 *P*= 0.496	*n=3* H_O=0.364 H_E=0.545 *P*= 0.02	*N=3* H_O=0.238 H_E=0.361 *P*=0.029	*n= 3* H_O=0.429 H_E=0.487 P= 0.757	*n=3* H_O=0.286 H_E=0.298 P= 0.448	*n=3* H_O=0.25 H_E=0.494 P= 0.021	*n=2* H_O=0.143 H_E=0.143 P=1.000	*n=4* H_O=0.571 H_E=0.524 P= 0.856	*n=3* H_O=0.286 H_E=0.512 P= 0.030	*n=3* H_O=0.647 H_E=0.572 P= 0.054	*n=3* H_O=0.316 H_E=0.351 P= 0.608	*n=4* H_O=0.6 H_E=0.655 P= 0.109	*n=1*	*n=2* H_O=0.429 H_E=0.363 P=1	*n=2* H_O=0.286 H_E=0.264 P=1	*n=3* H_O=0.383 H_E=0.406
Islay/Jura N=30	*n=3* H_O=0.56 H_E=0.496 P=0.695	*n=2* H_O=0.345 H_E=0.459 *P*=0.226	*n=3* H_O=0.435 H_E=0.580 *P= 0.005*	*n=3* H_O=0.4 H_E=0.524 *P= 0.013*	*n=3* H_O=0.182 H_E=0.312 *P= 0.108*	*n=2* H_O=0.588 H_E=0.471 *P= 0.591*	*n=4* H_O=0.4 H_E=0.490 *P= 0.121*	*n=4* H_O=0.696 H_E=0.662 *P= 0.918*	*n=2* H_O=0.348 H_E=0.394 *P= 0.607*	*n=5* H_O=0.421 H_E=0.561 *P= 0.052*	*n=3* H_O=0.4 H_E=0.371 *P=1.000*	*n=6* H_O=0.704 H_E=0.735 *P= 0.224*	*n=2* H_O=0 H_E=0.303 *P= 0.091*	*n=2* H_O=0.667 H_E=0.485 *P=1.000*	*n=2* H_O=0.833 H_E=0.682 *P=0.082*	*n=3.2* H_O=0.465 H_E=0.502
Arisaig N=10	*n=4* H_O=0.7 H_E=0.721 *P=0.654*	*n=2* H_O=0.5 H_E=0.521 *P=1.000*	*n=3* H_O= 0.6 H_E=0.563 *P=0.733*	*n=2* H_O=0.1 H_E=0.1 *P=1.000*	*n=2* H_O=0.1 H_E=0.1 *P=1.000*	*n=2* H_O=0.2 H_E=0.189 *P=1.000*	*n=3* H_O=0.60 H_E=0.468 *P=1.000*	*n=4* H_O=0.50 H_E=0.753 *P=0.420*	*n=2* H_O=0.40 H_E=0.505 *P=0.573*	*n=3* H_O=0.50 H_E=0.510 *P=1.000*	*n=3* H_O=0.50 H_E=0.416 *P=1.000*	*n=4* H_O=0.60 H_E=0.710 *P=0.657*	*n=1*	*n=2* H_O=0.20 H_E=0.337 *P=0.306*	*n=2* H_O=0.10 H_E=0.1 *P=1.000*	*n=2.714* H_O=0.4 H_E=0.400
Lismore N=24	*n=3* H_O=0.35 H_E=0.527 *P=0.133*	*n=2* H_O=0.5 H_E=0.485 *P=1.000*	*n=3* H_O=0.13 H_E=0.389 *P=0.0009*	*n=2* H_O=0.435 H_E=0.348 *P=0.538*	*n=2* H_O=*0.045* H_E=0.130 *P=0.070*	*n=3* H_O=0.273 H_E=0.385 *P=0.436*	*n=3* H_O=0.421 H_E=0.358 *P=1.000*	*n=4* H_O=0.478 H_E=0.571 *P=0.228*	*n=2* H_O=0.21 H_E=0.372 *P=0.071*	*n=4* H_O=0.385 H_E=0.545 *P=0.265*	*n=4* H_O=0.333 H_E=0.376 *P=0.631*	*n=4* H_O=0.813 H_E=0.724 *P=0.818*	*n=1*	*n=2* H_O=0.25 H_E=0.228 *P=1.000*	*n=2* H_O=0.25 H_E=0.344 *P=0.404*	*n=2.857* H_O=0.349 H_E=0.386
Skye N=14	*n=3* H_O=0.444 H_E=0.680 *P= 0.273*	*n=3* H_O=0.5 H_E=0.409 *P=1.000*	*n=3* H_O=0.692 H_E=0.551 *P=0.377*	*n=2* H_O=0.417 H_E=0.431 *P=1.000*	*n=2* H_O=0.286 H_E=0.264 *P=1.000*	*n=3* H_O=0.455 H_E=0.567 *P=0.424*	*n=4* H_O=0.286 H_E=0.267 *P=1.000*	*n=3* H_O=0.615 H_E=0.689 *P=0.604*	*n=3* H_O=0.385 H_E=0.578 *P=0.385*	*n=3* H_O=1.000 H_E=0.833 *P=1.000*	*n=3* H_O=0.231 H_E=0.342 *P=0.060*	*n=4* H_O=0.75 H_E=0.821 *P=0.772*	*n=1*	*n=2* H_O=0.286 H_E=0.254 *P=1.000*	*n=3* H_O=0.357 H_E=0.489 *P=0.044*	*n=2.92* H_O=0.479 H_E=0.478
Loch Sheildaig N=4	*n=3* H_O=0.5 H_E=0.464 *P=1.000*	*n=1*	*n=3* H_O=0.5 H_E=0.464 *P=1.000*	*n=2* H_O=0.25 H_E=0.25 *P=1.000*	*n=2* H_O=0.25 H_E=0.25 *P=1.000*	*n=1*	*n=1*	*n=2* H_O=0.5 H_E=0.571 *P=1.000*	*n=2* H_O=0.25 H_E=0.536 *P=0.428*	*n=1*	*n=2* H_O=0.25 H_E=0.25 *P=1.000*	*n=3* H_O=0.333 H_E=0.6 *P=0.198*	*n=1*	*n=1*	*n=1*	*n=2.375* H_O=0.354 H_E=0.226
Outer Hebrides N=18	*n=2* H_O=0.222 H_E=0.413 *P= 0.074*	*n=2* H_O=0.389 H_E=0.475 *P= 0.611*	*n=2* H_O=0.111 H_E=0.413 *P= 0.004*	*n=2* H_O=0.278 H_E=0.322 *P= 0.513*	*n=2* H_O=0.077 H_E=0.077 *P=1.000*	*n=2* H_O=0.077 H_E=0.471 *P= 0.004*	*n=3* H_O=0.529 H_E=0.469 *P=1.000*	*n=4* H_O=0.812 H_E=0.722 *P=0.755*	*n=3* H_O=0.294 H_E=0.533 *P=0.046*	*n=2* H_O=0.286 H_E=0.440 *P=0.442*	*n=3* H_O=0.444 H_E=0.398 *P=0.238*	*n=3* H_O=0.666 H_E=0.699 *P=1.000*	*n=2* H_O=0.055 H_E=0.055 *P=1.000*	*n=1* H_O=0.277 H_E=0.322 *P=0.513*	*n=3* H_O=0.444 H_E=0.452 *P=0.455*	*n=2.467* H_O=0.331 H_E=0.417
Pentland Firth N=8	*n=4* H_O=0.75 H_E=0.658 *P=0.736*	*n=2* H_O=0.5 H_E=0.4 *P=1.000*	*n=2* H_O=0.333 H_E=0.485 *P=1.000*	*n=2* H_O=0.167 H_E=0.167 *P=1.000*	*n=1*	*n=3* H_O=0.5 H_E=0.621 *P=0.213*	*n=1*	*n=3* H_O=0.429 H_E=0.582 *P= 0.624*	*n=3* H_O=0.333 H_E=0.667 *P= 0.134*	*n=2* H_O=0.286 H_E=0.264 *P=1.000*	*n=3* H_O=0.286 H_E=0.484 *P= 0.162*	*n=3* H_O=0.875 H_E=0.592 *P= 0.138*	*n=1*	*n=1*	*n=1*	*n=2.7* H_O=0.446 H_E=0.328
Orkney N=49	*n=3* H_O=0.545 H_E=0.598 *P= 0.591*	*n=3* H_O=0.327 H_E=0.336 *P= 0.018*	*n=3* H_O=0.292 H_E=0.284 *P=1.000*	*n=3* H_O=0.192 H_E=0.283 *P=0.021*	*n=2* H_O=0.229 H_E=0.248 *P=0.526*	*n=3* H_O=0.152 H_E=0.197 *P=0.025*	*n=4* H_O=0.227 H_E=0.209 *P=1.000*	*n=4* H_O=0.432 H_E=0.503 *P=0.620*	*n=3* H_O=0.522 H_E=0.629 *P=0.290*	*n=4* H_O=0.457 H_E=0.598 *P=0.019*	*n=3* H_O=0.422 H_E=0.475 *P=0.109*	*n=4* H_O=0.474 H_E=0.565 *P=0.052*	*n=3* H_O=0.08 H_E=0.079 *P=1.000*	*n=2* H_O=0.211 H_E=0.232 *P=0.493*	*n=2* H_O=0.378 H_E=0.489 *P=0.188*	*n=3.067* H_O=0.329 H_E=0.382
Shetland N=19	*n=2* H_O=0.0 H_E=0.533 *P= 0.200*	*n=1*	*n=2* H_O=0.263 H_E=0.235 *P=1.000*	*n=3* H_O=0.368 H_E=0.383 *P=1.000*	*n=2* H_O=0.263 H_E=0.309 *P=0.489*	*n=2* H_O=0.421 H_E=0.444 *P=1.000*	*n=3* H_O=0.263 H_E=0.421 *P=0.112*	*n=3* H_O=0.526 H_E=0.457 *P=0.619*	*n=3* H_O=0.474 H_E=0.579 *P=0.283*	*n=2* H_O=0.313 H_E=0.466 *P=0.274*	*n=3* H_O=0.368 H_E=0.522 *P=0.210*	*n=3* H_O=0.737 H_E=0.582 *P=0.505*	*n=1*	*n=1*	*n=1*	*n=2.545* H_O=0.363 H_E=0.329
Norway N=32	*n=5* H_O=0.516 H_E=0.680 *P= 0.187*	*n=3* H_O=0.033 H_E=0.158 *P= 0.001*	*n=3* H_O=0.156 H_E=0.255 *P= 0.030*	*n=3* H_O=0.531 H_E=0.536 *P= 0.645*	*n=3* H_O=0.419 H_E=0.423 *P= 0.185*	*n=2* H_O=0.531 H_E=0.504 *P=1.000*	*n=1*	*n=5* H_O=0.563 H_E=0.680 *P= 0.378*	*n=3* H_O=0.286 H_E=0.428 *P= 0.058*	*n=3* ***H_O*=0.0** ***H_E*=0.214** ***P=0.00002***	*n=4* H_O=0.548 H_E=0.668 *P= 0.358*	*n=6* H_O=0.655 H_E=0.776 *P= 0.151*	*n=1*	*n=1*	*n=1*	*n=3.63* H_O=0.385 H_E=0.355

Moray Firth N=41	*n=3* H_O=0.564 H_E=0.578 *P= 0.833*	*n=2* H_O=0.231 H_E=0.207 *P=1.000*	*n=2* H_O=0.237 H_E=0.212 *P=1.000*	*n=4* H_O=0.436 H_E=0.423 *P=0.137*	*n=3* H_O=0.385 H_E=0.355 *P=0.007*	*n=3* H_O=0.259 H_E=0.343 *P=0.161*	*n=3* H_O=0.4 H_E=0.345 *P=0.788*	*n=3* H_O=0.575 H_E=0.553 *P=0.670*	*n=3* H_O=0.474 H_E=0.616 *P=0.225*	*n=5* H_O=0.4 H_E=0.544 *P=0.043*	*n=3* H_O=0.344 H_E=0.50 *P=0.006*	*n=3* H_O=0.5 H_E=0.509 *P=0.710*	*n=1*	*n=3* H_O=0.28 H_E=0.365 *P=0.023*	*n=3* H_O=0.522 H_E=0.463 *P=0.279*	*n=3.071* H_O=0.400 H_E=0.404
Tay/ Eden N=36	*n=4* H_O=0.448 H_E=0.540 *P=0.037*	*n=4* H_O=0.071 H_E=0.105 *P=0.054*	*n=3* H_O=0.4 H_E=0.548 *P=0.021*	*n=4* H_O=0.417 H_E=0.414 *P=1.000*	*n=4* H_O=0.217 H_E=0.364 *P=0.035*	*n=2* H_O=0.083 H_E=0.223 *P=0.021*	*n=2* H_O=0.2 H_E=0.184 *P=1.000*	*n=4* H_O=0.485 H_E=0.490 *P=1.000*	*n=4* H_O=0.387 H_E=0.607 *P=0.003*	*n=3* H_O=0.292 H_E=0.324 *P=0.034*	*n=3* H_O=0.212 H_E=0.290 *P=0.017*	*n=4* H_O=0.593 H_E=0.674 *P=0.259*	***n=6*** ***H_O=0.158*** ***H_E=0.602*** ***P=0.0000***	*n=5* H_O=0.35 H_E=0.519 *P=0.0006*	*n=2* H_O=0.389 H_E=0.475 *P=0.611*	*n=3.6* H_O=0.313 H_E=0.424
Wash N=30	*n=4* H_O=0.48 H_E=0.551 *P=0.051*	*n=1*	*n=2* H_O=0.077 H_E=0.075 *P=1.000*	*n=3* H_O=0.5 H_E=0.494 *P=1.000*	*n=2* H_O=0.053 H_E=0.053 *P=1.000*	*n=2* H_O=0.25 H_E=0.296 *P=0.468*	*n=3* H_O=0.25 H_E=0.232 *P=1.000*	*n=4* H_O=0.76 H_E=0.68 *P=0.144*	*n=2* H_O=0.25 H_E=0.454 *P=0.057*	*n=4* H_O=0.15 H_E=0.458 *P=0.0004*	*n=3* H_O=0.429 H_E=0.542 *P=0.123*	*n=5* H_O=0.545 H_E=0.705 *P=0.002*	*n=2* H_O=0.125 H_E=0.125 *P=1.000*	*n=2* H_O=0.444 H_E=0.366 *P=1.000*	*n=2* H_O=0.75 H_E=0.5 *P=0.441*	*n=2.857* H_O=0.362 H_E=0.368
Blakeney N=14	*n=3* H_O=0.364 H_E=0.567 *P=0.222*	*n=1*	*n=2* H_O=0.083 H_E=0.083 *P=1.000*	*n=2* H_O=0.429 H_E=0.508 *P=0.621*	*n=1*	*n=1*	*n=1*	*n=3* H_O=0.571 H_E=0.624 *P=0.742*	*n=2* H_O=0.2 H_E=0.505 *P=0.080*	*n=3* H_O=0.4 H_E=0.511 *P=0.336*	*n=3* H_O=0.222 H_E=0.216 *P=1.000*	*n=3* H_O=0.667 H_E=0.562 *P=0.562*	*n=1*	*n=3* H_O=0.143 H_E=0.519 *P=0.0005*	*n=3* H_O=0.636 H_E=0.628 *P=0.513*	*n=2.7* H_O=0.372 H_E=0.315
Thames N=9	*n=2* H_O=0.429 H_E=0.495 *P=1.000*	*n=2* H_O=0.0 H_E=0.209 *P=0.059*	*n=1*	*n=2* H_O=0.556 H_E=0.425 *P=1.000*	*n=1*	*n=2* H_O=0.167 H_E=0.167 *P=1.000*	*n=2* H_O=0.111 H_E=0.111 *P=1.000*	*n=4* H_O=0.444 H_E=0.529 *P=0.712*	*n=2* H_O=0.111 H_E=0.425 *P=0.059*	*n=1*	*n=3* H_O=0.556 H_E=0.582 *P=1.000*	*n=1*	*n=2* H_O=0.143 H_E=0.143 *P=1.000*	*n=2* H_O=0.125 H_E=0.125 *P=1.000*	*n=2* H_O=0.375 H_E=0.525 *P=0.530*	*n=2.273* H_O=0.274 H_E=0.249
Chichester N=6	*n=2* H_O=0.6 H_E=0.467 *P=1.000*	*n=2* H_O=0.0 H_E=0.356 *P=0.112*	*n=2* H_O=0.0 H_E=0.303 *P=0.091*	*n=3* H_O=0.5 H_E=0.621 *P=0.213*	*n=1*	*n=2* H_O=0.4 H_E=0.356 *P=1.000*	*n=1*	*n=3* H_O=0.667 H_E=0.621 *P=1.000*	*n=2* H_O=0.0 H_E=0.303 *P=0.092*	*n=1*	*n=3* H_O=0.667 H_E=0.682 *P=1.000*	*n=1*	*n=2* H_O=0.6 H_E=0.467 *P=1.000*	*n=2* H_O=0.2 H_E=0.2 *P=1.000*	*n=2* H_O=0.6 H_E=0.467 *P=1.000*	*n=2.273* H_O=0.385 H_E=0.323
Normandy France N=12	*n=3* H_O=0.25 H_E=0.607 *P= 0.142*	*n=1*	*n=3* H_O=0.25 H_E=0.236 *P=1.000*	*n=2* H_O=0.5 H_E=0.464 *P=1.000*	*n=2* H_O=0.167 H_E=0.167 *P=1.000*	*n=2* H_O=0.2 H_E=0.556 *P=0.366*	*n=3* H_O=0.167 H_E=0.439 *P=0.090*	*n=5* H_O=0.833 H_E=0.772 *P=0.038*	*n=3* H_O=0.125 H_E=0.342 *P=0.066*	*n=2* H_O=0.667 H_E=0.533 *P=1.000*	*n=3* H_O=0.4 H_E=0.658 *P=0.122*	*n=3* H_O=0.25 H_E=0.75 *P=0.142*	*n=1*	*n=2* H_O=0.222 H_E=0.209 *P=1.000*	*n=2* H_O=0.4 H_E=0.533 *P=1.000*	*n=2.692* H_O=0.341 H_E=0.418
Dutch Wadden Sea N=12	*n=4* H_O=0.545 H_E=0.645 *P= 0.461*	*n=3* H_O=0.091 H_E=0.593 *P=0.0004*	*n=3* H_O=0.091 H_E=0.385 *P= 0.008*	*n=3* H_O=0.727 H_E=0.589 *P= 0.183*	*n=2* H_O=0.1 H_E=0.1 *P= 1.000*	*n=4* H_O=0.091 H_E=0.403 *P= 0.002*	*n=3* H_O=0.5 H_E=0.626 *P= 0.013*	*n=5* H_O=0.545 H_E=0.645 *P= 0.033*	*n=5* H_O=0.364 H_E=0.645 *P= 0.006*	*n=3* H_O=0.333 H_E=0.569 *P= 0.131*	*n=4* H_O=0.3 H_E=0.679 *P= 0.004*	*n=5* H_O=0.8 H_E=0.779 *P= 0.142*	*n=4* H_O=0.286 H_E=0.571 *P= 0.062*	*n=3* H_O=0.1 H_E=0.416 *P= 0.009*	*n=4* H_O=0.8 H_E=0.674 *P= 0.009*	*n=3.667* H_O=0.378 H_E=0.555
San Francisco, California[1] N=23	*n=5* H_O=0.625 H_E=0.726 *P= 0.133*	*n=3* H_O=0.136 H_E=0.280 *P= 0.027*	*n=6* H_O=0.478 H_E=0.705 *P=0.0007*	*n=6* H_O=0.783 H_E=0.723 *P= 0.445*	*n=4* H_O=0.435 H_E=0.592 *P= 0.291*	*n=4* H_O=0.727 H_E=0.735 *P= 0.326*	*n=10* H_O=0.682 H_E=0.867 *P= 0.006*	*n=7* H_O=0.739 H_E=0.748 *P= 0.962*	*n=5* H_O=0.609 H_E=0.664 *P= 0.071*	*n=7* H_O=0.632 H_E=0.836 *P= 0.009*	***n=5*** ***H_O=0.391*** ***H_E=0.744*** ***P=0.0001***	*n=6* H_O=0.826 H_E=0.754 *P= 0.264*	*n=3* H_O=0.077 H_E=0.335 *P= 0.005*	*n=6* H_O=0.810 H_E=0.765 *P= 0.676*	*n=3* H_O=0.714 H_E=0.528 *P= 0.122*	*n=5.33* H_O=0.576 H_E=0.667

Table 3. Genetic diversity measures for 20 putative populations of Harbour seals Phoca vitulina. (n) number of alleles, (HO) Observed and (HE) Expected Heterozygocity are shown for each population. Significance levels for Hardy-Weinberg equilibrium test after Bonferroni correction *P* = 0.00016 are highlighted in bold.

[1] NB This is sub-species, *Phoca vitulina richardsi* whereas UK harbour seals are *Phoca vitulina vitulina*

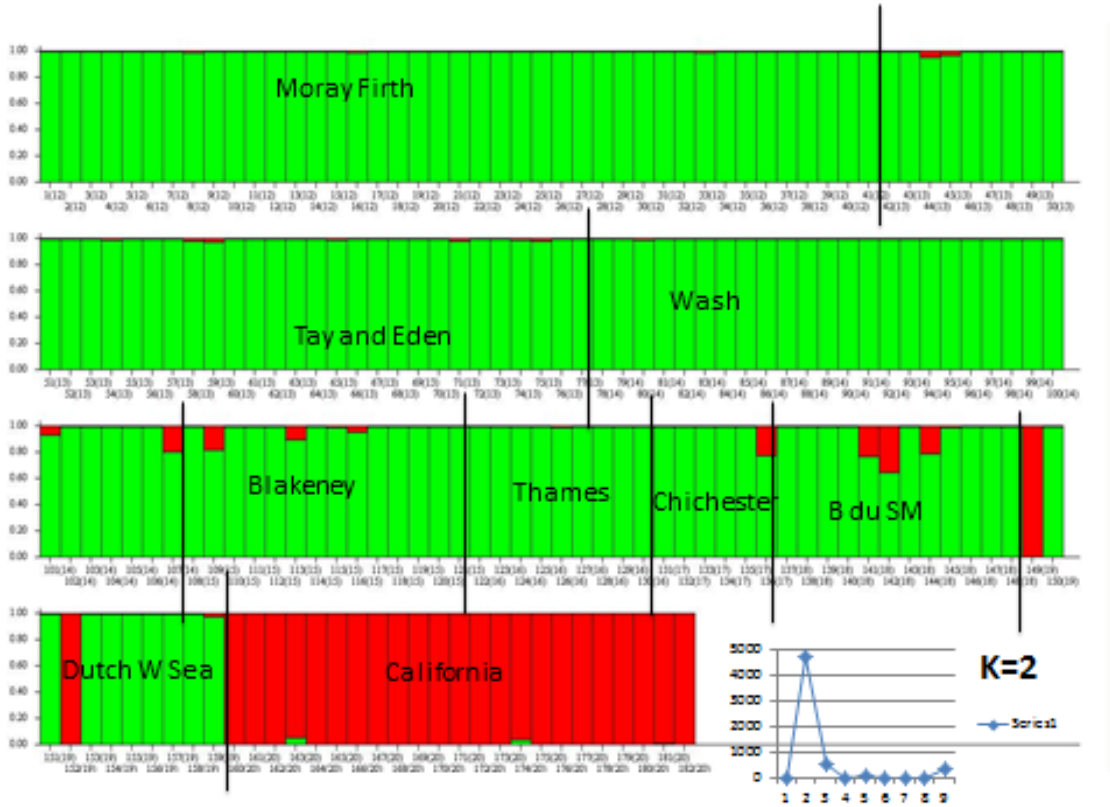


Figure 6. Barplot obtained with Structure 2.3.1 for a scenario of K=2. The Y-axis shows the likelihood of each individual's membership to a particular population. The putative populations shown in this analysis are: Moray Firth, Tay and Eden, the Wash, Blakeney, Thames, Chichester Harbour, Normandy, Dutch Wadden Sea, and California. The rate of change in K calculated with the Evanno method is shown in the right bottom corner, a clear peak in K=2 is observed.

The Californian population has been used in this study to have a parameter of comparison for a healthy and large population of harbour seals to be able to determine the status of the UK populations of the same species. Fig. 6 shows only two clusters as a result of our Bayesian analysis, each colour representing the population that a given individual animal is likely to belong to, thus each bar represents an individual DNA sample. The two clusters correspond to the Pacific and European harbour seals. This result shows us that despite a common ancestry these two populations have been isolated from each other long enough to show a deep divergence between them. This result is as expected as they are classified as different sub-species and serves to demonstrate the ability of our approach to separate these populations.

To focus on the fine-scale genetic differentiation of UK populations and its closer neighbours we eliminated the Pacific from the subsequent analyses (Fig. 7). The result of this analysis is a scenario with 3 populations (i.e. K=3). A clear separation between Scotland and England is observed as well as a different origin of some individuals from France and from the Tay and Eden populations.

The last sub-set included all the Scottish populations and the animals from Norway. Fig. 8 shows that the most likely scenario for these populations is K=3. A clear differentiation from the Scottish populations and the outgroup (Norway) is observed, along with a West/East general differentiation for Scotland but with individuals sharing information in both sides. This analysis is clearly well aligned with the current definition of the harbour seal Management Areas which have been assigned using ecological criteria (haul out and breeding sites) as shown in Fig. 9.

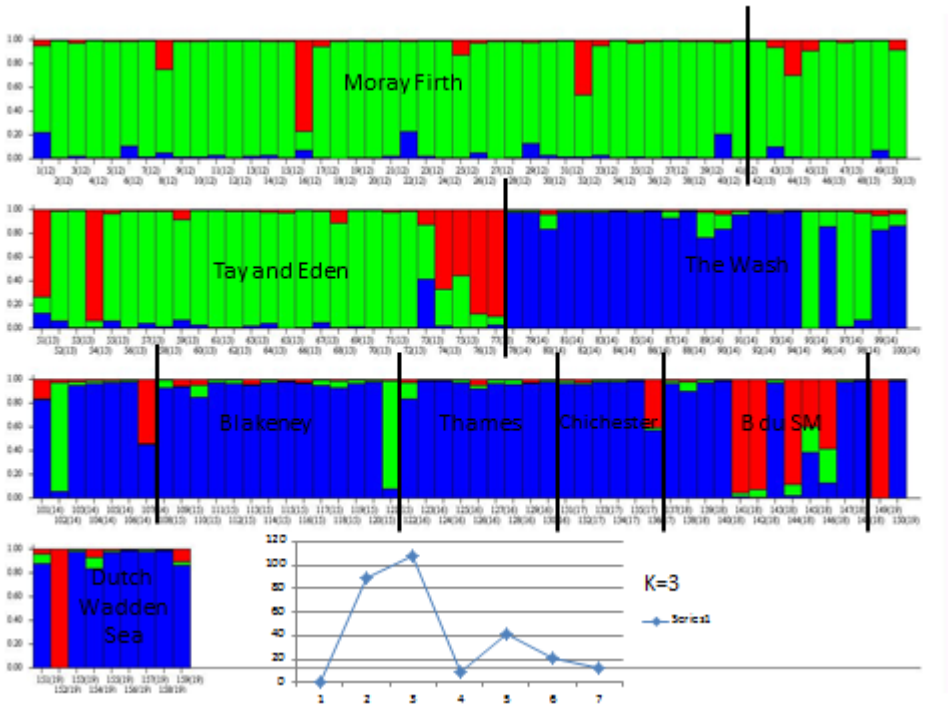
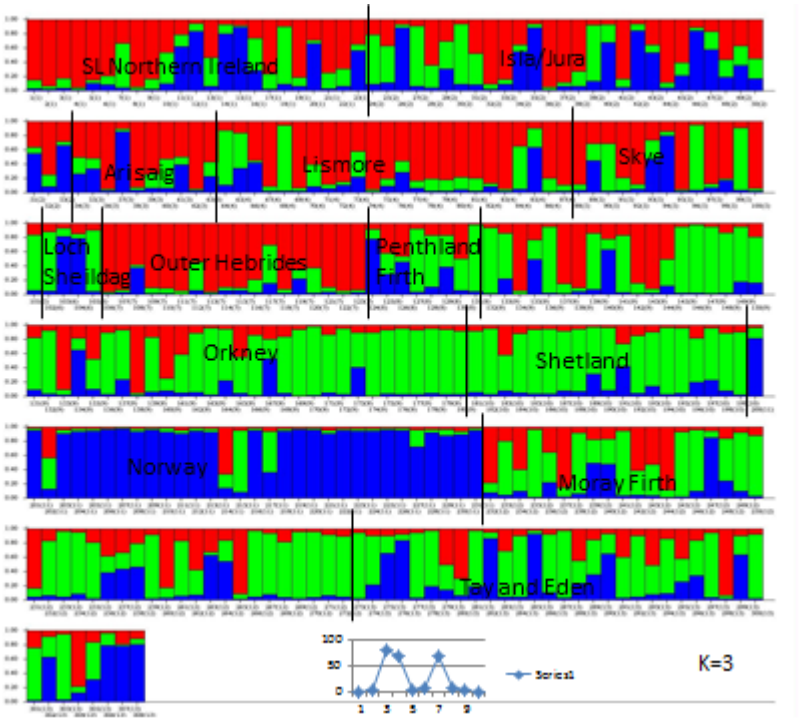


Figure 7. Barplot obtained with Structure 2.3.1 for a scenario of K=3. The Y-axis shows the likelihood of each individual's membership to a particular population. The putative populations shown in this analysis are: Moray Firth, Tay and Eden, the Wash, Blakeney, Thames, Chichester Harbour, Normandy and Dutch Wadden Sea. The rate of change in K calculated with the Evanno method is shown in the right bottom corner, a clear peak in K=3 is observed.



c

Figure 8. Barplot obtained with Structure 2.3.1 for a scenario of K=3. The Y-axis shows the likelihood of each individual's membership to a particular population. The putative populations shown in this analysis are: Strangford Lough SL, Northern Ireland, Isla/Jura, Arisaig, Lismore, Skye, Loch Sheildag, Outer Hebrides, Pentland Firth, Orkney, Shetland, Norway, Moray Firth and Tay/Eden. The rate of change in K calculated with the Evanno method is shown in the right bottom corner, a clear peak in K=3.

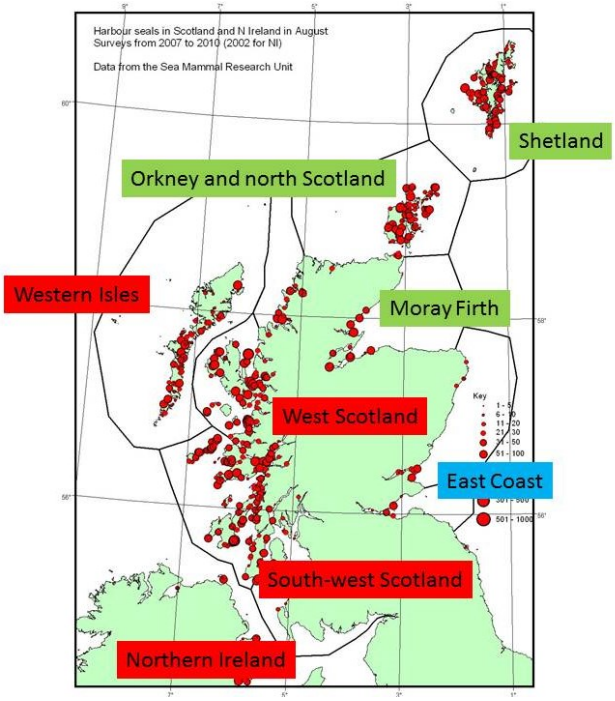


Figure 9. Map showing the harbour sela management regions and the putative populations from the final genetic analysis in different colours. This clusters the Western Isles with West and South-west Scotland and Northern Ireland. Shetland, Orkney and the Moray Firth with the East Coast remaining a separate population.

After this result we decided to re-calculate genetic diversity clustering together neighbouring populations that were similar in our Bayesian clustering analysis, along with its geographical distribution. Our last comparison set consisted of: Northern Ireland, West coast of Scotland, Pentland Firth/Orkney/Shetland, Norway, Moray Firth, Tay and Eden, England, France, Dutch Wadden Sea and California (Table 4.)

California showed the highest diversity values aspreviously, followed by the West Coast of Scotland, the Dutch Wadden Sea, Norway, Tay and Eden, the cluster formed by Pentland Firth/Orkney/Shetland, England, Moray Firth, Northern Ireland and Normandy.

In this analysis England showed 4 loci out of equilibrium, after Bonferroni correction (p= 0.00033), followed by the West Coast of Scotland with 2 loci out of equilibrium and only one in Norway. The higher number of loci out of equilibrium in these two populations (England and West Coast of Scotland) could be an indication of the Wahlund effect, which happens when a reduction in heterozygosity is caused by sub-structuring within the populations (Hartl and Clark 1997[2]). This result is expected as both populations are composed by several sub-populations pulled together by their genetic similarities.

Finally, to determine the differences in genetic differentiation among these ten clusters, the population differentiation indices (F_{ST} and D_{EST}) were calculated (Table 5). Due to small sample sizes and uninformative loci, not many pairwise comparisons were statistically significant.

The greatest differentiation, as expected, was for the significant pairwise comparisons with California; firstly with Pentland Firth/Orkney/Shetland ($F_{ST} = 0.4126$) , then with the West Coast of Scotland ($F_{ST} = 0.3910$), Tay and Eden ($F_{ST} = 0.37106$) and Dutch Wadden Sea having the smallest differences ($F_{ST} = 0.2887$). The West Coast of Scotland and the Dutch Wadden Sea show an intermediate level of differentiation ($F_{ST} = 0.1359$) and the last significant value is for West Coast of Scotland and Pentland Firth/Orkney/Shetland, ($F_{ST} = 0.0445$) that is the lowest value, suggesting a moderate amount of gene flow between these two clusters. D_{EST} values show higher values for the pairwise comparisons between California and Dutch Wadden Sea that resulted in a significant F_{ST} , but values for Pentland Firth/Orkney/Shetland and the West Coast of Scotland were similar.

4.2 Mitochondrial DNA

Results from this analysis will be provided in a Supplementary Report.

	Northern Ireland	West Coast of Scotland	PF/ Orkney/ Shetland	Norway	Moray Firth	Tay and Eden	England	France B du SM	Dutch Wadden Sea	California
OrrFCB	n=4 H_O=0.524 H_E=0.521 P=0.493	n=4 H_O=0.441 H_E=0.552 *P= 0.038*	n=4 H_O= 0.545 H_E=0.599 *P= 0.659*	n=5 H_O= 0.516 H_E= 0.679 *P= 0.184*	n=3 H_O=0.564 H_E=0.578 P= 0.832	n=4 H_O= 0.448 H_E=0.540 *P= 0.038*	n=4 H_O=0 .458 H_E=0.532 *P= 0.021*	n=3 H_O=0.250 H_E=0.607 *P=0.142*	*n=4* *H_O=0.545* H_E=0.645 *P= 0.461*	*n=5* *H_O=0.625* H_E=0.726 *P= 0.133*
Pvc78	n=3 H_O=0.363 H_E=0.545 P=0.019	n=3 H_O=0.411 H_E=0.456 *P= 0.579*	n=3 H_O=0.263 H_E=0.271 *P= 0.010*	n=3 H_O=0.333 H_E=0.157 *P= 0.001*	n=2 H_O=0.231 H_E=0.206 *P= 1.000*	n=4 H_O=0.071 H_E=0.105 *P= 0.057*	**n=2** ***H_O=0.000*** ***H_E=0.072*** ***P= 0.0003***	n=1	*n=3* *H_O=0.091* H_E=0.593 *P=0.0004*	*n=3* *H_O=0.136* H_E=0.280 *P= 0.027*
Lc28	n=3 H_O=0.238 H_E=0.361 *P=0.029*	**n=3** ***H_O=0.352*** ***H_E=0.506*** ***P= 0.00004***	n=3 H_O= 0.288 H_E=0.288 *P= 1.000*	n=3 H_O=0.156 H_E=0.254 *P= 0.029*	n=2 H_O=0.236 H_E=0.211 *P= 1.000*	n=3 H_O=0.400 H_E=0.547 *P= 0.021*	n=4 H_O=0.056 H_E=0.092 *P= 0.019*	n=3 H_O=0.250 H_E=0.235 *P=1.000*	*n=3* *H_O=0.091* H_E=0.385 *P= 0.008*	*n=6* *H_O=0.478* H_E=0.705 *P=0.0007*
Sgov11	n=3 H_O=0.428 H_E=0.486 *P=0.758*	n=3 H_O=0.348 H_E=0.377 *P= 0.001*	n=3 H_O=0.236 H_E=0.300 *P= 0.036*	n=3 H_O= 0.531 H_E=0.536 *P= 0.648*	n=4 H_O=0.435 H_E=0.423 *P= 0.140*	n=4 H_O=0.416 H_E=0.413 *P= 1.000*	n=4 H_O=0.491 H_E=0.513 *P= 0.028*	n=2 H_O=0.500 H_E=0.464 *P=1.000*	*n=3* *H_O=0.727* H_E=0.589 *P= 0.183*	*n=6* *H_O=0.783* H_E=0.723 *P= 0.445*
Lw11	n=3 H_O=0.285 H_E=0.298 *P= 0.449*	n=4 H_O=0.128 H_E=0.187 *P= 0.036*	n=2 H_O=0.216 H_E=0.245 *P= 0.318*	n=3 H_O=0.419 H_E=0.423 *P= 0.184*	n=3 H_O=0.384 H_E=0.355 *P= 0.007*	n=4 H_O=0.217 H_E=0.364 *P= 0.034*	n=2 H_O= .033 H_E=0.033 *P= 1.000*	n=2 H_O=0.167 H_E=0.167 *P=1.000*	*n=2* *H_O=0.1* H_E=0.1 *P= 1.000*	*n=4* *H_O=0.435* H_E=0.592 *P= 0.291*
Sgpv19	n=3 H_O=0.250 H_E=0.493 *P= 0.022*	**n=3** ***H_O=0.318*** ***H_E=0.507*** ***P= 0.00000***	n=4 H_O=0.275 H_E=0.343 *P= 0.0003*	n=2 H_O=0.531 H_E=0.503 *P= 1.000*	n=4 H_O=0.259 H_E=0.343 *P= 0.162*	n=2 H_O= 0.083 H_E=0.223 *P= 0.021*	n=2 H_O=0.250 H_E=0.267 *P= 0.564*	n=2 H_O=0.200 H_E=0.555 *P=0.365*	*n=4* *H_O=0.091* H_E=0.403 *P= 0.002*	*n=4* *H_O=0.727* H_E=0.735 *P= 0.326*
Lw20	n=2 H_O=0.142 H_E=0.142 *P=1.000*	n=6 H_O=0.440 H_E=0.412 *P= 0.239*	n=4 H_O=0.238 H_E=0.280 *P= 0.201*	n=1	n=3 H_O=0.400 H_E=0.344 *P= 0.786*	n=2 H_O=0.200 H_E=0.184 *P= 1.000*	n=3 H_O=0.119 H_E=0.114 *P= 1.000*	n=3 H_O=0.166 H_E=0.439 *P=0.092*	*n=3* *H_O=0.5* H_E=0.626 *P= 0.013*	*n=10* *H_O=0.682* H_E=0.867 *P= 0.006*
Hg6.3	n=4 H_O=0.571 H_E=0.523 P= 0.856	n=4 H_O=0.618 H_E=0.669 *P= 0.328*	n=4 H_O=0.457 H_E=0.498 *P= 0.760*	n=5 H_O= 0.562 H_E=0.680 *P= 0.376*	n=3 H_O=0.575 H_E=0.553 *P= 0.667*	n=4 H_O=0.485 H_E=0.489 P= 1.000	n=4 H_O=0.648 H_E=0.639 *P= 0.318*	n=5 H_O=0.833 H_E=0.771 *P=0.039*	*n=5* *H_O=0.545* H_E=0.645 *P= 0.033*	*n=7* *H_O=0.739* H_E=0.748 *P= 0.962*
Lc26	n=3 H_O=0.285 H_E=0.512 *P=0.029*	n=3 H_O=0.311 H_E=0.468 *P= 0.003*	n=3 H_O=0.492 H_E=0.618 *P= 0.070*	n=3 H_O=0.286 H_E=0.428 *P= 0.057*	n=3 H_O=0.474 H_E=0.616 *P= 0.225*	n=4 H_O=0.387 H_E=0.607 *P= 0.003*	**n=2** ***H_O=0.183*** ***H_E=0.436*** ***P= 0.00005***	n=3 H_O=0.125 H_E=0.342 *P=0.067*	*n=5* *H_O=0.364* H_E=0.645 *P= 0.006*	*n=5* *H_O=0.609* H_E=0.664 *P= 0.071*
Sgov2	n=3 H_O=0.647 H_E=0.572 *P=0.054*	**n**=6 H_O=0.400 H_E=0.581 *P= 0.00041*	n=4 H_O=0.396 H_E=0.529 *P= 0.003*	**n=3** ***H_O=0.000*** ***H_E=0.214*** ***P= 0.00003***	n=5 H_O=0.400 H_E=0.543 *P= 0.045*	n=3 H_O=0.291 H_E=0.323 *P= 0.032*	**n=4** ***H_O=0.200*** ***H_E=0.582*** ***P= 0.00001***	n=2 H_O=0.666 H_E=0.533 *P=1.000*	*n=3* *H_O=0.333* H_E=0.569 *P= 0.131*	*n=7* *H_O=0.632* H_E=0.836 *P= 0.009*
ZcwA12	n=3 H_O=0.316 H_E=0.352 *P= 0.603*	n=4 H_O=0.376 H_E=0.366 *P= 0.093*	n=4 H_O=0.394 H_E=0.491 *P= 0.002*	n=4 H_O=0.548 H_E=0.668 *P= 0.355*	n=3 H_O=0.343 H_E=0.549 *P= 0.007*	n=3 H_O=0.212 H_E=0.290 *P= 0.018*	n=3 H_O=0.442 H_E=0.537 *P=* 0.203	n=3 H_O=0.400 H_E=0.657 *P= 0.120*	*n=4* *H_O=0.3* H_E=0.679 *P= 0.004*	*n=5* *H_O=0.391* H_E=0.744 *P=0.0001*
Lw7	n=4 H_O=0.600 H_E=0.655 *P= 0.110*	n=6 H_O=0.696 H_E=0.718 *P= 0.083*	n=4 H_O=0.600 H_E=0.567 *P= 0.073*	n=6 H_O=0.655 H_E=0.775 *P= 0.154*	n=3 H_O=0.500 H_E=0.509 *P= 0.706*	n=4 H_O=0.592 H_E=0.673 *P= 0.279*	n=5 H_O=0.588 H_E=0.653 *P= 0.0007*	n=3 H_O=0.250 H_E=0.750 *P=0.141*	*n=5* *H_O=0.8* H_E=0.779 *P= 0.142*	*n=6* *H_O=0.826* H_E=0.754 *P= 0.264*
Lc18	n=1	n=3 H_O=0.021 H_E=0.063 *P= 0.011*	n=3 H_O=0.080 H_E=0.079 *P= 1.000*	n=1	n=1	**n=6** ***H_O=0.158*** ***H_E=0.602*** ***P= 0.00000***	n=2 H_O=0.192 H_E=0.177 *P= 1.000*	n=1	*n=4* *H_O=0.286* H_E=0.571 *P= 0.062*	*n=3* *H_O=0.077* H_E=0.335 *P= 0.005*
HI15	n=2 H_O=0.428 H_E=0.362 *P=1.000*	n=2 H_O=0.300 H_E=0.302 *P=1.000*	n=2 H_O=0.211 H_E=0.232 *P= 0.492*	n=1	n=3 H_O=0.280 H_E=0.365 *P= 0.024*	n=5 H_O=0.350 H_E=0.519 *P= 0.0006*	**n=4** ***H_O=0.222*** ***H_E=0.333*** ***P= 0.00003***	n=2 H_O=0.222 H_E=0.209 *P= 1.000*	*n=3* *H_O=0.1* H_E=0.416 *P= 0.009*	*n=6* *H_O=0.810* H_E=0.765 *P= 0.676*
Hg8.10	n=2 H_O=0.286 H_E=0.264 *P=1.000*	n=5 H_O=0.367 H_E=0.414 *P= 0.00043*	n=2 H_O= 0.378 H_E=0.488 *P= 0.188*	n=1	n=3 H_O=0.522 H_E=0.462 *P= 0.283*	n=2 H_O=0.388 H_E=0.475 *P= 0.613*	n=3 H_O=0.594 H_E=0.549 *P= 0.338*	n=2 H_O=0.400 H_E=0.533 *P= 1.000*	*n=4* *H_O=0.8* H_E=0.674 *P= 0.009*	*n=3* *H_O=0.714* H_E=0.528 *P= 0.122*
	n=3.0 H_O=0.383 H_E=0.435	n=3.933 H_O=0.368 H_E=0.438	n=3.267 H_O=0.338 H_E=0.388	n=3.636 H_O=0.385 H_E=0.468	n=3.071 H_O=0.400 H_E=0.496	n=3.6 H_O=0.313 H_E=0.424	n=3.2 H_O=0.298 H_E=0.372	n=2.69 H_O=0.341 H_E=0.482	*n=3.667* *H_O=0.378* *H_E=0.555*	*n=5.33* *H_O=0.576* *H_E=0.667*

Table 4. Genetic diversity measures for 10 populations of Harbour seals, *Phoca vitulina*. (n) number of alleles, (H_O) Observed and (H_E) Expected Heterozygocity are shown for each population. Significance levels for Hardy-Weinberg equilibrium test after Bonferroni correction P = 0.00033 are highlighted in bold.

Populations	Northern Ireland	West Coast of Scotland	Pentland Firth/Orkney/ Shetland	Norway	Moray Firth	Tay and Eden	England	Normandy	Dutch Wadden Sea	California
Northern Ireland		0.0178	0.1029	0.0974	0.0629	0.0172	0.1582	0.1240	0.1601	0.5683
West Coast Of Scotland	0.0384 NA		0.0591	0.0783	0.0555	0.2007	0.1058	0.0522	0.2988	0.6569
Pentland Firth, Orkney/ Shetland	0.0668 NA	**0.0445 P=0.00067**		0.1083	0.0137	0.1292	0.1592	0.1490	0.2779	0.6393
Norway	0.1823 NA	0.1608 NA	0.1669 NA		0.0476	0.0566	0.0593	0.0695	0.0336	0.5760
Moray Firth	0.0654 NA	0.0550 0.02511	0.0085 0.10533	0.1408 NA		0.0793	0.1394	0.0912	0.2055	0.5632
Tay and Eden	0.1021 NA	0.0571 0.00578	0.0555 0.00800	0.1823 NA	0.0578 0.03000		0.1305	-0.1676	0.0543	0.1389
England	0.1876 NA	0.1273 NA	0.1528 NA	0.1075 NA	0.1544 NA	0.1993 NA		0.0162	0.1451	0.6139
Normandy	0.1160 NA	0.0628 NA	0.0931 NA	0.0955 NA	0.0988 NA	0.1405 NA	0.0187 NA		-0.0069	0.4698
Dutch Wadden Sea	0.1573 NA	**0.1359 0.00044**	0.1835 0.00267	0.0754 NA	0.1778 P=0.01111	0.2087 P=0.00422	0.0565 NA	0.0287 NA		0.3374
California	0.3637 NA	**0.3910 P=0.00022**	**0.4126 P=0.00067**	0.3352 NA	0.3856 P= 0.00311	**0.3716 P= 0.00044**	0.4182 NA	0.3183 NA	**0.2887 P=0.00022**	

Table 5. Population differentiation pairwise comparisons. F_{ST} **values are show below the diagonal, P-values adjusted for multiple comparisons = 0.001111 obtained after :4500 permutations.** D_{EST} **values are shown above diagonal.**

5 Discussion and Conclusion

This study presents the first comprehensive analysis of harbour seal population structure and genetic diversity in Scottish waters. Nuclear molecular markers were employed to provide key population information to stakeholders, managers, and governmental agencies regarding the management and conservation of this species in Scotland.

Allelic diversity and heterozygosity are standard measures that assess the level of inbreeding which populations display as a reflection of their 'genetic health'. In this study we compared twenty different populations from Scotland, England, Northern Ireland, Norway, the Netherlands, France and California to get an insight into the status of Scottish harbour seals on a global scale.

In 1988 a severe outbreak of phocine distemper virus (PDV) killed approximately 50% of the European harbour seals (Harwood and Hall 1990). This event emphasized the importance of knowing the distribution of the genetic diversity and structure among the European populations in order to try to explain the differences in mortality between them. Previous studies found high to moderate levels of genetic diversity among European population using seven polymorphic microsatellites, but with almost half of this diversity driven by one single marker (Sgpv3), the remaining loci showed between 2 and 8 alleles (Goodman 1998). Twenty-four years later our study is investigating the patterns of genetic diversity in the survivors of this and the 2002 PDV epidemics.

First, we attempted to determine genetic diversity for each of the putative populations sampled (Table 3), but the effect of small sample sizes in several populations resulted in several monomorphic loci and a failure to calculate allelic richness for these. Nevertheless, the values obtained in this analysis: average number of alleles along with observed and expected heterozygosity (H_O, H_E) showed the highest values in California, followed by the Scottish and European populations. Despite the several arrangements of populations, separated or clustered together the highest diversity levels were found consistently in California, the Dutch Wadden Sea, Norway, East Scotland and Northern Ireland. If England is combined (Table 4) it shows the same amount of genetic diversity as these main populations, but if it is separated into sub-units it shows the lowest levels of genetic diversity along with France. The same occurs with the populations in Scotland that have very small sample sizes: Loch Sheildaig (N=4) and Pentland Firth (N=8). The putative populations with relatively good sample sizes and low levels of genetic diversity are Shetland (N=19) with an average number of alleles n=2.545 and H_O= *0.363,* and the Outer Hebrides (N=18) with an average number of alleles n=2.467 and H_O= 0.331, with N=19 and N=18 respectively. Compared to the previous results reported by Goodman (1998) for the Scottish East Coast (SEC), Scottish West Coast (SWC), Irish East Coast (IEC) and the Norwegian Coast (NOR) our overall H_O (observed heterozygosity) values are lower than Goodman's but when we looked at the 3 loci that we have in common for the same populations, the number of alleles and H_O for each locus (Sgpv10, Sgpv11 and Hg.6.3) are the same or slightly higher in our study. Compared to California, overall values of H_O in UK harbour seals are low (Table 4).

This is important because it has been widely shown that inbreeding, translated as very low levels of genetic diversity in wild populations is correlated with disease such as cancer (Acevedo-Whitehouse et al. 2003) and with susceptibility to pathogens such as parasites (Rijks et al. 2008) among others.

Population differentiation comparisons between the set of 10 populations resulted in mostly non-significant results due to the small samples sizes in some populations and the lack of informative loci combined. In addition samples sizes in this subset were not sufficiently large to estimate gene flow. Simulation studies have shown that the use of microsatellite data can lead to serious overestimates of gene flow unless population sample sizes are >50 and many loci (>20) have been investigated (Gaggiotti et al. 2004).

It has also been suggested that the rejection of panmixia given by significant values of F_{ST}, is not enough to determine population structure and assign management units (Taylor and Dizon 1999; Palsboll et al. 2007). For this reason it was decided to perform a Bayesian Clustering Analysis with Structure 2.3.1 to group the populations in a more natural way. The Evanno method used to determine highest hierarchical level of genetic differentiation differentiated the data set in two populations, California and a cluster representing Scotland, England, France and the Netherlands. As this method looks for the highest level of differentiation, the presence of a population from a different sub-species (California) underestimates the fine population structure that could be present within the second cluster (Fig. 6).

A second run of Structure was then performed without California and the Evanno method determined a value of K=3. This clustered the East coast Scottish populations together (Moray Firth, Tay and Eden) as well as the English populations (The Wash, Blakeney, Thames and Chichester), the Dutch Wadden Sea and Normandy together. A third run of Structure included all of Scotland and Norway, the analysis also showed a value of K=3 with the main clusters being: a) Norway, b) Northern Ireland, Isla/Jura, Arisaig, Lismore, Skye and Outer Hebrides and c) Loch Sheildaig, Pentland Firth, Orkney, Shetland, Moray Firth, Tay and Eden. Examining the Scottish populations alone indicated there might be some additional separation between the Tay and Eden compared to the other north and east coast groups.

Based on these clusters population differentiation pairwise comparisons among the ten populations showed highest and similar significant values between California and Pentland Firth/Orkney/Shetland, West Coast of Scotland and Tay and Eden. The smallest significant comparisons were between the Dutch Wadden Sea and California as well as between Pentland Firth/Orkney/Shetland and the West Coast of Scotland (Table 5). It was not unexpected to observe a higher genetic similarity among the Scottish populations but it was interesting to observe a higher genetic connectivity between California and the Dutch Wadden Sea. This probably comes from a shared ancestry that has been maintained in the Dutch Wadden Sea. This was also observed in the first Bayesian clustering analysis, where California separated from all the European populations but there were a couple of individuals in the Dutch Wadden Sea that fully matched the California population (Fig. 6). In this figure

a very small fraction of some Scottish individuals shared with the California population but a few individuals from France showed a shared ancestry between the two populations obtained under that model. The same French and Dutch individuals differentiated themselves from the others in the next analysis where California was eliminated (Fig. 7). In this scenario of 3 populations there is a clear separation between Scotland and a cluster comprising England, France and Dutch Wadden Sea, except for the individuals mentioned.

The difference between the harbour seals from California and those from the UK was not surprising, given that they are classified as a separate sub-species. However, the inclusion of this outgroup in the analysis illustrates the magnitude of the differences between completely isolated populations.

A number of harbour seal Management Areas have been assigned to the Scottish populations based on haul outs and breeding sites (SCOS, 2011). The result of the genetic analyses reported here clearly supports the designation and definition of these Areas. Some broader genetic clustering is apparent (e.g. North coast and Orkney with Shetland and Outer Hebrides with West Scotland Highland) but ecological separations based on haul out sites and associated local foraging areas are likely to be as important in the management of these populations as the maintenance of their genetic diversity.

6 References

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